

Flow Cytometry Checklist

CAP Accreditation Program



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Flow Cytometry Checklist



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ON-LINE CHECKLIST AVAILABILITY AND RESOURCES

Participants of the CAP accreditation programs may download the checklists from the CAP website (cap.org) by logging into e-LAB Solutions Suite. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

A repository of questions and answers and other resources is also available in e-LAB Solutions Suite under Accreditation Resources, Checklist Requirement Q & A.

SUMMARY OF CHECKLIST EDITION CHANGES

Flow Cytometry Checklist

09/22/2021 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
 - Modifications that may require a change in policy, procedure, or process for continued compliance; or
 - A change to the Phase
3. Deleted/Moved/Merged:
 - Deleted
 - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
 - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for on-site inspections and self-evaluations may not list all of these requirements.

NEW Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
FLO.30825	09/22/2021

REVISED Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
FLO.23325	06/04/2020
FLO.23737	09/22/2021
FLO.23800	09/22/2021
FLO.30840	09/22/2021
FLO.31000	06/04/2020

DELETED/MOVED/MERGED Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
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FLO.24275

09/21/2021

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a flow cytometry laboratory section or department.

Flow cytometry inspectors must be pathologists, clinical scientists or medical technologists who are actively involved with or have extensive recent experience in the practice of flow cytometry, are knowledgeable about current CAP Checklist and CLIA requirements, and have completed CAP Inspector Training. Inspectors should, to the greatest extent possible, be peers of the laboratory being inspected.

For laboratories performing only the interpretation component of flow immunophenotyping data (the flow technical component is performed at an outside flow laboratory), the following Flow Cytometry Checklist requirements apply: FLO.18385, FLO.23706, FLO.30640, FLO.30730, and FLO.30790. Additionally, requirements located in the All Common Checklist addressing proficiency testing, quality management, procedure manual, specimen rejection, and results reporting are applicable.

Laboratories not subject to US regulations: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).

PROFICIENCY TESTING

Inspector Instructions:



- Sampling of peer education records

FLO.18385 Peer Education Program

Phase I

For laboratories that perform only interpretations of flow immunophenotyping data, the laboratory participates in a peer education program in interpretive flow cytometry.

NOTE: This checklist item applies to laboratories that do not perform staining and acquisition of flow cytometry data, but which receive list mode files and/or representative dot plots from an outside laboratory for interpretation.

Programs dealing with analysis of flow data from hematolymphoid neoplasias and related benign conditions provide valuable educational opportunities for peer-performance comparisons. While not completely emulating the clinical setting involved in flow immunophenotyping, the peer data developed by these programs can provide a useful benchmark against which laboratory performance can be evaluated.

Evidence of Compliance:

- ✓ Records of enrollment/participation in an educational peer-comparison program for interpretive flow cytometry **OR** records for participation in a laboratory-developed program circulating cases with other laboratories or within the laboratory's own practice with records of peer review

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

QUALITY MANAGEMENT

REAGENTS

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of new antibody validation records • Sampling of new lot/shipment antibody and detection system reagent confirmation records
	<ul style="list-style-type: none"> • What procedure does your laboratory follow to ensure manufacturer's recommendations are followed regarding the use of kit reagents/controls? • How do you confirm the acceptability of new reagent lots?

Additional requirements are in the REAGENTS section of the All Common Checklist. Reporting requirements for use of analyte-specific reagents and other reagents used in laboratory-developed tests are included in the All Common Checklist (COM.40850).

FLO.23250 Reagent Usage Phase II

The recommendations of the manufacturer for the proper use of reagents and controls in kit procedures are followed.

Evidence of Compliance:

- ✓ Written procedure consistent with manufacturer's instructions **OR** records of method validation if alternative procedures are used

REFERENCES

- 1) Caldwell CW. Analyte-specific reagents in the flow cytometry laboratory. *Arch Pathol Lab Med*. 1998;122:861-864

FLO.23275 Antibody Validation Phase II

The laboratory has recorded the initial validation of new antibodies, prior to use in patient diagnosis.

NOTE: Antibodies used are validated on the cell sub-population of interest in the context of the antibody combination used in an assay.

****REVISED** 06/04/2020**

FLO.23325 New Reagent Lot/Shipment Confirmation of Acceptability

Phase II

The performance of new lots/shipments of antibody and reagents (eg, lysing agents, permeabilization reagents) is compared with old lots/shipments before or concurrently with being placed into service and records retained.

