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## Flow Cytometry Checklist

CAP Accreditation Program





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# Flow Cytometry Checklist



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## SUMMARY OF CHECKLIST EDITION CHANGES

### Flow Cytometry Checklist 07/11/2011 Edition

The following requirements have been added, revised, or deleted in this edition of the checklist, or in the two editions immediately previous to this one.

If this checklist was created for a reapplication, on-site inspection or self-evaluation it has been customized based on the laboratory's activity menu. The listing below is comprehensive; therefore some of the requirements included may not appear in the customized checklist. Such requirements are not applicable to the testing performed by the laboratory.

*Note: For revised checklist requirements, a comparison of the previous and current text may be found on the CAP website. Click on Laboratory Accreditation, Checklists, and then click the column marked Changes for the particular checklist of interest.*

#### NEW Checklist Requirements

| <u>Requirement</u> | <u>Effective Date</u> |
|--------------------|-----------------------|
| FLO.30605          | 06/17/2010            |

#### REVISED Checklist Requirements

| <u>Requirement</u> | <u>Effective Date</u> |
|--------------------|-----------------------|
| FLO.20100          | 07/11/2011            |
| FLO.22050          | 07/11/2011            |
| FLO.23050          | 07/11/2011            |
| FLO.24230          | 07/11/2011            |
| FLO.24475          | 07/11/2011            |
| FLO.24650          | 07/11/2011            |
| FLO.30290          | 06/17/2010            |
| FLO.30320          | 07/11/2011            |

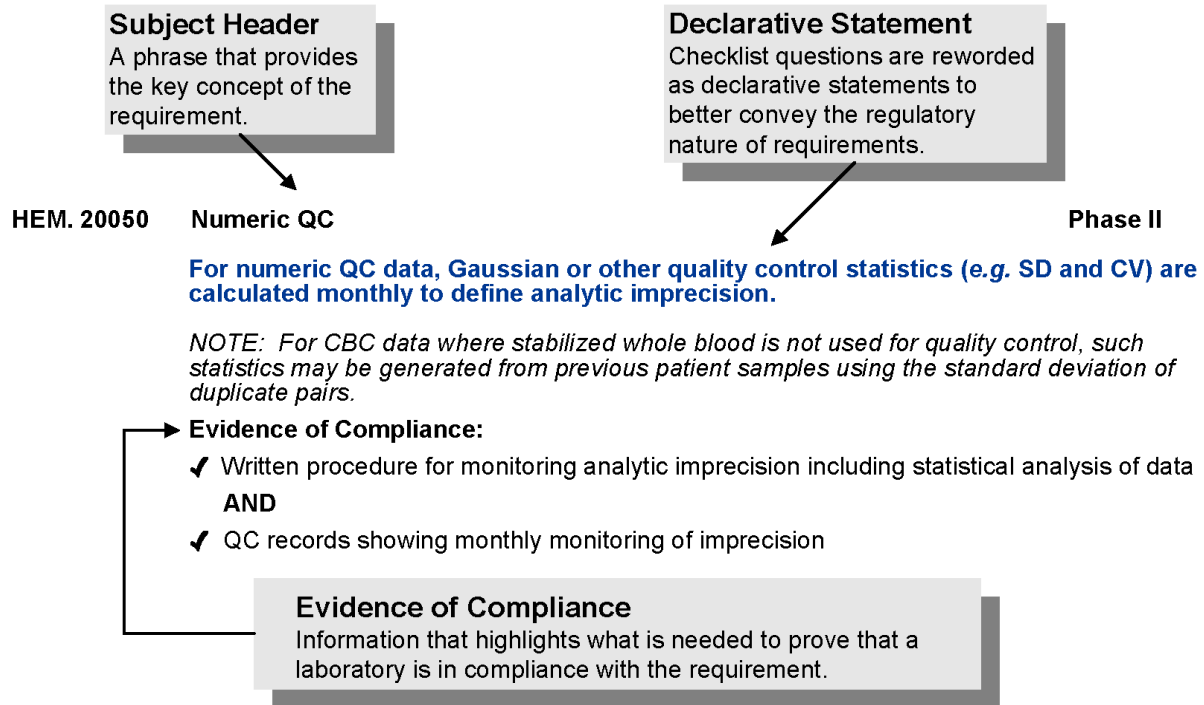
#### DELETED Checklist Requirements

| <u>Requirement</u> | <u>Effective Date</u> |
|--------------------|-----------------------|
| FLO.05075          | 07/10/2011            |
| FLO.10150          | 07/10/2011            |
| FLO.10180          | 07/10/2011            |
| FLO.10210          | 07/10/2011            |
| FLO.10260          | 07/10/2011            |
| FLO.13540          | 07/10/2011            |
| FLO.16770          | 07/10/2011            |
| FLO.20020          | 07/10/2011            |
| FLO.20050          | 07/10/2011            |
| FLO.21000          | 07/10/2011            |
| FLO.21100          | 07/10/2011            |
| FLO.21125          | 07/10/2011            |
| FLO.21150          | 07/10/2011            |
| FLO.21210          | 07/10/2011            |
| FLO.21220          | 07/10/2011            |

