

**2006 Bethesda International Consensus Recommendations on
the Immunophenotypic Analysis of Hematolymphoid Neoplasia
by Flow Cytometry: Recommendations for Training and
Education to Perform Clinical Flow Cytometry**

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Abstract

As clinical flow cytometry practices continue to expand and the immunophenotyping for leukemia and lymphoma becomes more widespread, the need for defined guidelines for training of medical professionals is imperative. Standards of expected knowledge and skills are necessary to ensure reliable test results as well as provide direction to those who are considering adding flow cytometry to their clinical laboratory practice. Before now, no clear guidelines have been established for defining the areas of responsibility, education and training standards, and credentials that would be required to perform clinical flow cytometry for leukemia and lymphoma. As part of the 2006 Bethesda Consensus conference, a committee was formed to address this need and provide recommendations for training and education. The committee included laboratory professionals from private, public, and university hospitals as well as large reference laboratories that routinely operate clinical flow cytometry laboratories with an emphasis on lymphoma and leukemia immunophenotyping. This document represents the work of the committee. Categories of work responsibility are defined and the requisite education, training, and credentials, as well as measurement methods for assessing competency for each area of responsibility are provided. Additional recommendations are included that promote creating a specialty certification in flow cytometry, establishing benchmarks for training technologists and interpreters, and offer suggestions for minimum levels of experience to direct a clinical flow cytometry laboratory.

Key terms: lymphoma, leukemia, flow cytometry, education and training, credentials

Introduction

This document addresses issues as they pertain to education and training of personnel who routinely perform clinical flow cytometry applications on lymphoma and leukemia cases. Clinical Flow Cytometry acquisition and analysis is by definition¹ a high-complexity assay. As flow cytometry practices continue to expand, the need for defined guidelines for training of medical professionals is imperative. Errors in flow cytometry sample acquisition or data analysis can lead to misleading and unreliable results. Standards of expected knowledge and skills would hopefully contribute to more reliable results as well as provide direction to those who are considering adding flow cytometry to their clinical laboratory practice. No clear consensus has been established to provide guidance as to who should perform the testing, what training is necessary to insure high quality results, how successful completion of training could be determined, and who should train others. This document is intended to offer suggestions or a *proposed position statement* instead of representing a broad consensus opinion. The committee that developed these guidelines consisted of representatives from private, public, reference, and university-

based clinical laboratories. It was peer reviewed by laboratory professionals primarily from the United States and Canada as well as some European countries.

The committee's goals consisted of (1) defining training and education resources available for clinical flow cytometry (2) define the levels of responsibility, education and training necessary for consistent performance of flow cytometry testing of lymphomas and leukemias (3) describe a model for flow cytometry education, including measurable training methods and procedures (4) make recommendations for obtaining as well as renewing credentials for those who perform testing.

Training and Education Resources

Formal classroom training and coursework like that found in medical technology programs is a typical starting point for many laboratorians who receive instruction in several clinical laboratory disciplines including flow cytometry. Successful completion usually qualifies the individual to take a licensing or board exam like that of the American Society of Clinical Pathologists (ASCP).

Medical school graduates usually enter pathology residencies and fellowships in hematopathology programs that include several disciplines including flow cytometry, histology, cytology, immunohistochemistry, and molecular genetics. However, all of the formal approaches currently seem very limited for training in clinical flow cytometry. **Instrument manufacturer training programs** are another resource that is oftentimes available upon purchase of the equipment by the laboratory. Although it does not completely satisfy all the procedures required for starting up and performing lymphoma and leukemia testing, it does offer a good foundation for learning instrument operation and using analytical software.

On the Job training is usually part of every laboratory's training program. Oftentimes it includes procedures and protocols that are specific to the institution or hospital's needs and rarely may include some classroom work along with hands-on experience. The training is usually conducted by an experienced laboratorian and there are often regulatory requirements and evaluation criteria that have to be met before the individual is allowed to report results. Job experience still is the primary method of training clinical flow cytometrists.

On-line or self-guided training programs: internet-based training is becoming an increasingly popular resource for learning new procedures and theory without requiring travel or actual classroom attendance. These programs may also be beneficial for Continuing Education purposes as well as introducing new procedures to a laboratory. Self-guided programs offer convenience and access to information and technology that would otherwise be unavailable

locally. Though convenient and highly useful, they are not generally sufficient to fully prepare someone to perform all procedures or replace person-to-person training with actual samples.