NOTE: Parallel staining is required to control for variables such as disparity in the lots of detection reagents or instrument function. Comparing the results of the new reagent run in parallel on the same fresh control (patient or normal) is recommended to ensure that the new reagent lot provides a clinically comparable result to the old reagent lot. Testing the new reagent on a standardized control with defined acceptance criteria is also acceptable.

Before using a new lot of antibody, it must be compared with the previous lot with defined acceptance criteria from old lot to new lot. If the antibody is used in combination with others in a cocktail, the new cocktail should be similarly compared with the old. Only when there is agreement between the individual old and new lots, or the old and new cocktail, may the new lot of antibody or the new cocktail be placed into routine use.

RECORDS

Inspector Instructions:



- Record retention policy (gated dot plots/histograms)

FLO.23706 Record Retention - Flow Cytometry

Phase II

Flow cytometry data for evaluation of hematolymphoid neoplasias, PNH, and congenital immunodeficiency evaluations are retained for at least 10 years. Routine lymphocyte subset and CD34+ enumeration data are retained for at least two years.

NOTE: Stored data must include raw listmode data and final interpretation. Storage of gated data is encouraged but not required.

If the laboratory responsible for the interpretation component (interpretation only flow cytometry) does not retain the data locally, it must ensure that the data are being retained for the full retention period, such as with an agreement with the laboratory performing the flow cytometry technical component (see ANP.29670).

Evidence of Compliance:

- ✓ Written record retention policy **AND**
- ✓ Data files with or without gated dot plots and histograms **OR**
- ✓ Written agreement with laboratory performing technical component for data storage

REFERENCES

- 1) CAP Policy PP, Retention of Laboratory Records and Materials

CONTROLS AND STANDARDS

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of QC policies and procedures (includes acceptable control type/frequency for each flow cytometric application) • Sampling of QC records
	<ul style="list-style-type: none"> • How do you determine when quality control is unacceptable and when corrective actions are needed? • How does your laboratory establish or verify acceptable QC ranges?
	<ul style="list-style-type: none"> • Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory procedure for corrective action

****REVISED** 09/22/2021**

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

Phase II

For qualitative assays (eg, leukemia/lymphoma analysis), negative and positive staining patterns of the residual normal cell population are evaluated each day of patient testing. Cellular control material is assayed at least monthly.

NOTE: Residual normal cells function as negative and positive controls. The staining patterns of normal cells must be reviewed and evaluated daily against defined criteria established by the laboratory for acceptability. This evaluation must be recorded each day of patient testing.

Cellular control materials can be derived from residual patient material, commercial sources, cryopreserved cells, or other antigen-validated material. There must be written guidelines defining acceptable performance records of the evaluation of the actual periodic performance. Both positive and negative cell populations must be defined for each antibody/stain used.

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.

Evaluation of instrument performance such as optical alignment, fluorochrome standards, and laser (see Instruments and Equipment section) is integral to the QC process and hence must be performed and recorded each day of patient testing.

Evidence of Compliance:

- ✓ Written procedure for QC requirements and performance for each test **AND**
- ✓ Records of QC results

****REVISED** 09/22/2021**

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

Phase II

For quantitative assays, at least two levels of positive cellular controls are analyzed 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:

- ✓ Written procedure for QC requirements and performance for each test **OR**
- ✓ 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

An acceptable control range is established or verified for each lot of control material.

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

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

Evidence of Compliance:

- ✓ Written procedure to establish or verify control ranges **AND**
- ✓ Records for control range establishment or verification of each lot

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Evaluation of Precision Performance of Quantitative Measurement Methods; 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

There are records of corrective action taken when control results exceed defined acceptability limits.

NOTE: 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.

FLO.24250 QC Handling Phase II

Control specimens are tested in the same manner and by the same personnel as patient samples.

NOTE: QC specimens must be analyzed by personnel who routinely perform patient testing - this does not imply that each operator must perform QC daily, so long as each instrument and/or test system has QC performed at required frequencies, and all analysts participate in QC on a regular basis. To the extent possible, all steps of the testing procedure must be controlled.

Evidence of Compliance:

- ✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(d)(8)]

FLO.24300 QC Confirmation of Acceptability Phase II

The results of controls are reviewed for acceptability before reporting results.

Evidence of Compliance:

- ✓ Written policy stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Records of control result approval

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(f)]

FLO.24475 Monthly QC Review Phase II

Quality control data are reviewed and assessed at least monthly by the laboratory director or designee.

NOTE: The review of quality control data must be recorded and include follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month should be reviewed when the tests are performed.