|           |            |
|-----------|------------|
| FLO.21250 | 07/10/2011 |
| FLO.24100 | 07/10/2011 |
| FLO.25000 | 07/10/2011 |
| FLO.25050 | 07/10/2011 |
| FLO.30380 | 07/10/2011 |
| FLO.30390 | 07/10/2011 |
| FLO.30400 | 07/10/2011 |
| FLO.30500 | 07/10/2011 |
| FLO.30599 | 07/10/2011 |
| FLO.31450 | 07/10/2011 |
| FLO.50000 | 07/10/2011 |
| FLO.50050 | 07/10/2011 |
| FLO.50100 | 07/10/2011 |
| FLO.50150 | 07/10/2011 |
| FLO.50200 | 07/10/2011 |
| FLO.50250 | 07/10/2011 |
| FLO.50300 | 07/10/2011 |
| FLO.50350 | 07/10/2011 |
| FLO.50400 | 07/10/2011 |
| FLO.50450 | 07/10/2011 |
| FLO.50550 | 07/10/2011 |
| FLO.50600 | 07/10/2011 |
| FLO.50650 | 07/10/2011 |
| FLO.50700 | 07/10/2011 |
| FLO.50750 | 07/10/2011 |

## UNDERSTANDING THE CAP ACCREDITATION CHECKLIST COMPONENTS

To provide laboratories with a better means to engage in and meet their accreditation requirements, the CAP has enhanced the checklist content and updated its design. New components containing additional information for both the laboratory and inspectors include Subject Headers, Declarative Statements and Evidence of Compliance. See below for a definition of each new feature as an example of how they appear in the checklists.



### Using Evidence of Compliance (EOC)

This component, which appears with several checklist requirements, is intended to:

- 1 Assist a laboratory in preparing for an inspection and managing ongoing compliance
- 2 Drive consistent understanding of requirements between the laboratory and the inspector
- 3 Provide specific examples of acceptable documentation (policies, procedures, records, reports, charts, etc.)

In addition to the Evidence of Compliance listed in the checklist, other types of documentation may be acceptable. Whenever a policy/procedure/process is referenced within a requirement, it is only repeated in the Evidence of Compliance if such statement adds clarity. All policies/procedures/processes covered in the CAP checklists must be documented. A separate policy is not needed for each item listed in EOC as it may be referenced in an overarching policy.





## HOW TO INSPECT USING R.O.A.D INSPECTION TECHNIQUES (Read, Observe, Ask, Discover)

CAP has streamlined the inspection approach used during onsite inspections and is now offering guidance to inspectors by providing assessment techniques to facilitate a more efficient, consistent, and effective inspection process. Specific inspector instructions are listed at the beginning of a grouping of related requirements.

Rather than reviewing each individual requirement, CAP inspectors are encouraged to focus on the Inspector Instructions for a grouping of related requirements. Once an area of concern has been identified through "Read," "Observe," "Ask," "Discover," or a combination thereof, inspectors are encouraged to "drill down" to more specific requirements, when necessary and review more details outlined in the Evidence of Compliance statements. If a requirement is non-compliant, circle the requirement number to later list on the Inspector Summation Report. Inspectors may also make notes in the margins of the checklist document.

Inspector Instructions and Icons used to evaluate a laboratory's performance now appear in several areas throughout the Inspector Checklists. Please note that all four R.O.A.D elements are not always applicable for each grouping, or sections of related requirements.

### Inspector Instructions:

|  |   |
|--|---|
|  <p><b>READ</b></p>      | <p><b>READ</b>/review a sampling of laboratory documents. Information obtained from this review will be useful as you observe processes and engage in dialogue with the laboratory staff.<br/>(Example of the complimentary inspector instructions for Quality Management/Quality Control General Issues section appearing across checklists):</p> <ul style="list-style-type: none"> <li>• Sampling of QM/QC policies and procedures</li> <li>• Incident/error log and corrective action</li> </ul>  |
|  <p><b>OBSERVE</b></p>  | <p><b>OBSERVE</b> laboratory practices by looking at what the laboratory personnel are actually doing and note if practice deviates from the documented policies/procedures.<br/>(Example)</p> <ul style="list-style-type: none"> <li>• Observe the settings/QC range limits established in the laboratory LIS/HIS to ensure that the laboratory's stated ranges are accurately reflected</li> </ul>  |
|  <p><b>ASK</b></p>      | <p><b>ASK</b> open-ended, probing questions that start with phrases such as "tell me about..." or "what would you do if..." This approach can be a means to corroborate inspection findings that were examined by other techniques, such as Read &amp; Observe. Ask follow-up questions for clarification. Include a variety of staff levels in your communication process.<br/>(Example)</p> <ul style="list-style-type: none"> <li>• As a staff member, what is your involvement with quality management?</li> <li>• How do you detect and correct laboratory errors?</li> </ul>                                  |
|  <p><b>DISCOVER</b></p> | <p><b>DISCOVER</b> is a technique that can be used to "drill down" or further evaluate areas of concern uncovered by the inspector. "Follow the specimen" and "teach me" are two examples of Discovery. Utilizing this technique will allow for the discovery of pre-analytic, analytic, and post-analytic processes while reviewing multiple requirements simultaneously.<br/>(Example)</p> <ul style="list-style-type: none"> <li>• Select several occurrences in which QC is out of range and follow documentation to determine if the steps taken follow the laboratory policy for corrective action</li> </ul> |