Continuing Education Workshops and Seminars are another useful method for training flow cytometry fundamentals or even learning advanced procedures but rarely sufficient to completely prepare an individual to perform diagnostic testing of lymphoma and leukemia cases. When additional training is desired this resource can many times be a good way to update skills as well as learn new procedures.

Flow Cytometry Testing Responsibilities

Several unique responsibilities are found in laboratories that process specimens for lymphoma and leukemia. Each particular task requires its own procedures and protocols with knowledge that is sometimes specific to flow cytometry and at other times may resemble many other clinical laboratory disciplines. An instructor, lab director, medical director, supervisor or senior technologist with considerable experience and expertise usually conducts the training. They train new employees or students to understand the theory of the application, the steps to performing the procedures, and make determinations of training proficiency using specific indicators of progress also known as *competency*. The expertise and experience required to train may vary depending on educational resources, academic or instruction program availability, and access to a sufficient flow cytometry caseload that includes a broad spectrum of hematopathological specimens. Many of these training programs are used to prepare an individual to take board exams or satisfy other credentialing agency requirements.

Levels of Responsibility Found in Clinical Flow Cytometry Laboratories

Key Operator: In a clinical institution this person is usually a medical technologist or laboratory technician. The key operator routinely performs specimen setup, makes or composes antibody panels, and in some situations may also perform instrument operation and limited data analysis.

Advanced Operator (Analyst): The analyst may have some or all of the responsibilities as those listed for the Key Operator. In a clinical institution, they are usually medical technologists or others who have received additional training in complex and multicolor listmode data analysis using analytical software programs. In some situations they may contribute to the interpretation of results. Completion of a flow cytometer and software training program is an additional resource that contributes to their educational background. Some key operators and analysts may receive training and certification as part of a military medical training program. In European and other countries, special schools and institutions teach and train specific laboratory disciplines as well as other medical laboratory sciences. Many countries offer certification as laboratory technicians to students who successfully complete a laboratory training program, however, specific and comprehensive certification in clinical flow cytometry with an emphasis on lymphoma and leukemia applications is not available.

Instructor / Trainer : Usually an advanced medical technologist, supervisor, PhD., and M.D. or other experienced individual with flow cytometry-specific teaching responsibilities. They may be certified as specialists or qualified to teach and train by local licensing agencies such as a state licensing board. Postgraduate studies with an emphasis on teaching is a highly recommended credential for these individuals and completion of an instrument and software training program is also highly recommended. The Education and Training consensus committee recommends that in the U.S. and Canada, trainers of new laboratorians or students to perform flow cytometry testing of lymphoma and leukemia specimens should be licensed and/or board certified medical technologists with a four-year degree and have a minimum of 2 years of clinical flow cytometry

experience. Military medical training with certification similar to what is offered in programs specifically for becoming a laboratory professional should also qualify as being an acceptable credential.⁴

Interpreter : An advanced and experienced individual usually a PhD or pathologist, particularly the latter if a diagnosis is included in the patient testing report. Case review and correlation with morphology as well as clinical and laboratory findings is often used as a training method for developing proficiency and the knowledge required to interpret data. Training at this level is typically provided in the context of a graduate degree program for PhDs or post-graduate residency and fellowship training for MDs, often with supplemental on the job training. Regulatory requirements oftentimes dictate who can diagnose versus who can interpret (for example, in the US the Clinical Laboratory Improvement Act (CLIA) dictates that only MDs , DOs, or PhDs can direct non-forensic and non-anatomic laboratories)¹.

Suggested Training and Education Models

Each area in the clinical flow cytometry laboratory requires specific training. Much of the training and education is common to all but some areas require additional or even advanced training. The additional training required for clinical flow cytometry applications usually includes five well-defined areas:

- The Theory and Applications of Clinical Flow Cytometry Testing.
- Specimen Triage and Antibody Panel Selection.
- Flow Cytometer and Reagent Quality Control, Instrument Operation and Sample Acquisition.
- Listmode Data Analysis.
- Interpretation and Reporting of Results.

Key Operator Training

Theory and Applications of Flow Cytometry Testing: core knowledge of flow cytometry theory, principles and applications. Teaching that includes fundamental practices along with flow cytometry applications is usually found in all training programs. Training should include either lecture or tutorial instruction along with hands-on time in the laboratory to fully orientate the new user.