Evidence of Compliance:

- ✓ Records of QC review including follow-up for outliers, trends or omissions

INSTRUMENTS AND EQUIPMENT

FLOW CYTOMETERS

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

	<ul style="list-style-type: none"> • 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
	<ul style="list-style-type: none"> • 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?

FLO.25150 Optical Alignment

Phase II

There are procedures for monitoring of optical alignment (where applicable) and instrument reproducibility at least daily (or after each time the flow cytometer is restarted), and there are records of this monitoring.

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.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 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 calibration; and the results are recorded for quality control purposes.

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

Evidence of Compliance:

- ✓ Written procedure for calibration using appropriate fluorochrome standards with records of results

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

There is a written procedure for assay specific optimization of voltages.

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:

- ✓ Procedure for voltage optimization **AND**
- ✓ 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.

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

Procedures are established for determining appropriate color compensation settings.

NOTE: For two or more 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.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition.* CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 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**

For laser instruments, there are written procedures to ensure acceptable and constant laser performance.

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

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:

	<ul style="list-style-type: none"> • 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)
	<ul style="list-style-type: none"> • How have you established or verified reference intervals? • 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?

FLO.30430 Specimen Integrity

Phase II

There is a written procedure to verify specimen integrity.

NOTE: The yield of T lymphocytes from blood samples is affected by a number of factors. If specimens are not processed immediately after collection, the laboratory should verify that its anticoagulant, holding temperature and preparation method maintain specimen integrity. Selective loss of cell subpopulations and/or the presence of dead cells may lead to spurious results. Routine viability testing is not necessary on specimens of whole blood that are analyzed within 24 hours of drawing. Analyses on older samples are possible if the laboratory has verified the absence of statistical differences between the fresh and aged specimen phenotype fractions being evaluated.

Evidence of Compliance:

- ✓ Records of specimen evaluation (eg, viability results) as applicable

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.30450 Specimen Storage

Phase II

Specimens are stored appropriately after initial processing.

NOTE: As one example, paraformaldehyde (0.5%) fixation of stained cells preserves cellular integrity and fluorescence for up to five days. Caution must be exercised in utilizing this procedure, as fluorescence may be diminished with some reagents and cytometers.

Evidence of Compliance:

- ✓ Written procedure for specimen storage

REFERENCES

- 1) American Society for Microbiology. *Manual of clinical immunology*, 4th ed. Washington, DC: ASM, 1992:940
- 2) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

FLO.30460 Gating Technique

Phase II

Appropriate gating techniques are used to select the cell population for analysis.

NOTE: This may involve a combination of light scatter and/or fluorescence measurements. This is particularly important if the cell samples have a low lymphocyte count and/or a relatively high monocyte-granulocyte count. Lymphocyte gates may be verified using linear forward angle light scatter and 90-degree side scatter, and/or by using monoclonal antibodies to markers, such as CD45 and CD14.

REFERENCES

- 1) CDC Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus; 2003 Jan 31 vol 52, (rr02); 1-13

FLO.30470 Gate Purity

Phase II

Results of lymphocyte subset analysis are corrected for gate purity as appropriate.

NOTE: When >5% non-lymphocyte events are included in a gate, results must be corrected for the proportion of contaminating cells. One method uses low side scatter and bright CD45 fluorescence for identification of lymphocytes, where an assumption is made that the only cells meeting these criteria are lymphocytes, and therefore the lymphocyte purity of the gate is close to 100%. Other methods may also be appropriate, and must be recorded.

Evidence of Compliance:

- ✓ Written procedure defining method for correction of results for gate purity

REFERENCES

- 1) National Institute for Allergy and Infectious Diseases/Division of AIDS. Revised 3 color supplement to flow cytometry guidelines, sec 5.02
- 2) 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.30480 Markers/Cursors

Phase II

There is a procedure to set markers (cursors) to distinguish fluorescence negative and fluorescence positive cell populations.

NOTE: Each laboratory must have a set of objective criteria to define the appropriate placement of markers (cursors) to delineate the population of interest. Isotypic controls may not be necessary in all cases, and cursor settings for the isotype control may not be appropriate for all markers. Cursor settings must be determined based on the fluorescence patterns from the negative and positive populations for CD3, CD4 and CD8.

REFERENCES

- 1) CDC Guidelines for Performing Single-Platform Absolute CD4+ T-Cell Determinations with CD45 Gating for Persons Infected with Human Immunodeficiency Virus; 2003 Jan 31 vol 52, (rr02); 1-13
- 2) Sreenan JJ, *et al.* The use of isotypic control antibodies in the analysis of CD3+ and CD3+, CD4+ lymphocyte subsets by flow cytometry. Are they really necessary? *Arch Pathol Lab Med.* 1997;121:118-121

FLO.30550 Reference Intervals Established

Phase II

The report includes an established or verified reference interval for blood lymphocyte subsets appropriate for the age of the patient.