## INTRODUCTION

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

*An inspection of a laboratory section, or department will include the discipline-specific checklist(s), the Laboratory General Checklist, and the All Common Checklist (COM).*

*In response to the ongoing request to reduce the redundancy within the Accreditation Checklists, the CAP accreditation program is introducing the All Common Checklist.*

*The purpose of the All Common Checklist is to group together requirements that were redundant in Laboratory General and the discipline-specific checklists. Therefore, the CAP centralized all requirements regarding: proficiency testing, procedure manuals, test method validation, and critical results into one checklist, the COM checklist.*

*Note for non-US laboratories: Checklist requirements apply to non-US laboratories unless the checklist items contain a specific disclaimer of exclusion.*

## 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 which do not perform staining and acquisition of flow cytometry data, but which receive [1] list mode files and/or [2] 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 documentation of peer review




## REFERENCES

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

## QUALITY MANAGEMENT AND QUALITY CONTROL

### GENERAL ISSUES

#### Inspector Instructions:

|   |  |
|---|--|
|    | <ul style="list-style-type: none"> <li>• Sampling of QM policies and procedures</li> <li>• QM/QC program, including pre-analytic, analytic and post-analytic monitor records and corrective action when indicators do not meet threshold</li> <li>• Incident/error log and corrective action</li> <li>• Records of high school graduate high complexity test review by supervisor</li> </ul> |
|   | <ul style="list-style-type: none"> <li>• How do you evaluate data on the incident/error log? How do you determine appropriate corrective action?</li> <li>• As a staff member, what is your involvement with quality management?</li> <li>• How do you detect and correct laboratory errors?</li> </ul>  |
|  | <ul style="list-style-type: none"> <li>• Follow an incident identified on the incident/error log and follow actions including notification and resolution</li> <li>• Select several problems identified by the QM plan and follow tracking and corrective action. Determine if the methods used led to discovery and effective correction of the problem.</li> </ul>                         |

#### FLO.20000 Documented QM/QC Plan

Phase II

#### The flow cytometry laboratory has a written quality management/quality control (QM/QC) program.

*NOTE: The program must ensure quality throughout the preanalytic, analytic, and post-analytic (reporting) phases of testing, including patient identification and preparation; specimen collection, identification, preservation, transportation, and processing; and accurate, timely result reporting. The program must be capable of detecting problems in the laboratory's systems, and identifying opportunities for system improvement. The laboratory must be able to develop plans of corrective/preventive action based on data from its QM system.*

*All QM requirements in the Laboratory General Checklist pertain to the flow cytometry laboratory.*

## REFERENCES

- 1) D'Hautcourt JL. Quality control procedures for flow cytometric applications in the hematology laboratory. *Hematol Cell Ther.* 1996;38:467-470
- 2) Gratama JW, et al. Quality control of flow cytometric immunophenotyping of haematological malignancies. *Clin Lab Haem.* 1999;21:155-160

**\*\*REVISED\*\*** 07/11/2011**FLO.20100 Unusual Laboratory Results****Phase II**

**There is a documented system in operation to detect and correct significant clerical and analytical errors, and unusual laboratory results, in a timely manner.**

*NOTE: One common method is review of results by a qualified person (technologist, supervisor, pathologist, laboratory director) before release from the laboratory, but there is no requirement for supervisory review of all reported data for single analyte tests that do not include interpretation. All tests that include an interpretation must be reviewed by the laboratory director or qualified designee before release from the laboratory. In computerized laboratories, there should be automatic "traps" for improbable results. The system for detecting clerical errors, significant analytical errors, and unusual laboratory results must provide for timely correction of errors, i.e. before results become available for clinical decision making. For confirmed errors detected after reporting, corrections must be promptly made and reported to the ordering physician or referring laboratory, as applicable.*

*Each procedure must include a listing of common situations that may cause analytically inaccurate results, together with a defined protocol for dealing with such analytic errors or interferences. This may require alternate testing methods; in some situations, it may not be possible to report results for some or all of the tests requested.*

*The intent of this requirement is NOT to require verification of all results outside the reference (normal) range.*

**Evidence of Compliance:**

- ✓ Record of review of results **OR** records of consistent implementation of the error detection system(s) defined in the procedure **AND**
- ✓ Records of timely corrective action of identified errors

**FLO.20200 Supervisory Result Review****Phase II**

**In the absence of on-site supervisors, the results of tests performed by personnel are reviewed by the laboratory director or general supervisor within 24 hours.**

*NOTE: The CAP does NOT require supervisory review of all test results before or after reporting to patient records. Rather, this requirement is intended to address only that situation defined under CLIA for "high complexity testing" performed by trained high school graduates qualified under 42CFR493.1489(b)(5) when a qualified general supervisor is not present.*

**Evidence of Compliance:**

- ✓ Written policy defining the review process and personnel whose results require review **AND**
- ✓ Records of result review for specified personnel




