Every patient deserves the GOLD STANDARD...

Flow Cytometry Checklist

CAP Accreditation Program
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# Flow Cytometry Checklist

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ON-LINE CHECKLIST AVAILABILITY

Participants of the CAP accreditation programs may download the checklists from the CAP website (www.cap.org) by logging into e-LAB Solutions. 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.

SUMMARY OF CHECKLIST EDITION CHANGES
Flow Cytometry Checklist
07/28/2015 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 listing of requirements below is 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

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INTRODUCTION

This checklist is used on 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 leukemia/lymphoma immunophenotyping data (the flow technical component is performed at an outside flow laboratory), the following Flow Cytometry Checklist requirements apply: FLO.18385, FLO.23675, FLO.23706, FLO.30605, 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.

Note for non-US laboratories: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist.

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 for leukemias and lymphomas, the laboratory participates in a peer education program in interpretive flow cytometry of hematolymphoid neoplasia.

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 leukemia/lymphoma 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
QUALITY MANAGEMENT AND QUALITY CONTROL

REAGENTS

Inspector Instructions:

- Sampling of new antibody validation records
- Sampling of new lot/shipment antibody and detection system reagent confirmation records
- 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.

**NEW** 04/21/2014

FLO.23250  Reagent Usage

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


**NEW** 04/21/2014

FLO.23275  Antibody Validation

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.

**NEW** 04/21/2014

FLO.23325  New Reagent Lot/Ship ment Confirmation of Acceptability

The performance of new lots/shipments of antibody and detection system reagents is compared with old lots/shipments before or concurrently with being placed into service and records maintained.
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 cocktail, it is recommended to compare each individual antibody by using a side scatter vs. fluorescence plot and having defined acceptance criteria from old lot to new lot. Only when there is agreement between the individual old and new lots should the cocktail be made and then tested against the old cocktail.

RECORDS AND REPORTS

Inspector Instructions:

- Sampling of patient reports (includes disclaimer when Class I ASR's are used)
- Record retention policy (gated dot plots/histograms)

FLO.23675 ASR Report Phase II

If patient testing is performed using Class I analyte-specific reagents (ASR's) obtained or purchased from an outside vendor, the patient report includes the disclaimer required by federal regulations.

NOTE: ASR's are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.

An ASR is the active ingredient of a laboratory-developed test system.

This checklist requirement concerns Class I ASR's. Class I ASR's are not subject to preclearance by the US Food and Drug Administration (FDA) or to special controls by FDA. Most ASR's are Class I. Exceptions include those used by blood banks to screen for infectious diseases (Class II or III), or used to diagnose certain contagious diseases (e.g. HIV infection and tuberculosis) (class III).

If the laboratory performs patient testing using Class I ASR's, federal regulations require that the following disclaimer accompany the test result on the patient report: "This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the US Food and Drug Administration."

The CAP recommends additional language, such as "FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing."

The disclaimer is not required for tests using reagents that are sold in kit form with other materials or an instrument, nor reagents sold with instructions for use.

The laboratory must establish the performance characteristics of tests using Class I ASR's.

REFERENCES
Flow Cytometry Checklist 07.28.2015


**REVISED** 07/28/2015

FLO.23706 Record Retention Phase II

Gated dot plots and histograms are retained for at least 10 years. List mode files that include analysis and gates are acceptable.

NOTE: The intent of this checklist requirement is retention of gated dot plots and histograms of hematolymphoid neoplasias, CD34 stem cell records, and congenital immunodeficiency evaluations for 10 years. Paper copies of gated dot plots and histograms are not required as long as the information is available electronically (e.g., .pdf, .tiff, .jpeg files).

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

CONTROLS AND STANDARDS

Inspector Instructions:

READ

- Sampling of QC policies and procedures (includes acceptable control type/frequency for each flow cytometric application)
- Sampling of QC records

ASK

- 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?