NOTE: Age- and sex-specific reference intervals must be determined by laboratory, if feasible. For example, a reference interval can be verified by testing samples from 20 healthy representative individuals; if no more than two results fall outside the proposed reference interval, that interval can be considered verified for the population studied (refer to CLSI guideline EP28-A3, referenced below). If this is not possible or practical, then the laboratory must evaluate the use of published data for its own reference intervals, and retain records of this evaluation.

Evidence of Compliance:

- ✓ Patient result reported with reference interval, as applicable and record of completed reference Interval study **OR** records of verification of manufacturer's stated interval when

reference interval study is not practical (eg, unavailable normal population) **OR** other methods approved by the section director

REFERENCES

- 1) Knight JA. Laboratory issues regarding geriatric patients. *Lab Med.* 1997;28:458-461
- 2) Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline.* 3rd ed. CLSI Document EP28-A3c. Clinical and Laboratory Standards Institute, Wayne, PA, 2010.

CD34 STEM CELL ENUMERATION

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of CD34 analysis policies and procedures • Sampling of CD34 records (events counted)
	<ul style="list-style-type: none"> • How does your laboratory record CD34 cellular viability? • How does your laboratory gate to define the population of CD34+ cells? • What class of anti-CD34 monoclonal antibodies does your laboratory use, and how are they conjugated?

FLO.30564 CD34 Cellular Viability - Apheresis and Cord Blood Products

Phase II

The laboratory measures the viability of CD34 positive cells in samples aliquoted at the time of processing of hematopoietic progenitor cell, apheresis products, and cord blood products.

NOTE: CD34 cell viability testing of cord blood products must be done on a sample aliquoted prior to the addition of cryoprotectant. For any hematopoietic progenitor cell product, CD34 cell viability testing during or after storage should be considered as an additional quality control measure. The viability dye 7-amino actinomycin-D (7-AAD) yields excellent results in this analysis. The viability assay must be performed using a flow cytometric method with the viability dye included in the same tube with the CD34 and CD45 monoclonal antibodies for the CD34+ viability determination. Estimates of total cellular viability (for example, trypan blue exclusion) may not be used as an alternative because the method can overestimate the viability of the CD34 stem cell population.

REFERENCES

- 1) Owens M, Loken M. Peripheral blood stem cell quantitation, In *Flow Cytometry Principles for Clinical Laboratory Practice*. New York, NY: Wiley-Liss, 1995:111-127
- 2) Keeney M., et al. Single platform flow cytometry absolute CD34+ cell counts based on the ISHAGE guidelines. *Cytometry.* 1998; 34:61-70
- 3) Hubl W, et al. Measurement of absolute concentration and viability of CD34+ cells in cord blood and cord blood products using fluorescent beads and cyanine nucleic acid dyes. *Cytometry.* 1998; 34:121-127
- 4) Gratama J, et al. Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. *Cytometry.* 1998;34:128-145
- 5) Lee S., et al. Post thaw viable CD34+ cell count is a valuable predictor of haematopoietic stem cell engraftment in autologous peripheral blood stem cell transplantation. *Vox Sang Feb:* 2008; 94:46-152
- 6) Reich-Slotky R., et al. Determining post-thaw CD34+ cell dose of cryopreserved haematopoietic progenitor cells demonstrates high recovery and confirms their integrity. *Vox Sang* 2008; May 94(4):351-357
- 7) Ruiz-Argüelles GJ, Ruiz-Argüelles A, Pérez-Romano B, et al. Filgrastim-mobilized peripheral-blood stem cells can be stored at 4 degrees and used in autografts to rescue high-dose chemotherapy. *Am J Hematol.* 1995; 48(2):100-3.
- 8) Kao GS, Kim HT, Daley H, et al. Validation of short-term handling and storage conditions for marrow and peripheral blood stem cell products. *Transfusion.* 2011; 51(1):137-47.

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:

- ✓ Written policy defining the use of appropriate class of monoclonal antibodies **AND**
- ✓ 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:

- ✓ Written policy defining the minimum number of CD34+ events for analysis **AND**
- ✓ Records of number of events counted

REFERENCES

- 1) Sutherland DR, Anderson L, Keeney M, et al. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. *J Hematotherapy*. 1996;3:213-226
- 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).