## 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):7182 [42CFR493.1463(a)(3) and 42CFR493.1463(c)]:7183 [42CFR493.1489(b)(1) and 42CFR493.1489(b)(5)]

## SPECIMEN COLLECTION AND HANDLING

### Inspector Instructions:

- Sampling of flow cytometry specimen collection and handling policies and procedures
- Sampling of specimen rejection records/log

|   |   |
|---|---|
|  |   |
|  | <ul style="list-style-type: none"> <li>• Sampling of flow cytometry specimens (labeling)</li> </ul>   |
|  | <ul style="list-style-type: none"> <li>• What is your course of action when you receive unacceptable/sub-optimal flow cytometry specimens?</li> </ul> |

**FLO.20500 Specimen Collection Manual Phase II**

**There is a documented procedure describing methods for patient identification, patient preparation, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing, consistent with good laboratory practice.**

**FLO.22000 Specimen Identity/Integrity Phase II**

**Procedures are adequate to verify sample identity and integrity (includes specimens of blood, body fluids and tissues).**

**Evidence of Compliance:**

- ✓ Specimen collection and handling procedure **AND**
- ✓ Patient collection and processing records

**\*\*REVISED\*\* 07/11/2011**

**FLO.22050 Specimen Rejection Criteria Phase II**

**There are documented criteria for the rejection of unacceptable specimens or the special handling of sub-optimal specimens.**

*NOTE: This requirement does not imply that all "unsuitable" specimens are discarded or not analyzed. If for example, improper storage hemolyzes a sample and hemolysis interferes with testing, there must be a mechanism to notify clinical personnel responsible for patient care. If the treating physician desires the result, then the laboratory must note the condition of the sample on the report. Some or all tests may not be analytically valid on such a specimen. The laboratory may wish to record that a dialogue was held with the physician, when such occurs.*

**Evidence of Compliance:**

- ✓ Defined specimen acceptability criteria **AND**
- ✓ Records of rejected/unacceptable specimens

**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):7183 [42CFR493.1249(a) and (b)]
- 2) National Institute for Allergy and Infectious Diseases/Division of AIDS guidelines for flow cytometric immunophenotyping, version 1.0, Jan 1993, sec 1.06

**FLO.22100 Disposition of Unacceptable Specimens****Phase II**




**The disposition of all unacceptable specimens is documented in the patient report and/or quality management records.**

*NOTE: This information is essential to proper patient test management and to the laboratory quality management program.*

## REAGENTS

The laboratory has the responsibility for ensuring that all reagents, calibrators and controls, whether purchased or prepared by the laboratory, are appropriately reactive. The verification of reagent performance is required and must be documented. Any of several methods may be appropriate, such as direct analysis with reference materials, parallel testing of old vs. new reagents, and checking against routine controls. The intent of the requirements is for new reagents to be checked by an appropriate method and the results recorded before patient results are reported. Where individually packaged reagents/kits are used, there should be criteria established for monitoring reagent quality and stability, based on volume of usage and storage requirements. Processing of periodic "wet controls" to validate reagent quality and operator technique is a typical component of such a system.

### Inspector Instructions:

|   |  |
|---|--|
|   | <ul style="list-style-type: none"> <li>• Sampling of test procedures for reagent handling</li> <li>• Sampling of new reagent/shipment verification records</li> <li>• Sampling of ambient temperature logs (if reagents stored at ambient temperature)</li> </ul>  |
|  | <ul style="list-style-type: none"> <li>• Sampling of reagents (expiration date, labeling, storage)</li> </ul>  |
|  | <ul style="list-style-type: none"> <li>• How do you store reagents and controls used in test procedures?</li> <li>• How do you verify new reagent lots?</li> <li>• What process does your laboratory follow to ensure manufacturer's recommendations are followed regarding the use of reagents/controls in kit procedures?</li> <li>• What are your laboratory's criteria for mixing components from one lot number of reagent kit with components from another lot number of kit?</li> <li>• How does your laboratory manage and control reagent inventory?</li> </ul> |

**FLO.23000 Reagent Labeling****Phase II**

**Reagents, calibrators, cellular controls, and solutions are properly labeled, as applicable and appropriate, with the following elements.**

1. **Content and quantity, concentration or titer**
2. **Storage requirements**
3. **Date prepared or reconstituted by laboratory**

#### 4. Expiration date

*NOTE: The above elements may be recorded in a log (paper or electronic), rather than on the containers themselves, providing that all containers are identified so as to be traceable to the appropriate data in the log. While useful for inventory management, labeling with "date received" is not routinely required. There is no requirement to routinely label individual containers with "date opened"; however, a new expiration date must be recorded if opening the container changes the expiration date, storage requirement, etc.*

##### Evidence of Compliance:

- ✓ Written policy defining elements required for reagent labeling

##### 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):7164 [42CFR493.1252(c)]

**\*\*REVISED\*\***

**07/11/2011**

**FLO.23050**

**Reagent Expiration Date**

**Phase II**

**All reagents are used within their indicated expiration date.**

*NOTE: The laboratory must assign an expiration date to any reagents that do not have a manufacturer-provided expiration date. The assigned expiration date should be based on known stability, frequency of use, storage conditions, and risk of deterioration.*