Specimen Triage and Antibody Panel Selection

Specimen triage and antibody panel selection includes specimen transport, handling and preparation, knowledge of supplies and solutions, selection and preparation of antibody panels, reagent quality control, safety, and familiarity with standard medical laboratory practices.

Quality Control, Instrument Operation and Sample Acquisition

Quality control, instrument operation and sample acquisition includes safety procedures, alignment and optics, fluidics, color compensation, the use of bead and biological controls, recognition of quality results, knowledge of corrective actions for out-of-range results, routine maintenance and troubleshooting. Instrument manufacturer training programs are a highly useful resource for learning these fundamental procedures.

Limited Listmode Data Analysis

The Key Operator should have practical knowledge of peripheral blood lymphocyte assays such as CD4 counts or lymphocyte profiles that include major mature (B's, T's, NK's) lymphocyte studies, quality control, and laboratory practices from a standpoint of less complex testing than that required with lymphoma and leukemia samples.

Suggested Credentials for Key Operators (U.S. / Canada): licensed medical technologist or medical laboratory technician. Military training with certification should also qualify as being an

acceptable credential. If available, certification by a specific and comprehensive flow cytometry program is highly recommended.

Advanced Operator (Analyst) Training

Analysts in many cases receive the same training as Key Operators and it is highly recommended that they have previous experience in those responsibilities. This can be beneficial since training of flow cytometry theory and principles is a fundamental requirement for all levels of responsibility. The analyst should be familiar with technical issues and performance characteristics of flow cytometry reagents and antibodies that are commonly used in lymphoma and leukemia applications. Knowledge of instrument operation and sample acquisition is essential and should be included in advanced operator training. Besides knowing flow cytometry theory and principles, training to become an Advanced Operator Analyst should include the following:

Analytical Procedures: sufficient knowledge to analyze listmode data using appropriate software. This requirement may be facilitated by successful completion of software or instrument manufacturer training programs. In addition, the training should include general knowledge of technical analysis procedures such as light scatter and fluorescence gating, fluorochrome excitation/emission, color compensation, and a substantial understanding of the immunophenotyping of lymphoma and leukemia specimens.

Situational Analysis: the analyst should be able to recognize normal or reactive populations from abnormal, aberrant or unexpected populations. In many cases the population in question may be discrete and may be present at relatively low abundance (0.1-1.0%). Knowing how to identify and differentiate these populations should be included in the training.

Experience gained by case review: analyst training should include analysis of at least 100 cases and encompass a variety of routine and atypical presentations often found in the evaluation

for neoplasia. Results should be reviewed for accuracy by the trainer / instructor or other qualified individual prior to analyzing actual cases that are to be reported. A comparison to other ancillary laboratory results such as morphology reports, immunohistochemical stains and cytogenetic lab results, if available, can be a highly useful way to measure training proficiency.

Suggested Credentials for Analysts (U.S. / Canada): licensed medical technologist. Military training with certification should also qualify as being an acceptable credential. Having the American Society of Clinical Pathology Qualification in Flow Cytometry (QCYM) or similar type certification is also recommended if and when such credentialing programs become more comprehensive and reflect different levels of competency. For example in the US, the National Credentialing Agency for Laboratory Personnel offers certification for the Clinical Laboratory Specialist in Cytogenetics (CLSpCG) and a Certified Laboratory Specialist in Molecular Biology (CLSpMB) as well as, although generic, certification for Clinical Laboratory Supervisor (CLSup).

Instructor /Trainer Training

Instructors should be familiar with the complete training requirements for Key Operators and Advanced Operators (Analysts). Having several years of experience in performing procedures and analysis of lymphoma and leukemia flow cytometry cases is vitally important and strongly recommended. Successful completion of advanced instrument and software training programs is also recommended. Post-graduate degrees especially in teaching, laboratory sciences, or medical specialties are highly desirable.

Suggested Credentials for Instructors / Trainers (U.S. / Canada): licensed medical technologists with 2 or more years of experience in clinical flow cytometry who have personally reviewed and analyzed many different types of lymphoma and leukemia cases should have knowledge sufficient to train Key Operators and Advanced Operator (Analysts). Though a few laboratories have directed training programs for becoming instructors, hands-on experience of

actual patient cases is essential and a minimum of 100 cases is recommended. Many laboratories have specific requirements and regulations for training but are often times insufficient in the subspecialty of clinical flow cytometry. Post graduate degrees such as master's degrees, MD's, or Ph.D.'s are desirable and acceptable qualifications for training responsibilities.