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, PA 19087-1898 USA, 2007
- 2) Sutherland DR, Anderson L, Keeney M, et al. Towards a worldwide standard for CD34+ enumeration? *J. Hematotherapy*. 1997;6:85-89

LEUKEMIA AND LYMPHOMA

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of leukemia/lymphoma immunophenotyping policies and procedures • Sampling of patient reports and histograms (to include abnormal cell immunophenotype, interpretive comments, etc.)
	<ul style="list-style-type: none"> • 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?

FLO.30610 Cellular Viability

Phase II

There is a policy for determining when the percentage of viable cells in each test specimen should be 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.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 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:

- ✓ Written procedure to select appropriate antibodies, where applicable **AND**
- ✓ 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 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 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

FLO.30670 Cell Concentrations**Phase II**

Cell concentrations are adjusted for optimal antibody staining.

Evidence of Compliance:

- ✓ Written procedure for adjusting cell concentrations to ensure 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 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

FLO.30720 Immunoglobulin Staining**Phase II**

There are written procedures to ensure that immunoglobulin staining is intrinsic and not extrinsic (cytophilic).

NOTE: The requirement is to ensure that the immunoglobulin light chain analysis includes only light chain synthesized by B cells (intrinsic light chain). Many cell types will bind serum immunoglobulin nonspecifically via Fc receptors (including B cells). To ensure that immunoglobulin staining detected by flow cytometry is intrinsic (on B cells) rather than cytophilic, a pan-B cell marker (eg, CD19, CD20) may be included in the same tube as one or both anti-light chain reagents. The inclusion of both lambda and kappa light chain reagents in the same tube allows a clear delineation of non-specific binding, even on B cells.

Evidence of Compliance:

- ✓ Written procedure for method to ensure intrinsic immunoglobulin 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 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

FLO.30730 Abnormal Cell Distinction**Phase II**

There are procedures established for distinguishing abnormal cells of interest from normal cells, based on their light scatter and fluorescence properties.

NOTE: Generally, both neoplastic and non-neoplastic cells are acquired in any gate used for acquisition. Attempts must be made to distinguish them at the time of analysis. Appropriate procedures include use fluorescent antibodies, fluorescent dyes, light scatter measurements, or any combination thereof to select out the relevant cell subpopulation for further analysis. Morphologic evaluation is also a valuable parameter to improve analysis.

Laboratories performing interpretation only of immunophenotyping data from an outside facility (ie, technical flow laboratory) must ensure that appropriate gating techniques are used. There

must be a process by which individuals interpreting the results can provide feedback on the appropriateness of the gating techniques used. Records of such feedback and corrective action taken when problems are identified may be incorporated into the laboratory's quality management system.

REFERENCES

- 1) Muirhead KA, *et al.* Methodological considerations for implementation of lymphocyte subset analysis in a clinical reference laboratory. *Ann NY Acad Sci.* 1986;468:113-127
- 2) American Society for Microbiology. Manual of clinical immunology, 4th ed. Washington, DC: ASM, 1992
- 3) Sun T, *et al.* Gating strategy for immunophenotyping of leukemia and lymphoma. *Am J Clin Pathol.* 1997;108:152-157
- 4) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition.* CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007
- 5) Macon WR, Salthany KE. T-cell subset analysis of peripheral T-cell lymphomas by paraffin section immunohistology and correlation of CD4/CD8 results with flow cytometry. *Am J Clin Pathol.* 1998;109:610-617
- 6) Dunphy CH. Combining morphology and flow cytometric immunophenotyping to evaluate bone marrow specimens for B-cell malignant neoplasms. *Am J Clin Pathol.* 1998;109:625-630

FLO.30760 Cell Population Distinction

Phase II

There is a procedure to distinguish fluorescence-negative and fluorescence-positive cell populations.

NOTE: This does not imply that a separate negative control sample must be run. It is possible to coordinate panels of monoclonal antibodies to compare the binding of monoclonal antibodies of the same subclass that typically have mutually exclusive patterns of reactivity of subsets of hematopoietic cells. In this way, test antibodies may also double as control reagents.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition.* CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007

FLO.30790 Final Report

Phase II

The final report includes information about the immunophenotype of the abnormal cells, if identified, and comments necessary to facilitate the interpretation.

NOTE: Clinical information and available pathologic material should be reviewed to select appropriate antibodies. In cases of suspected hematolymphoid neoplasia direct morphologic correlation of all applicable sample types should be performed when possible and clinically appropriate. In cases involving leukemia and lymphoma phenotyping, correlation should be made between the immunologic and pathologic results. The flow histograms, rather than just the percentage of positive cells, should be reviewed by the interpreting pathologist in difficult cases. The peak channel and shapes of the curves may be helpful in identifying clonal populations.