*For laboratories not subject to US regulations, expired reagents may be used only under the following circumstances: 1. The reagents are unique, rare or difficult to obtain; or 2. Delivery of new shipments of reagents is delayed through causes not under control of the laboratory. The laboratory must document validation of the performance of expired reagents in accordance with written laboratory policy. Laboratories subject to US regulations must not use expired reagents.*

##### Evidence of Compliance:

- ✓ Written policy for evaluating reagents lacking manufacturer's expiration date

##### 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):7164 [42CFR493.1252(d)]

**FLO.23100**

**Reagent Storage**

**Phase II**

**Reagents are stored as recommended by the manufacturer.**

*NOTE: Reagents must be stored as recommended by the manufacturer to prevent environmentally-induced alterations that could affect test performance. If ambient temperature is indicated, there must be documentation that the defined ambient temperature is maintained and corrective action is taken when tolerance limits are exceeded.*

##### Evidence of Compliance:

- ✓ Records of reagent storage consistent with manufacturer's instructions, including refrigerator, freezer and room temperature monitoring, as applicable

**FLO.23150**

**New Reagent Lot Verification**

**Phase II**

**New reagent lots and/or shipments are checked against old reagent lots or with suitable reference material before or concurrently with being placed in service.**

*NOTE: The purpose is to determine that the new lot or shipment of test reagent gives a clinically comparable result to the old reagent. This may be accomplished by comparing the results of the old and new reagent tested in parallel on the same fresh control (patient or normal) or testing just*

*the new reagent on a standardized control with defined mean and reference range. This does not imply the need to compare the new reagent lot to a negative control.*

**Evidence of Compliance:**

- ✓ Written procedure for the verification of new lots and shipments prior to use **AND**
- ✓ Records of verification of new reagents/shipments

**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 accuracy evaluation 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.23300 Reagent Kit Components**

**Phase II**

**If there are multiple components of a reagent kit, the laboratory only uses within kit lot components of reagents unless otherwise specified by the manufacturer.**

*NOTE: If there are multiple components of a reagent kit, the laboratory must use components of reagent kits only with other kits that are in the same lot number unless otherwise specified by the manufacturer or accuracy/equivalency is verified by the laboratory.*

**Evidence of Compliance:**

- ✓ Written documentation defining allowable exceptions for mixing kit components from different lots

**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):7164 [42CFR493.1252(d)]

## 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 of 1988 (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

- 1) Department of Health and Human Services, Food and Drug Administration. Medical devices; classification/reclassification; restricted devices; analyte specific reagents. Final rule. *Fed Register*. 1997(Nov 21);62243 [21CFR809 and 864]
- 2) Caldwell CW. Analyte-specific reagents in the flow cytometry laboratory. *Arch Pathol Lab Med*. 1998;122:861-864
- 3) Graziano. Disclaimer now needed for analyte-specific reagents. *CAP Today*. 1998;12(11):5-11
- 4) U.S. Department of Health and Human Services, Food and Drug Administration. Analyte Specific Reagents; Small Entity Compliance Guidance. <http://www.fda.gov/cdrh/oivd/guidance/1205.html>, February 26, 2003
- 5) Shapiro JD and Prebula R.J. FDA's Regulation of Analyte-Specific Reagents. Medical DeviceLink, February 2003. <http://www.deviceLink.com/mddi/archive/03/02/018.html>

## FLO.23706 Record Retention

Phase II

### **Gated dot plots and histograms are retained for at least 10 years.**

*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). List mode data files with embedded gates and analysis regions are also acceptable.*

#### REFERENCES

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

## CONTROLS AND STANDARDS




*Controls are samples that act as surrogates for patient specimens. They are periodically processed like a patient sample to monitor the ongoing performance of the entire analytic process.*

*Most quantitative tests are traditionally monitored with 2 levels of liquid control material (procedural control). This is done at a frequency within which the accuracy and precision of the measuring system is expected to be stable (based upon manufacturer's recommendations), but at least each day that patient testing is performed. The daily use of two levels of liquid control may NOT be required for certain test systems, where the daily use of instrument and/or electronic controls is demonstrably sufficient to validate that calibration status is maintained within acceptable limits.*



The daily use of 2 levels of instrument and/or electronic controls as the only QC system is acceptable only for unmodified test systems cleared by the FDA and classified under CLIA as "waived" or "moderate complexity." The laboratory is expected to provide documentation of its validation of all instrument-reagent systems for which daily controls are limited to instrument and/or electronic controls, and the inspector will review these data to assess the adequacy of the QC system. This documentation must include the Federal complexity classification of the testing system AND data showing that calibration status is monitored.

