CLS Entry Level Competencies in Flow Cytometry

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OBJECTIVE: To define entry level competencies in flow cytometry for CLS generalists.

DESIGN: Flow cytometry practitioners completed an electronic survey. Of 134 respondents, 131 met the desired demographics and were analyzed.

SETTING: Links to the survey were mailed to 3 listservs (Medlab-L, CLSEduc, Purdue Cytometry) and 2 email groups (ASCLS and AMLI). Participants completed the survey on-line.

PARTICIPANTS: The target population was flow cytometry practitioners who had experienced CLS education, earned certification and practiced at least one year in flow. Survey instructions asked participants not to complete the survey if they did not meet the demographic criteria.

MAIN OUTCOME MEASURES: A competency was deemed important at entry level if ≥50% of respondents agreed.

RESULTS: There was strong consensus (62-87%) that entry level CLS generalists should be able to 1) perform HIV CD4/CD8 monitoring, 2) gate cell populations using forward/side scatter and CD45/bright/dim markers and 3) evaluate specimen acceptability. Concepts to understand included leukemia immunophenotyping, quality control and instrument principles (61-83%). Most respondents (74%) felt that memorization of the leukemic CD panels was unnecessary. However, survey results indicated that the markers and cell type associations to memorize are CD3, CD4, CD8, CD19/20, CD34, CD45 and light chains. Hands-on experience with instruments was not identified as critical.

CONCLUSION: CLS educational programs can deliver almost all flow cytometry content in the didactic portion of the curriculum and can restrict CD marker memorization to a limited list. At minimum, HIV monitoring via CD4/CD8 counts and concepts of leukemia immunophenotyping should be included.

ABBREVIATIONS: AMLI- Association of Medical Laboratory Immunologists, ASCLS- American Society of Clinical Laboratory Science, ASCP- American Society of Clinical Pathology, CD – Cluster of differentiation, CLS - Clinical Lab Science, CLSEduc-Clseduc@list.apsu.edu, CLSI - Clinical Laboratory Standards Institute, DNA- Deoxyribonucleic acid, EDTA- Ethylenediaminetetraacetic acid, HIV- Human Immunodeficiency Virus, HLA- Human Leukocyte Antigen, Medlab-L- Medlab-L@listserv.buffalo.edu, NCA – National Credentialing Agency for Laboratory Personnel, PNH – Paroxysmal Nocturnal Hemoglobinuria, Purdue Cytometry - Cytometry@lists.purdue.edu.

INDEX TERMS: CLS - Clinical Lab Science, Entry Level Competency, Flow Cytometry.

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INTRODUCTION

In recent years, the scope of practice for clinical

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laboratory scientists (CLS) has expanded to include flow cytometry. The job analysis for CLS entry level tasks completed in 2008 by the National Credentialing Agency for Laboratory Personnel (NCA) identified for the first time that CLS practitioners expect new graduates to have some knowledge and skill with flow cytometry.[1,2] However, most CLS programs do not have a flow cytometer for use in student labs or affiliates that perform this type of analysis. Nonetheless, as questions on this topic are incorporated into certification examinations, it is essential that educators identify elements that should be included in the CLS curriculum.

The purpose of CLS generalist certification exams is to identify those individuals with minimum entry level competency in the field. Thus, certification examination questions should reflect entry level skills in flow cytometric analyses rather than those of an experienced practitioner. The task descriptions in the NCA Detailed Content Outline for CLS were necessarily broad, general concepts. This is also true of the content outline for the Medical Laboratory Scientist certification examination administered by the American Society of Clinical Pathology Board of Certification (ASCP BOC). These task lists contain little detail about what specifically students should know or be able to perform. This lack of specificity could cause exam items to exceed minimum entry level competency. For example, the task "correlate patient's results to available information" could be as simple as recognizing that an HIV patient should have a low CD4 count or as complex as evaluating a panel of several leukemic markers against microscopic morphology. To construct a CLS flow cytometry curriculum to optimize student outcomes, more detail is needed.