Reporting requirements for use of analyte-specific reagents and other reagents used in laboratory-developed tests are included in the All Common Checklist (COM.40850).

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition.* CLSI document H43-A2 (ISBN 1-56238-635-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007
- 2) Nguyen AND, *et al.* A relational database for diagnosis of hematopoietic neoplasms using immunophenotyping by flow cytometry. *Am J Clin Pathol.* 2000;113:95-106
- 3) 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
- 4) 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.
- 5) Sever C, Abbott CL, de Baca ME, *et al.* Bone marrow synoptic reporting for hematologic neoplasms: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med;* 2016;140(9):932-49.

RARE EVENT FLOW CYTOMETRIC ASSAYS

FLO.30800 Rare Event Flow Cytometric Assays Phase II

For rare event flow cytometric assays, the lower limit of enumeration has been validated.

NOTE: The detection of rare events may occur in assays, such as Paroxysmal Nocturnal Hemoglobinuria (PNH) clone testing or minimal residual disease (MRD) testing. Analytic sensitivity of the lower detection limit should be validated by performing dilutional studies using known patient or suitable reference material, such as proficiency testing material.

For high sensitivity paroxysmal nocturnal hemoglobinuria (PNH) testing (detecting and reporting clones less than or equal to 1%), validations to establish the lower limit of enumeration (quantitation) are required for red blood cells, granulocytes, and monocytes.

REFERENCES

- 1) Sutherland DR, Illingworth A, Marinov I, et al. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 2 - reagent selection and assay optimization for high sensitivity testing. *Clin Cytom.* 2018; 94(1):23-48.

FLO.30820 Rare Event Flow Cytometric Assays Phase I

For rare event flow cytometric assays, the lower limit of enumeration is included in the diagnostic report.

NOTE: When performing rare event testing, including minimal residual disease (MRD) on low cellularity samples, the number of events needed to achieve the laboratory's validated lower limit of enumeration/sensitivity may not be able to be collected. In these cases (especially when the interpretation is negative), laboratories should clearly state in the diagnosis report that the paucicellular sample may have reduced analytical sensitivity.

****NEW** 09/22/2021**

FLO.30825 Rare Event Flow Cytometric Assays - Sample Carryover Phase II

For all rare event assays (including MRD testing and PNH testing), the laboratory evaluates the assay for carryover and has processes to reduce possible sample carryover to a level below the detection limit of the assay.

NOTE: The laboratory must perform formal assessment of carryover during validation of the assay and reassess carryover after major maintenance or repair of the instrument.

Highly sensitive assays may be affected significantly by sample carryover introduced during sample processing or during data acquisition. Methods for reducing this possibility include:

- *Running blank tubes containing deionized water, PBS, or similar non-cellular fluid prior to sample tubes to monitor for sample carryover*
- *Monitoring unstained or isotype control tubes for prior stained sample carryover*
- *Monitoring pipetting or sample processing instrumentation for possible carryover using blank tubes following samples.*

Evidence of Compliance:

- ✓ Procedures detailing methods used to reduce carryover **AND**
- ✓ Records of carryover studies performed at validation and following major maintenance/repair

FLO.30830 Rare Event Flow Cytometric Assays - MRD Testing Phase I

For minimal residual disease (MRD) testing (including B lymphoblastic leukemia, acute myeloid leukemia, plasma cell myeloma, and mature lymphoid disorders), the method appropriately separates normal populations from residual disease.

NOTE: Examples include:

- *B lymphoblastic leukemia - distinguish hematogones (normal immature B-cell precursors) from B lymphoid blasts*
- *Acute myeloid leukemia - distinguish normal myeloid blasts from disease-associated/neoplastic blasts*
- *Plasma cell myeloma - distinguish neoplastic from non-neoplastic plasma cells*
- *Non-Hodgkin lymphoma - separate normal B cells from abnormal/neoplastic B cells (or T cells if assay targets T cell non-Hodgkin lymphoma)*

Evidence of Compliance:

- ✓ Validation data showing normal and abnormal population analysis **OR**
- ✓ Procedures detailing methods used to distinguish normal from abnormal and sample patient data from both normal and abnormal cases

