Inspector Instructions:

READ	• Sampling of optical alignment/laser output checks • Sampling of procedures for optical alignment, calibration, color compensation, and laser checks • Sampling of calibration records with fluorochrome standards
ASK	• How does your laboratory monitor instrument reproducibility? • How does your laboratory ensure each fluorochrome is appropriately calibrated? • How does your laboratory determine appropriate color compensation settings? • How is carryover mitigated for rare event assays and paucicellular specimens?

FLO.25150 Optical Alignment — Phase II

The laboratory monitors optical alignment (where applicable) and instrument reproducibility at least daily, or after each time the flow cytometer is restarted.

NOTE: Verifying reproducibility of instrument performance is an essential element of quality assurance within the laboratory. Instrument performance must be monitored under the same conditions used to run test samples.

Evidence of Compliance:

✓ Records for monitoring optical alignment (where applicable) and instrument reproducibility

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.30250 Fluorochrome Standards — Phase II

Appropriate standards for each fluorochrome (*eg,* fluorescent beads) are run each day that the instrument is used as part of the quality control to verify instrument performance, and the results are recorded and reviewed.

NOTE: These steps are necessary to optimize the flow system and the optics of the instrument.

Evidence of Compliance:

✓ Records of results, with corrective action when quality control beads fail

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.30255 Voltage Settings — Phase II

The laboratory optimizes the voltages for each specific assay.

NOTE: Voltage settings for each PMT must be optimized in order to maximize the resolution (signal-to-noise ratio) and place the antigen-negative and antigen-positive populations visibly "on -scale" for analysis. This is particularly important for dimly expressed antigens as well as visualization of antigen-negative populations. Appropriate voltage settings must be determined during validation of the specific assay; however, timing and frequency of monitoring on-going performance is at the discretion of the laboratory director.

Evidence of Compliance:

✓ Records demonstrating voltage optimization

REFERENCES

1) Illingworth A. ICCS Quality and Standards Committee. Instrument optimization - Adjusting PMT voltages and compensation on a Beckman Coulter System [module]. March 13, 2017.
2) Griffin ML, Batchelder J, Hoffman B, et al; ICCS Quality and Standards Committee. Instrument Optimization for BD FACSCanto Instruments - Creating Application Settings for White Blood Cells using Lyse/Wash, or Lyse/No Wash methods [module]. May 3, 2017.
3) Perfecto SP, Ambrozak D, Nguyen R, Chattopadhyay P, Roederer M. Quality Assurance for polychromatic flow cytometry. *Nat Protoc.* 2006;1(3):1522-30.
4) Maecker HT and Trotter J. Flow Cytometry Controls, Instrument Setup and the Determination of Positivity. *Cytometry Part A.* 2006; 69(9):1037-42.

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FLO.30260 Color Compensation Settings **Phase II**

The laboratory determines and monitors appropriate color compensation settings.

NOTE: For multi-color analysis there must be a procedure to ensure that cells co-labeled with more than one fluorescent reagent can be accurately distinguished from cells labeled only with one reagent. Cells stained with mutually exclusive antibodies bearing the relevant fluorochromes or singly-stained cell samples for each fluorochrome may be used to establish appropriate compensation settings. Alternatively, beads labeled with the appropriate fluorochromes may be used; these form the basis of some automated instrument setup. When beads are used, it is important that an unstained "negative" bead that approximates the autofluorescence of unstained cells is included.

Compensation settings must be determined during validation of each assay and may vary with different antibody combinations. Compensation settings must be reviewed periodically to ensure selected settings remain appropriate. Laboratories must have a documented process for checking compensation at defined intervals (such as after routine preventative maintenance or repair to the flow cytometer as these may alter the compensation). The procedure must include appropriate acceptance criteria and actions to be taken when those criteria are not met. The time interval for review of the compensation setting is at the discretion of the laboratory director.

Evidence of Compliance:

✓ Records of periodic review of color compensation settings and corrective action

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) Byrd T, Carr KD, Norman JC, Huye L, Hegde M, Ahmed N. Polystyrene microspheres enable 10-color compensation for immunophenotyping of primary human leukocytes. *Cytometry A.* 2015 Nov;87(11):1038-46. doi: 10.1002/cyto.a.22717. Epub 2015 Jul 22.

FLO.30270 Laser Performance **Phase I**

The laboratory ensures acceptable and constant laser instrument performance.

NOTE: For some instruments, current is a better gauge of laser performance than is power output, which may be relatively constant.

Evidence of Compliance:

✓ Records of laser performance checks

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FLO.30275 Carryover Mitigation **Phase II**

The laboratory has a process to evaluate and mitigate against carryover when applicable, including rare event assays (MRD, PNH) and paucicellular specimens.

NOTE: The laboratory must perform formal assessment of carryover during validation for assays or specimens that are particularly susceptible to it, and reassess carryover after major maintenance or repair of the instrument. Rare event assays (including MRD and PNH testing) and paucicellular specimens (such as CSF) may be affected significantly by sample carryover