Interpreter Training

At its most basic level, interpretation of flow cytometric findings may be provided relative to the information inherent in the data alone, i.e. population identification, lineage assignment, etc. This is commonly done when access to supplemental information is limited, as occurs in a reference laboratory setting, or in laboratories having interpreters whose experience and expertise is more technical than clinical. In this regard, the Centers for Medicare and Medicaid Services (CMS) in the US has recently created a new National Provider Identifier (NPI) code for non-MD lab director taxonomy⁵. This technical interpretation typically results in a more descriptive assessment of the sample contents rather than a complete diagnosis. Understanding the clinical significance of flow cytometric findings requires integration of this information in the context provided by historical, clinical, morphologic, immunohistochemical, cytogenetic, molecular and other laboratory or radiographic observations. This task is commonly performed by a physician, usually a board-certified pathologist or ideally a hematopathologist, and results in an integrated diagnosis.

Interpreters serve as the ultimate gatekeepers of quality for the laboratory, as they are the individuals who evaluate the net result of the laboratory's work and are responsible for verification of the final report. Consequently, their training needs to encompass all areas of activity in the laboratory to the extent that they impact the quality of the data being interpreted.

Instrument and Reagent Performance and Data acquisition: Every interpreter should receive documented training in the technical and procedural issues that impact the evaluation of flow cytometry data. This training of necessity should develop an understanding of the principles of

instrument set-up and daily quality control, color compensation, reagent evaluation and performance characteristics, specimen preparation methods, and instrument operation. This is not to say the interpreter must be able to perform these tasks in the manner of a key operator, but they should be able to recognize when problems in these areas are impacting data quality and be able to assist in guiding troubleshooting. The interpreter should be familiar with the performance characteristics of the procedures and reagents on normal and abnormal specimens by examining sufficient representative data from the laboratory collected under the conditions that the interpreter will be evaluating prior to issuing clinical reports. If the practitioner performing the interpretation is unable to make this assessment, then they are not prepared to evaluate data generated by these procedures. Participation by the trainee in the preparation and acquisition of data for each of the major types of clinical samples, e.g. blood, marrow, lymph node, is a useful way to improve their understanding of the technique.

Analytical Procedures: The interpreter must acquire the ability to personally analyze the primary flow cytometry data when necessary using appropriate software. The independent interpretation of data apart from an analysis suggested by technical staff is required. The inability to provide an independent assessment of data is a common source of error and is an unacceptable clinical practice. Even if not exercised in every case, the ability to perform this function is a prerequisite to an adequate understanding of the processes involved in the evaluation of flow cytometry data.

Experience gained by case review: The training of interpreters should include independent analysis of a large number of cases that encompass a variety of routine and atypical presentations often found in the evaluation for neoplasia; a minimum of 100 cases is recommended. Results should be reviewed for accuracy by the trainer / instructor or other qualified individual and documentation of performance maintained. A comparison to other ancillary laboratory results such as morphology reports, immunohistochemical stains and cytogenetic lab results, if available, can be a highly useful way to measure training proficiency.

Suggested Credentials for Interpreters (U.S. / Canada): The interpreter should have a PhD or MD with documentation of appropriate training in flow cytometric data interpretation. Due to the wide variation in the quality of flow cytometric training provided in graduate, residency and fellowship programs, board certification in pathology or even hematopathology does not necessarily equate to an expertise in interpreting clinical flow cytometry data and specific documentation of training activities performed should be evaluated. The interpreter should also participate in flow cytometry proficiency testing as well as documented continuing medical education with an emphasis on clinical flow cytometry applications

Competency

Measuring training and education can be accomplished by several different methods. Adequate training is required prior to allowing an individual to process and report results in typical laboratory settings and many of these training assessments can also be applied in a clinical flow cytometry laboratory. Training and education competency should be measured upon completion of initial training, annually, as part of training review required by regulatory agencies, and re-evaluation of previously trained personnel whose competency completions have expired or to evaluate new procedures added since the last checklist was completed.