REFERENCES

- 1) Flores-Montero J, Sanoja-Flores L, Paiva B, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia*. 2017; 31(10):2094-2103.
- 2) Rawstron AC, Paiva B, Stetler-Stevenson M. Assessment of minimal residual disease in myeloma and the need for a consensus approach. *Cytometry B Clin Cytom*. 2016; 90(1):21-5.
- 3) 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):40-6.
- 4) Gupta S, Devidas M, Loh ML, et al. Flow cytometric vs morphologic assessment of remission in childhood acute lymphoblastic leukemia: A report from the Children's Oncology Group (COG). *Leukemia*. 2017; Dec 18. doi: 10.1038/leu.2017.341.
- 5) Keeney M, Wood BL, Hedley BD, et al. A QA program for MRD testing demonstrates that systematic education can reduce discordance among experienced interpreters. *Cytometry B Clin Cytom*. 2017; May 5. doi: 10.1002/cyto.b.21528.
- 6) Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: consensus document from ELN MRD Working Party. *Blood*. 2018 Jan 12. pii:blood-2017-09-801498.
- 7) Lacombe F, Campos L, Allou K, et al. Groupe d'Etude Immunologique des Leucemies (GEIL). Prognostic value of multicenter flow cytometry harmonized assessment of minimal residual disease in acute myeloblastic leukemia. *Hematol Oncol*. 2017; Dec 7. doi: 10.1002/hon.2488.
- 8) Botcher S, Ritgen M, Kneba M. Flow cytometric MRD detection in selected mature B-cell malignancies. *Methods Mol Biol*. 2013; 971:149-74.

****REVISED** 09/22/2021**

FLO.30840 PNH Testing

Phase I

The laboratory has a written procedure describing how to separate normal populations from abnormal PNH clones.

NOTE: For RBC analysis, the procedure must include an appropriate panel of antibodies/reagents and methods for distinguishing normal type I RBCs from PNH type II and type III clones. This typically applies to larger RBC clones with well-defined clusters of RBC populations. Minor clones (equal or less than 1%) may not show distinct separation between the type II and type III populations and one gate for the combined RBC PNH clone is sufficient.

Assay specific PMT settings must be established (see FLO.30255). For the red cell assay, unstained red blood cells are appropriate. For white cell assays, the method should include an appropriate panel of antibodies/reagents to distinguish normal from abnormal PNH clones in the populations of interest (granulocytes and/or monocytes). For PMT settings, it is important to ensure the unstained lymphocyte population is clearly on scale. Some WBC samples may show type II populations; current consensus is that these cells should be included in total "PNH neutrophils".

Evidence of Compliance:

- ✓ Written procedure indicating the appropriate panel of antibodies and reagents being used and the method used to distinguish normal cells from abnormal PNH clones

REFERENCES

- 1) Sutherland DR, Illingworth A, Marinov I, et al. ICCS/ESCCA consensus guidelines to detect GPI-deficient cells in paroxysmal nocturnal hemoglobinuria (PNH) and related disorders part 2 - reagent selection and assay optimization for high sensitivity testing. *Clin Cytom*. 2018; 94(1):23-48.

DNA CONTENT AND CELL CYCLE ANALYSIS

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of DNA analysis policies and procedures (includes reference to established methodology and list of acceptable neoplasms for DNA analysis) • Sampling of specimen evaluation records • Sampling of DNA analysis linearity and QC records • Sampling of sub-optimal/specimen rejection records/log
	<ul style="list-style-type: none"> • What is your laboratory's course of action when unacceptable or sub-optimal specimens are received? • How does your laboratory ensure debris and aggregates are excluded from consideration? • How does your laboratory ensure that the analysis contains neoplastic cells of interest? • How does your laboratory ensure detection of DNA aneuploidy?

****REVISED** 06/04/2020**

FLO.31000 Neoplastic Cell Content

Phase II

There are procedures to ensure that specimens processed for DNA content and cell cycle analysis contain neoplastic cells of interest.

NOTE: It is critical that specimens submitted for flow cytometric analysis are representative samples of the neoplastic disorder being characterized. In specimens in which no population of abnormal DNA content is detected, it is especially important to demonstrate that neoplastic cells are present in the sample run through the flow cytometer. This generally requires microscopic evaluation of the specimen by a qualified pathologist.

Evidence of Compliance:

- ✓ Written procedure for verifying the presence of neoplastic cells **AND**
- ✓ Records of specimen evaluation

FLO.31010 Cellular Debris

Phase II

There are written procedures to account for cellular debris and aggregates.