DISCOVER

- 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

FLO.23737 QC - Reagents/Stain Phase II

The performance of reagents and staining procedures are verified by the use of positive controls.

NOTE: The source (type) of positive control(s) and their frequency of evaluation will vary by the particular flow cytometric application. The frequency should be: 1) each day of analysis for lymphocyte subset and CD34+ stem cell measurements, regardless of whether one- or two-platform methods are used; and 2) at least monthly for leukemia/lymphoma immunophenotyping.

For single platform measurements of CD4+ lymphocyte and CD34+ stem cell concentrations, and for dual platform measurements of CD34+ stem cell concentrations, two levels of control are needed (see the next checklist requirement, below). For dual platform measurements of lymphocyte subsets (CD4+ lymphocytes), one level of positive control is sufficient.

The source of control material should be 1) external positive controls (e.g. normal or commercial control(s)) for lymphocyte subset, CD34+ stem cell quantitations, and leukemia/lymphoma samples; or 2) internal positive controls only for leukemia/lymphoma samples. Such internal
control cells are the variable numbers of residual normal cells in the patient's sample. Like the external controls, there must be written guidelines defining objective criteria for acceptable performance of the internal controls and written records of the evaluation of the actual periodic performance.

When antigen positive cells are not readily available through commercial controls or patient materials, then the laboratory director must implement an equivalent procedure to meet the positive control requirements (e.g. CD1a, CD103). This may include cryopreserved or fresh cell lines, patient material or antigen validated material. At a minimum, there must be testing of rare antigens performed at least semiannually.

Evidence of Compliance:
✓ Written procedure for QC requirements and performance for each test AND
✓ Records of QC results

FLO.23800 QC - Single/Dual Platform Tests Phase II
For single platform quantitative tests (e.g. CD4+, CD34+ cell concentrations), and dual platform quantitation of CD34+ stem cell concentrations, at least two levels of positive cellular controls are analyzed at least daily (or each time the flow cytometer is restarted) to verify the performance of reagents, preparation methods, staining procedures and the instrument.

NOTE: One of the levels of these controls should be at (or near) clinical decision levels (e.g. low CD34). Examples would be a low CD4+ lymph count of 200 cell/µL in a HIV+ individual, or a 5 – 20 CD34+ stem cells/µL concentration in the peripheral blood of an individual being readied for peripheral stem cell pheresis. When commercial controls are not available, then the laboratory director must implement an equivalent procedure to meet the two-level requirement. Control testing is not necessary on days when patient testing is not performed.

Evidence of Compliance:
✓ Written procedure for QC requirements and performance for each test OR validation records of an alternate/equivalent procedure when commercial controls are not available
✓ Records of QC results

REFERENCES

FLO.23925 QC Range Verification Phase II
A statistically valid target mean and range are established or verified for each lot of control material.

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

Evidence of Compliance:
✓ Written procedure to establish or verify control ranges AND
✓ Records for control range verification of each lot

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

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, recognizing that pre-analytic and post-analytic variables may differ from those encountered with patients.

Evidence of Compliance:
✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES

FLO.24300 QC Confirmation of Acceptability

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

NOTE: It is implicit in quality control that patient test results will not be reported when controls do not yield acceptable results.

Evidence of Compliance:
✓ Defined QC tolerance limits and records of confirmation of acceptable QC results

REFERENCES

**REVISED** 04/21/2014
FLO.24475 Monthly QC Review

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:

- 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
- 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

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

FLO.30250 Fluorochrome Standards

Appropriate standards for each fluorochrome, (e.g. 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

FLO.30260 Color Compensation Settings

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 are the proper reference material for establishing appropriate compensation settings.

REFERENCES

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FLO.30270 Laser Current Phase I
For laser instruments, there are written procedures to ensure acceptable and constant laser current.