### Inspector Instructions:

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

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 documentation 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 and patient material.*

**Evidence of Compliance:**

- ✓ Written procedure defining QC requirements 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 2 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/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. When commercial controls are not available, then the laboratory director must implement an equivalent procedure to meet the two-level requirement. This alternative must be deemed acceptable by the laboratory inspection team through LAP. Control testing is not necessary on days when patient testing is not performed.*

**Evidence of Compliance:**

- ✓ Written procedure defining QC requirements for each test **OR** records of validation of an alternate/equivalent procedure when commercial controls are not available
- ✓ 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 QC Range Validation****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 defining methods used to establish or verify control ranges **AND**
- ✓ Records for control range verification of each lot

**\*\*REVISED\*\* 07/11/2011****FLO.24230 QC Corrective Action****Phase II**

**There is documentation 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 process 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**

- 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 Verification Phase II**

**The results of controls are verified 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 unacceptable results.*

**Evidence of Compliance:**

- ✓ Defined QC tolerance limits and records of verification of acceptable 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*. 2003(Jan 24):7166 [42CFR493.1256(f)]

**\*\*REVISED\*\* 07/11/2011  
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 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 with documented follow-up for outliers, trends or omissions

**\*\*REVISED\*\* 07/11/2011  
FLO.24650 Comparability of Instrument/Method**

**Phase II**

**If the laboratory uses more than one instrument/method to test for a given analyte, the instruments/methods are checked against each other at least twice a year for correlation of results.**

*NOTE: This requirement applies to tests performed on the same or different instrument makes/models or by different methods. This comparison must include all nonwaived instruments/methods. The laboratory director must establish a protocol for this check.*

Quality control data may be used for this comparison for tests performed on the same instrument platform, with both control materials and reagents of the same manufacturer and lot number.

Otherwise, the use of human samples, rather than stabilized commercial controls, is preferred to avoid potential matrix effects. The use of pooled patient samples is acceptable since there is no change in matrix. In cases when availability or pre-analytical stability of patient specimens is a limiting factor, alternative protocols based on QC or reference materials may be necessary but the materials used should be validated (when applicable) to have the same response as fresh human samples for the instruments/methods involved.

This checklist requirement applies only to instruments/methods accredited under a single CAP number.

#### Evidence of Compliance:

- ✓ Written procedure for performing instrument/method correlation including criteria for acceptability **AND**
- ✓ Records of correlation studies reflecting performance at least twice per year with appropriate specimen types

#### REFERENCES




- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24):5236 [42CFR493.1281(a)]
- 2) Miller WG, Ereik A, Cunningham TD, et al. Commutability limitations influence quality control results with different reagent lots. *Clin Chem*. 2011;57:76-83

## INSTRUMENTS AND EQUIPMENT

### Flow Cytometers

A variety of instruments and equipment are used to support the performance of analytical procedures. All instruments and equipment should be properly operated, maintained, serviced, and monitored to ensure that malfunctions of these instruments and equipment do not adversely affect the analytical results. The procedures and schedules for instrument maintenance must be as thorough and as frequent as specified by the manufacturer.

#### Inspector Instructions:

|  |   |
|--|---|
|  <p>READ</p>    | <ul style="list-style-type: none"> <li>• Sampling of instrument(s) policies and procedures</li> <li>• Sampling of instrument maintenance logs and repair records</li> <li>• Sampling of optical alignment/laser output checks</li> </ul>  |
|  <p>OBSERVE</p> | <ul style="list-style-type: none"> <li>• Instrument records (promptly retrievable)</li> </ul>   |
|  <p>ASK</p>     | <ul style="list-style-type: none"> <li>• How does your laboratory monitor instrument reproducibility?</li> <li>• How does your laboratory ensure each fluorochrome is appropriately calibrated?</li> <li>• How does your laboratory determine appropriate color compensation settings?</li> </ul> |

|  |                                      |                 |
|--|--------------------------------------|-----------------|
| <b>FLO.25100</b>   | <b>Function Checks</b>               | <b>Phase II</b> |
| <b>Appropriate function checks are performed for all instruments prior to testing patient samples.</b>   |                                      |                 |
| <i>NOTE: There must be a schedule and procedure at the instrument for appropriate function checks. These may include (but are not limited to) electronic, mechanical and operational checks. The procedure and schedule must be as thorough and as frequent as specified by the manufacturer. Function checks should be designed to check the critical operating characteristics to detect drift, instability, or malfunction, before the problem is allowed to affect test results. All servicing and repairs must be documented.</i> |                                      |                 |
| <b>FLO.25150</b>   | <b>Optical Alignment</b>             | <b>Phase II</b> |
| <b>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 is documentation of this monitoring.</b>  |                                      |                 |
| <i>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.</i>  |                                      |                 |
| REFERENCES   |                                      |                 |
| 1) Clinical and Laboratory Standards Institute (CLSI). <i>Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition</i> . 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   |                                      |                 |
| <b>FLO.25200</b>   | <b>Instrument Troubleshooting</b>    | <b>Phase II</b> |
| <b>Instructions are provided for minor troubleshooting and repairs of instruments (such as manufacturer's service manual).</b>   |                                      |                 |
| <b>FLO.25250</b>   | <b>Instrument Function Checks</b>    | <b>Phase II</b> |
| <b>Instrument maintenance, service and repair records (or copies) are promptly available to, and usable by, the technical staff operating the equipment.</b>   |                                      |                 |
| <i>NOTE: The effective utilization of instruments by the technical staff depends upon the prompt availability of maintenance, repair, and service documentation (copies are acceptable). Laboratory personnel are responsible for the reliability and proper function of their instruments and must have access to this information. Off-site storage, such as with centralized medical maintenance or computer files, is not precluded if the inspector is satisfied that the records can be promptly retrieved.</i>                  |                                      |                 |
| <b>FLO.25300</b>   | <b>Instrument Maintenance Review</b> | <b>Phase II</b> |
| <b>Records of maintenance are kept and reviewed at least monthly by the person in charge of technical operations of the flow cytometry laboratory.</b>   |                                      |                 |
| <b>Evidence of Compliance:</b>   |                                      |                 |
| ✓ Instrumentation records including documentation of review and corrective action  |                                      |                 |