The following methods are recommended for determining training adequacy in flow cytometry laboratories that perform lymphoma and leukemia testing:

1. **Board Exams:** any of a number of different examinations could be given for Key Operators and Advanced Operators (Analysts) in similar format to those for obtaining an ASCP medical technologist generalist or specialty certificate or the NCALP (National Credentialing Agency for Laboratory Personnel) for specialists in cytogenetics or molecular biology as well as supervisors. Medical board exams similar to those given for MDs in hematopathology or

Molecular Pathology, the American Board of Medical Laboratory Immunologists (ABMLI) for MDs or PhDs , directors of Cytogenetic Labs by the American College of Medical Genetics (ACMG) for MDs or PhDs, or those available for MD or PhD Clinical Molecular Biology lab directors (American Board of Biophysics or the National Credentialing Agency for Laboratory Personnel) are examples of programs in the US that should also be considered with specific modifications and emphasis on flow cytometry interpretation and practices. Periodic renewal of certification for Key Operators, Advanced Operators (Analysts) and Interpreters should also be considered to ensure maintaining competency in a rapidly evolving discipline such as clinical flow cytometry.

2. Proficiency Testing: similar to that utilized by CAP surveys and ASCP Check samples. Specimens are treated like actual patient samples and they require the same testing procedures and protocols used in the laboratory. These are useful tools to evaluate training and can be used to compare results with other laboratories that practice similar procedures. Flow cytometry list-mode data cases have recently been added to the CAP survey menu and these can be useful to not only assess competency but also provide continuing education credits to the individual upon successful completion.

3. Institution and /or Laboratory Specific Competency Checklists: that include line item listings of specific training steps for procedures with successful completion signed by both the trainer and trainee. These checklists are training tools designed primarily for Key Operators and Advanced Users (Analysts). Checklists can also be used for re-evaluation of previously trained personnel whose competency completions have expired or to evaluate new procedures added since the last checklist was completed. (See Appendix A: "*Flow Cytometry Competency Checklist*"). Personnel competency assessment policies and procedures are required for any laboratory that performs nonwaived testing according to the Clinical Laboratory Improvement Act (CLIA)¹.(In-blue added to previous text 10/18/2007).

4. Perform List Mode Analysis of Several Lymphoma / Leukemia Specimen Types:

include typical, atypical, obvious as well as subtle (0.1% to 1%) malignant populations and normal / reactive examples. To document competency on an annual basis, Analysts should build a case portfolio that includes at least 20 or more cases that encompass the spectrum of hematopathological malignancies that are frequently submitted for flow cytometry testing.

5. Cross-reference flow testing results: with results performed in other labs or review cases that have had conformational testing such as performed by immunohistochemistry or cytogenetics. This method of measuring competency is also a useful procedure to monitor quality control of flow cytometry results.

6. On-line Proficiency testing: using available continuing education exercises by certifying agencies such as the CAP or ASCP for example, could be used to assess new, previously trained employees or annual re-evaluation of current employees (CAP checklist requirement)².

Additional Certification, Training and Education Recommendations

Currently, the ASCP does not offer a specialty certificate in flow cytometry. Instead, a Qualification in Cytometry, "QCYM", is available for Key Operators and Advanced Operators (Analysts) and is obtained after meeting education and experience requirements. Prior to November, 2006, applicants with flow cytometry experience had to successfully complete 6 "wet" case studies including peripheral blood T-Cell subsets, a lymphoma case, a leukemia case, and DNA ploidy quantitation. Each case had to be setup, acquired, analyzed, and reported by the person applying for the certification, usually (but not always) a medical technologist. A jury or panel of experts graded the cases and successful applicants were granted Qualification status. As of January, 2007 the 6 case studies have been replaced by the successful completion of a 50 question on-line examination.³ Renewal of the QCYM requires 30 hours of Continuing Education between renewal dates (5 years).

Although a step in the right direction, the Education and Training committee believes that a specialty in clinical Flow Cytometry ("SFC"), should be considered by the ASCP or other certifying organizations. Such organizations should consider exams for the Key Operators level and another for the Advanced Operators (Analyst) level of experience. This would promote advanced education and training as well as recognize certain standards of measurable knowledge, especially in the area of lymphoma and leukemia immunophenotyping. The flow cytometry specialty would be granted after successful completion of either a proctored exam and / or case studies, that would focus on lymphoma, leukemia, and reactive cases similar to those required for the certification. In addition, specialty applicants should be required to have at least 3 years of experience working in a CAP or Joint Commission (JC) accredited clinical flow cytometry laboratory (if residing in the US). As the specialty certification implies expertise, continuing education requirements between renewal dates should continue to insure up-to-date familiarity with technical advancements and applications.