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

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PIPETTING DEVICES

Inspector Instructions:
- Pipette calibration procedure
- Sampling of pipette/dilutor checks

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**REVISED** 07/28/2015
FLO.30415 Pipette Accuracy Phase II
Pipettes used for quantitative dispensing are checked for accuracy and reproducibility before being placed in service and at defined intervals (at least annually), and results recorded.

NOTE: Pipette checks must be performed following manufacturer’s instructions, at minimum, and as defined in laboratory procedure. Such checks are most simply done gravimetrically. Alternative approaches include spectrophotometry and the use of commercial kits. The frequency of checks depends on how the pipettor is used: for materials requiring high precision and accuracy (such as internal standards), quarterly checks are appropriate. Less frequent checks may be appropriate for other materials. Computer software is useful where there are many pipettes, and provides convenient records. For analytic instruments with integral automatic pipettors, this checklist requirement applies, unless such checks are not practical for the end-user laboratory. Manufacturers’ recommendations should be followed.

REFERENCES
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 have you established or verified reference ranges?
● 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

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 (e.g. viability results) as applicable

REFERENCES
FLO.30450 Specimen Storage  
**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**

FLO.30460 Gating Technique  
**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  
**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 this 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

FLO.30480 Markers/Cursors  
**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

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 (normal values) 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 range study OR records of verification of manufacturer’s stated range when reference range study is not practical (e.g. 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

CD34 STEM CELL ENUMERATION

Inspector Instructions:

READ

• Sampling of CD34 analysis policies and procedures
• Sampling of CD34 records (events counted)

ASK

• 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
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

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

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

LEUKEMIA AND LYMPHOMA

Inspector Instructions:

- Sampling of leukemia/lymphoma immunophenotyping policies and procedures
- Sampling of patient reports and histograms (to include abnormal cell immunophenotype, interpretive comments, etc.)

- 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.30605 Immunophenotyping Data Phase II

If flow leukemia/lymphoma immunophenotyping data from an outside facility (i.e. a technical flow laboratory) are interpreted, the laboratory ensures the following:

1. The technical flow laboratory’s panel of monoclonal antibodies are sufficiently comprehensive to address the clinical problem under consideration
2. The technical flow laboratory uses appropriate gating techniques
3. The final report includes information about the immunophenotype of normal and abnormal cells and includes comments necessary to facilitate the interpretation
4. Gated dot plots and histograms are retained for 10 years. List mode files that include analysis gates are acceptable.

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

FLO.30640 Appropriate Antibodies Phase II

The laboratory uses antibodies appropriate for the clinical situation.

NOTE: The panel of monoclonal antibodies employed must be sufficiently comprehensive to address the clinical problem under consideration. 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.

Evidence of Compliance:
✓ Written procedure to select appropriate monoclonal antibodies

REFERENCES

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

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 (e.g. 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
Abnormal Cell Distinction

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.

**REFERENCES**


Cell Population Distinction

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**


Final Report

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.

**REFERENCES**

RARE EVENT FLOW CYTOMETRIC ASSAYS

**NEW** 07/28/2015

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.

**NEW** 07/28/2015

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.

DNA CONTENT AND CELL CYCLE ANALYSIS

Inspector Instructions:

- 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

- 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?

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 an anatomic or clinical pathologist.

Evidence of Compliance:

✓  Written procedure for verifying the presence of neoplastic cells AND
✓  Records of specimen evaluation
Cellular Debris

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

DNA Content Linearity

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

Procedure Manual

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.

Specimen Treatment

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, (e.g. ethidium and propidium iodide) bind to RNA. Prior treatment with RNase eliminates artificial 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

Neoplasm DNA Analysis Criteria

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

Histogram Acceptability Criteria

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

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

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
The person in charge of bench testing/section supervisor in flow cytometry has education equivalent to that of an associate’s degree (or beyond) in a chemical, physical or biological science or medical technology and at least 4 years experience (one of which is in flow cytometry) under a qualified section director.

Evidence of Compliance:
✓ Records of qualifications including degree or transcript, current license (if required) and work history in related field