**FLO.30250 Fluorochrome Standards Phase II**

**Appropriate standards for each fluorochrome, (e.g. fluorescent beads), are run each day that the instrument is used as part of the calibration process; 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 documentation 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.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 are the proper reference material for establishing appropriate compensation settings.*

## 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.30270 Laser Current Phase I**

**For laser instruments, there are procedures in place 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.*

**FLO.30280 Calibration/Laser Review Phase II**

**The results of instrument calibration and laser output checks (where appropriate) are reviewed monthly by the person in charge of technical operations of the flow cytometry laboratory.**

**Evidence of Compliance:**

- ✓ Records of follow-up for any outliers or trends, as applicable

## Temperature-Dependent Equipment

### Inspector Instructions:

- Sampling of temperature logs (refrigerator, freezer, water bath, heat blocks)



**\*\*REVISED\*\***    **06/17/2010**  
**FLO.30290**    **Temperature Checks**

**Phase II**

**Temperatures are checked and recorded daily for each of the following types of equipment.**

- 1. Water baths**
- 2. Incubators (where temperature control is necessary for a procedure)**
- 3. Refrigerators and freezers**

*NOTE: Temperature-dependent equipment containing reagents and patient specimens must be monitored daily, as equipment failures could affect accuracy of patient test results. Items such as water baths and heat blocks used for procedures need only be checked on days of patient testing.*

*The two acceptable ways of recording temperatures are: 1) recording the numerical temperature, or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually, or using a graphical recording device). The identity of the individual recording the temperature(s) must be documented (recording the initials of the individual is adequate).*

*The use of automated (including remote) temperature monitoring systems is acceptable, providing that laboratory personnel have ongoing immediate access to the temperature data, so that appropriate corrective action can be taken if a temperature is out of the acceptable range. The functionality of the system must be documented daily.*

**\*\*REVISED\*\***    **07/11/2011**  
**FLO.30320**    **Temperature Range**

**Phase II**

**Acceptable ranges have been defined for all temperature-dependent equipment with documentation of corrective action when values exceed these ranges.**

**Evidence of Compliance:**

- ✓ Temperature log or record with defined acceptable range **AND**
- ✓ Evidence of corrective action

## Thermometers

### Inspector Instructions:



- Records of traceability to NIST standards

- What is your laboratory's course of action prior to using non-certified thermometers?

**FLO.30360 Thermometric Standard Device****Phase II**

**An appropriate thermometric standard device of known accuracy is available. (guaranteed by manufacturer to meet NIST Standards.)**

*NOTE: Thermometers should be present on all temperature-controlled instruments and environments and checked daily. Thermometric standard devices should be recalibrated or recertified prior to the date of expiration of the guarantee of calibration.*

**Evidence of Compliance:**

- ✓ Thermometer certificate of accuracy

**FLO.30370 Non-Certified Thermometers****Phase II**

**All non-certified thermometers in use are checked against an appropriate thermometric standard device before use.**

**Evidence of Compliance:**

- ✓ Written procedure defining criteria for verification of non-certified thermometers **AND**
- ✓ Records of verification prior to being placed in service

## Automatic Pipetting Devices

### Inspector Instructions:



- Automatic pipette calibration procedure
- Sampling of pipette/dilutor checks

**FLO.30415 Automatic Pipettes****Phase II**

**Automatic pipettes used for quantitative dispensing are checked for accuracy and reproducibility before being placed in service and at least annually, with documentation of results.**

*NOTE: 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 provide convenient documentation. 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**

- 1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab.* 1994;12(7):8-9



- 2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab*. 1994;12(9):16-17
- 3) Perrier S, et al. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem*. 1995;41:S183
- 4) Bray W. Software for the gravimetric calibration testing of pipets. *Am Clin Lab*. Oct 1995
- 5) CLSI. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline*. CLSI Document GP31-A. (ISBN 1-56238-697-2). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2009
- 6) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist*. 1999;13(12):14
- 7) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News*. 1999;31(13):20-22
- 8) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med*. 2000;31:478-479

## 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

Phase II

**There is a procedure in place to document 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

- 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 5 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 validated using linear forward angle light scatter and 90-degree side scatter, or using CD45-FITC and CD14-PE monoclonal antibodies.*

REFERENCES

- 1) National Institute for Allergy and Infectious Diseases/Division of AIDS guidelines for flow cytometric immunophenotyping, ver 1.0, Jan 1993