NOTE: Cellular debris can affect measurements of S-phase fraction, and aggregates can alter ploidy assessments; these need to be excluded from consideration. DNA analysis software programs generally provide options for debris subtraction and doublet discrimination. Each laboratory should incorporate such methods into their procedures. Confirmation with fluorescent microscopic examination of the stained nuclear suspension may provide additional documentation of cellular aggregates.

Evidence of Compliance:

- ✓ Written procedure for method(s) to account for debris and aggregates

FLO.31020 DNA Content Linearity

Phase II

Criteria are established for determining acceptable linearity for DNA content measurement using cells or particles of known relative fluorescence.

FLO.31050 Procedure Manual **Phase II**

The staining and analytical procedures described in the procedure manual are based upon established methodology (reference cited).

NOTE: Many different variables need to be controlled to ensure proper stoichiometry of dye binding to DNA. Therefore, it is essential that procedures adopted by a laboratory are based on published work.

FLO.31100 Specimen Treatment **Phase II**

Specimen treatment with nucleic acid dye includes treatment with RNase if the dye is not specific for DNA.

NOTE: Certain dyes used to stain fixed cells, (eg, ethidium and propidium iodide) bind to RNA. Prior treatment with RNase eliminates artifactual broadening of the DNA content distributions that would result from fluorescence of complexes of the dye with RNA.

Evidence of Compliance:

- ✓ Written procedure for specimen treatment with RNase

REFERENCES

- 1) Shapiro HA. Practical flow cytometry. New York, NY: Alan R. Liss, 1985

FLO.31150 Neoplasm DNA Analysis Criteria **Phase I**

There are written criteria that specify the type of neoplasms acceptable for DNA analysis.

NOTE: The laboratory should show evidence that it restricts analysis to those neoplasms for which the literature supports significant independent prognostic significance for DNA ploidy and/or S-phase analysis.

REFERENCES

- 1) DNA cytometry consensus conference. *Cytometry*. 1993;14:471-500
- 2) Henson D, et al. College of American Pathologists Conference XXVI on clinical relevance of prognostic markers in solid tumors. Summary. *Arch Pathol Lab Med*. 1995;119:1109-1112

FLO.31200 Histogram Acceptability Criteria **Phase II**

There are written criteria for acceptability of histograms for interpretation.

FLO.31300 Nucleic Acid-Specific Dye Concentration **Phase II**

The concentration of nucleic acid-specific dye has been determined to be a saturating concentration.

NOTE: Standard techniques use an excess concentration of fluorochrome since concentrations below saturation will make the cells appear hypoploid.

Evidence of Compliance:

- ✓ Written procedure to determine the nucleic acid-specific stain concentration

REFERENCES

- 1) Shapiro HA. Practical flow cytometry. New York, NY: Alan R. Liss, 1985

FLO.31350 G0/G1 Peak **Phase II**

Control cells of known DNA content are run with each specimen or batch of specimens to establish an acceptable CV for the G0/G1 peak and to determine the DNA index.

NOTE: Repetitive analysis of the reference cells allows reference intervals to be established to determine an acceptable range of results. This can be used as a control for DNA staining and instrumental parameters used in the analysis.

Evidence of Compliance:

- ✓ Written procedure for use of control cells for DNA analysis **AND**
- ✓ Records of QC results

REFERENCES

- 1) Hiddemann W, *et al.* Convention on nomenclature for DNA cytometry. *Cytometry*. 1984;5:445-446

FLO.31400 Aneuploid Cell Population ID

Phase II

Analytical criteria are established for identification of an aneuploid cell population in the test specimen.

NOTE: The ability to detect DNA aneuploidy by flow cytometric measurement depends upon the resolution of the DNA measurements, usually assessed by the coefficient of variation (CV) of the peaks. CVs should be reported for all clinical studies. The range of CVs is highly dependent on the tissue type and the way it is prepared. Histograms observed for clinical specimens often represent complex overlapping patterns because most tumor specimens contain a mixture of tumor cells, stromal cells, and inflammatory cells. Analysis of control cells is necessary to establish the CV for a normal diploid, G0/G1 peak. Periodic review of the CVs for control cells is necessary to ensure adequate functioning of the analytic procedure.

An international workshop recommended that cells (or nuclei) should be termed as having an "abnormal DNA stemline" or "DNA aneuploidy" when at least two separate G0/G1 peaks are demonstrated.

REFERENCES

- 1) Hiddemann W, *et al.* Convention on nomenclature for DNA cytometry. *Cytometry*. 1984;5:445-446
- 2) Coon JS, *et al.* Advances in flow cytometry for diagnostic pathology. *Lab Invest*. 1987;57:453-479