In regards to education and training, there are currently no established programs specifically and solely designed for the training of Key Operators in clinical flow cytometry labs in contrast to those limited programs established for clinical cytogenetics labs and clinical molecular biology labs. Advanced Operators (Analysts) in clinical flow cytometry usually gain experience and training from a background as Key Operators. Similarly, there are no programs that solely train Interpreters. Education and training by pathology residency programs as well as fellowships in hematopathology for flow cytometric testing of hematopoietic malignancies varies from program to program. Stipulation of actual content for such education and training programs would be desirable. A model could also be adopted as by the American College of Medical Genetics (ACMG) for directors of clinical cytogenetic labs that requires working in an approved lab training program followed by a two year assistant directorship position.

The CAP flow cytometry checklist in the US² requires the “person in charge of flow cytometry have education equivalent to that of an MT(ASCP) and at least one year’s experience in flow cytometry under a qualified director”. This is a step in the right direction but probably inadequate for flow cytometry labs reporting highly complex diagnostic test results for leukemia and lymphoma. Defining what "in charge" means would also be useful. The Education and Training consensus committee recommends that this requirement be increased to at least 2 years of experience in clinical flow cytometry under a qualified director.

Conclusion

A description of education and training requirements of professionals to perform lymphoma and leukemia testing using flow cytometry is important for insuring quality results. These recommendations are designed to facilitate those who train and those who are to be trained. The details of how to meet many of these training and education requirements have been cited and it is the desire of the Education and Training consensus committee that these guidelines be a starting point for others to add additional suggestions. Review of the recommendations may actually present opportunities for professional societies such as the Clinical Cytometry Society, the European Society for Clinical Cell Analysis, the American Society of Hematology, the American Society of Clinical Pathologists, the College of American Pathologists and others to play a larger role in determining education and training requirements. Although the committee that created this document primarily included examples of training in a United States or Canadian laboratory, it is hoped that additional international input will afford more universal acceptance. As stated earlier, this document is intended to offer suggestions or a proposed position statement contrary to representing a broad consensus opinion.

References

1. **Clinical Laboratory Improvement Amendments of 1988**, Dept. of Health and Human Services, Form CMS-116. Web address: <http://www.fda.gov/cdrh/CLIA/index.html>

2. **Commission on Laboratory Accreditation, Flow Cytometry Checklist**, FLO.4000, 2005 College of American Pathologists.

3. **American Society of Clinical Pathology**, web address:
http://www.ascp.org/Certification/CertifyingExaminations/qual_about/qcym_about.aspx

4. **U.S. Army Department of Recruiting, Operations and Professional Development**, Nashville, TN.

5. **National Provider Standards, Centers for Medicare and Medicaid Services**, web address: <http://www.cms.hhs.gov/NationalProvIdentStand>

(see following page for Attachment A))

Appendix A: Flow Cytometry Competency Checklist for Key Operators and Analysts (Example)

<i>Instructions</i>	<i>New Employees</i>	<i>Annual Evaluation</i>		
1. Read Each Procedure	YES	YES		
2. Perform each item using any one or more Check Methods.	YES	NO		
3. Both Trainer and Trainee Initial Upon Completion.	YES	YES		
4. Perform only the items in BOLD PRINT.	NO	YES		
Competency Validation: Use one or more of the Check Methods listed.				
CHECK METHODS				
	Direct Observation	Problem Solving	Proficiency Testing	Trainer / Trainee/ Date
SPECIMEN HANDLING				
1. Has read the procedure.				
2. Follows Standard Precautions.				
3. Performs Safety Steps listed in Specimen Handling.				
4. Knows general specimen requirements for each panel/specimen.				
5. Familiar with Criteria for Rejection of specimens.				
SUPPLIES AND SOLUTIONS				
1. Has read the procedure.				
2. Can prepare different solutions as necessary.				
3. Can reconstitute 7-AAD or other dyes for use in Viability Panels.				
SPECIMEN SETUP: LYMPHOMA / LEUKEMIA				
1. Has read the procedure.				
2. Knows screening procedure for screening all lymphoma-leukemia samples for viability, concentration, and panel determination.				
3. Knows when to consult interpreter about panel selection.				
4. Can make CytoCentrifuge preparations and smears.				
5. Can perform or submit samples for Wright Giemsa or H&E Staining				
6. Knows steps for assaying Body Fluids (including CSF).				
7. Can prepare single cell suspensions using automated or manual method.				

