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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.
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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

introduced during sample processing or during data acquisition. The definition of a paucicellular sample may vary with sample type and composition, and the procedure must include such a definition per the discretion of the laboratory director (for example, a total cell count of 200 or less for a CSF sample).

The assay procedure must define limits for carryover during assessment and include methods for reducing possible carryover, if detected. Appropriate corrective action must be taken when defined carryover limits are exceeded. Methods for reducing this possibility include (but are not limited to):

- *Cleaning the instrument prior to running an assay*
- *Washing or flushing the sample probe between sample tubes*
- *Use of a blank tube (eg, tube containing cell-free liquid (PBS, dH20, diluent) or an unstained control tube prior to running a specimen).*

Evidence of Compliance:

✓ Records of carryover studies performed at validation and following major maintenance/repair

REFERENCES

1) 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.
2) Oldaker TA, Wallace PK, Barnett D. Flow cytometry quality requirements for monitoring of minimal disease in plasma cell myeloma. *Cytometry B Clin Cytom*. 2016;90(1):40-46.

PROCEDURES AND TEST SYSTEMS

NOTE: Reticulocyte quantification by flow cytometry is separately covered in the Hematology and Coagulation Checklist.

IMMUNOPHENOTYPING

Inspector Instructions:

	• Select a representative assay and follow the entire process from specimen receipt to final result reporting • If problems are identified during the review of immunophenotyping procedures, further evaluate the laboratory's responses, corrective actions and resolutions.

BLOOD LYMPHOCYTE SUBSET ENUMERATION

Inspector Instructions:

	• Sampling of lymphocyte subset analysis policies and procedures (includes procedure describing method to set markers (cursors) to distinguish between negative and positive fluorescence cell populations)
	• How does your laboratory ensure specimen integrity? • How are specimens stored after initial processing? • How does your laboratory validate lymphocyte gates? • How are results of lymphocyte subset analysis corrected for gate purity?