**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 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 documented.*

**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) National Institute of Allergy and Infectious Diseases/Division of AIDS flow cytometry guidelines, sec 3.09B and 5.03A
- 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 (normal values) must be determined by laboratory, if feasible. For example, a reference interval can be validated by testing samples from 20 healthy representative individuals; if no more than 2 results fall outside the proposed reference interval, that interval can be considered validated for the population studied (refer to CLSI guideline C28-A3, referenced below). If this is not possible or practical, then the laboratory should carefully evaluate the use of published data for its own reference intervals, and retain documentation 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 laboratory director

**REFERENCES**

- 1) Knight JA. Laboratory issues regarding geriatric patients. *Lab Med.* 1997;28:458-461
- 2) Clinical Laboratory and Standards Institute (CLSI). *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition* CLSI Document C28-A3c (ISBN 1-56238-682-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2008

## CD34 Stem Cell Enumeration

### Inspector Instructions:

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

**FLO.30564 CD34 Cellular Viability****Phase I**

**There is a procedure in place to document CD34 cellular viability, where applicable.**

*NOTE: Viability testing is not necessary on peripheral blood or apheresis specimens that are stained and analyzed within 4 hours of drawing (harvesting). Cord blood, bone marrow and samples more than four hours old should have total cellular viability evaluated as a minimum by dye exclusion. Analyses on these older samples are possible if the laboratory has verified the absence of clinically significant differences in CD34+ cells between the fresh and aged specimens. The viability dye 7-amino actinomycin-D (7-AAD) has been reported to provide excellent results in this analysis.*

**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;34:128-142

**FLO.30571 Apheresis Specimen Handling****Phase I**

**For apheresis samples stored more than 4 hours, an aliquot of the product is taken immediately before processing for freezing (autologous donors) or infusion (allogeneic donors).**

*NOTE: For apheresis samples stored for more than 4 hours before CD34 cell analysis, an aliquot of the product should be taken and tested for CD34+ cell numbers and viability immediately before infusion or processing for freezing. The viability assessment 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 a precise determination of CD34+ cell viability. Estimates of total cellular viability (for example, trypan blue exclusion) may not be used as an alternative, as they may overestimate the viability of the much smaller (and more fragile) CD34 stem cell population. When for clinical reasons it is necessary to perform CD34 cell analysis on specimens stored for more than 4 hours, the report should include the number of hours elapsed prior to analysis of the specimen and a statement that laboratory viability testing is a reflection of the ability of CD34 cells to survive and proliferate in vivo.*

**Evidence of Compliance:**

- ✓ Written procedure for apheresis specimen handling

**REFERENCES**

- 1) Keeney M, et al. Single platform flow cytometry absolute CD34+ cell counts based on the ISHAGE guidelines. *Cytometry*.1998;34:61-70

**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 procedure defining 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 procedure defining 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:



- 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?

**\*\*NEW\*\***

06/17/2010

**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. Procedures must be in place to ensure that viable cells are analyzed. 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 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 defining use of appropriate monoclonal antibodies

## 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**

**Methods are established to ensure that immunoglobulin staining is intrinsic and not extrinsic (cytophilic).**

*NOTE: The requirement is directed towards ensuring 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 defining 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.*

## 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, Salhany 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. Ideally, direct morphologic correlation should be done. Such review is appropriate for bone marrow samples, solid tissues, and blood in cases of suspected hematolymphoid neoplasia. 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

- 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

## 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 methods 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 defining method for verifying the presence of neoplastic cells **AND**
- ✓ Records of specimen evaluation

**FLO.31010 Cellular Debris****Phase II**

**There are methods in place 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 defining method 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, (e.g. 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 documented 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 documented criteria for acceptability of histograms for interpretation.**

**FLO.31250 Specimen Rejection Criteria Phase II**

**There are documented criteria for specimen rejection for DNA content analysis.**

**Evidence of Compliance:**

- ✓ Written procedure defining criteria for specimen rejection and handling of sub-optimal specimens **AND**
- ✓ Records of specimen rejection **AND**
- ✓ Records noting the conditions of sub-optimal specimens

**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 defining controls used 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
- 2) NCCLS. *Laboratory Statistics—Standard Deviation; A Report*. NCCLS document EP13-R (ISBN 1-56238-277-2). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1995

**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

## PERSONNEL

### Inspector Instructions:



- Documentation of supervisor's education and experience

**FLO.40000 Personnel - Technical Operations**

**Phase II**

**The person in charge of technical operations in flow cytometry has education equivalent to that of an MT(ASCP) and at least 4 years experience (one of which is in flow cytometry) under a qualified director.**

**Evidence of Compliance:**

- ✓ Records of qualifications including degree or transcript, certification/registration, current license (if required) and work history in related field

## LABORATORY SAFETY

*The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the flow cytometry laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.*

### Inspector Instructions:



- Sampling of procedures for safety information

**FLO.50850 Safety Manual**

**Phase II**

**The procedure manuals contain information for safe handling of clinical samples and instruments with regard to infectious biohazard risks, cryogenic substances, carcinogenic dyes, and lasers.**