	CHECK		METHODS	
	Direct Observation	Problem Solving	Proficiency Testing	Trainer / Trainee/ Date
INTRACELLULAR ANTIGEN ASSAYS				
1. Has read the procedure.				
2. Can perform sample set-up using special permeabilization reagents.				
3. Can properly perform sample setup of TdT, MPO, cyCD3, cyCD79a, and other intracellular Assays.				
4. Can properly perform sample setup of other Intracellular assays.				
INSTRUMENT OPERATION				
1. Has read the procedure.				
2. Can perform Daily Maintenance according to Checklist.				
3. Able to prepare fluidics and examine flow cell.				
4. Can perform system clean manually.				
5. Can clean the air filter.				
6. Knows how to change the sheath filter.				
7. Complies with Laser and Biohazard precautions/procedure.				
INSTRUMENT QC				
1. Has read the procedure.				
2. Runs alignment beads, checks CV, Peak Channel and graphs results.				
3. Can troubleshoot out-of-range-results.				
4. Files Printout in Daily QC folder.				
5. Performs instrument compensation, fluidics, laser power, PMTs, time delays, and other instrument QC using standard beads.				
6. Can troubleshoot out-of-range results.				
LYMPHOMA / LEUKEMIA SCREENING PROCEDURES				
1. Has read the procedure.				
2. Handles all specimens using Universal Precautions.				
3. Can Acquire Gate tube (example: CD19-33-45-7AAD).				
4. Can determine cell Concentration/Viability & identify subpopulations by				

procedure.		CHECK	METHODS	
	Direct Observation	Problem Solving	Proficiency Testing	Trainer / Trainee/ Date
LYMPHOMA / LEUKEMIA SCREENING PROCEDURES CONT.				
5. Able to set up all Leukemia and Lymphoma Panels.				
6. Understands Viability testing using 7AAD or other dyes as well as specimen rejection criteria.				
7. Can recognize the population in question using light scatter and/or or fluorescence gating.				
8. Knows when to consult Advanced Operator(Analyst) or Interpreter about questionable samples.				
LYMPHOMA / LEUKEMIA SAMPLE ACQUISITION				
1. Has read the procedure.				
2. Can Perform Autoacquisition of panels using instrument operation procedure.				
3. Can Perform Manual Acquisition using instrument operation procedure.				
4. Can acquire add-on tubes/markers to panels previously acquired.				
LISTMODE FILE ANALYSIS (ANALYST)				
LYMPHOMA PANELS				
1. Has read the <i>Kappa / Lambda Analysis</i> procedure.				
2. Can perform proper gating of Kappa/ Lambda assay.				
3. Can interpret K/L results for monoclonality; recognizes reactive populations.				
4. Has read the <i>Lymphoma Panels Analysis</i> procedure.				
5. Can edit a multigraph.				
6. Can re-gate if necessary.				
7. Can write up all Lymphoma panel results.				
8. Understands common phenotypes of typical lymphomas including CLL, FCC, MCL, MZL, HCL.				
9. Successfully analyze 10 different lymphoma (positive) and 5 reactive cases using correct gates and analysis tools.				

	CHECK		METHODS	
	Direct Observation	Problem Solving	Proficiency Testing	Trainer / Trainee/ Date
LEUKEMIA PANELS				
1. Has read the <i>Leukemia Analysis Procedure</i> .				
2. Understands and can demonstrate proper gating of population in question.				
3. Can perform leukemia panel analysis using appropriate software program.				
4. Knows how to regate on different populations.				
5. Knows the difference and how to calculate % <i>Total</i> and % <i>Gated</i> populations.				
6. Can record results correctly on the Lymphoma./Leukemia Worksheet.				
7. Understands common phenotypes of typical leukemias including AML, AMML, B-ALL, T-ALL, Biphenotypic / bilineal leukemias.				
8. Can perform acquisition and analysis of all miscellaneous lymphoma and leukemia panels (Multiple Myeloma, PNH, Intracellular Antigens, etc.)				
9. Successfully analyze 10 different leukemia cases using correct gates and analysis tools.				
MISC. LYMPHOMA / LEUKEMIA PANELS				
1. Successfully analyze 5 different Multiple Myeloma cases.				
2. Correctly identify cytoplasmic light chain monoclonality.				
2. Successfully analyze 5 different Minimum Residual Disease (< 0.1-1.0%) cases.				