MATERIALS AND METHODS

Using the NCA CLS Detailed Content Outline, textbooks on immunology and hematology and Clinical Laboratory Standards Institute (CLSI) documents,[1-11] we designed a survey that included a gamut of flow cytometry tasks and competencies spanning entry level to advanced practice. In this way, we hoped to define the limits of a good curriculum.

The target population for this survey completed a baccalaureate program in CLS and earned certification. Further, the ideal respondent had at least a year or more work experience in flow cytometry. We wanted respondents familiar with the depth and breadth of a CLS program as well as the entry level skills that are crucial. To avoid bias in the results from respondents with little experience in flow cytometry, our instructions on the survey were very specific regarding our desired participant. Recipients were asked not to respond to the survey if they did not meet these criteria. Survey questions related to participant demographics were very detailed in order to probe participant qualifications and inclusion in data.

Also in the instructions, participants were advised that 1) the survey was voluntary and anonymous, 2) the data gathered from this survey would be presented in aggregate at professional meetings and/or in publications, 3) participants would not be identifiable, 4) the study authors had no financial interest to disclose related to the survey and 5) adverse effects from the survey could be reported to phone numbers provided. The administration of the survey was approved by the standard Institutional Review Board protocols at both Salisbury University and Virginia Commonwealth University.

After the survey was piloted by four flow cytometry practitioners, minor adjustments were made and the survey was posted to www.surveymonkey.com (see Appendix for complete survey). The link to the survey was mailed to three listservs (Clseduc@list.apsu.edu, Medlab-L@listserv.buffalo.edu, and Cytometry@lists.purdue.edu). The American Society of Clinical Laboratory Science (ASCLS) and the Association of Medical Laboratory Immunologists (AMLI) kindly included a request to participate in the survey and a link to the survey in an email to their members. Responses were collected for 40 days.

Responses were analyzed as aggregate data and reported as descriptive statistics. Recommendations for the inclusion in the CLS curriculum of tasks to satisfy entry-level competencies were based on an arbitrary value of 50% or higher agreement in the responses of the survey for each task. Written comments from the survey respondents were reviewed to detect repeated

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themes or recommendations not included in the survey questions.

RESULTS

A total of 134 people responded to the survey. Three were eliminated, one of whom had no flow cytometry experience and two of whom indicated that their highest degree was at the associate's level and who did not answer any of the survey questions. Answers were required to demographics questions; however, participants were permitted to skip other items. We expected, for example, that some participants might work primarily with leukemia phenotyping and would not want to answer items related to other types of testing. When determining the aggregate response to an item, percentages were calculated based on the number of people who responded to the item, not the total number of participants in the survey.

Since actual flow cytometry practitioners were the target population, there was concern about a number of respondents who indicated that they work at educational institutions. Since some large medical centers are also simultaneously educational institutions, we did not want to automatically exclude data from these respondents. The survey software allowed us to apply "filters" to the data to include or exclude various populations, which allowed for the comparison of responses from the total sample with those from the total sample minus the respondents from educational institutions. Since major differences were not observed, the sample size was maintained at 131 for the analysis.

The majority of survey participants had bachelor's degrees (67.2%) and many had master's and doctorates (Figure 1). Some form of certification (NCA, ASCP, specialty) had been earned by 77.9% of participants, and the median years of service in medical lab science and flow cytometry was 20 and 10 years, respectively. Participants worked primarily in large institutions, with 57.3% from hospitals of 300 or more beds, but significant numbers were employed in research, educational institutions and reference labs (Figure 2).

We asked participants to identify those tasks that they have performed to assess breadth of flow cytometry experience and identify non-practitioners. At least 90% of participants indicated that they have processed samples, operated instruments and performed preventive maintenance, quality control, instrument optimization, troubleshooting, data analysis and interpretation. Even managerial tasks such as laboratory quality assurance and compliance issues have been performed by 89.3% of this group, so indeed experienced practitioners composed the sample of 131 usable surveys.

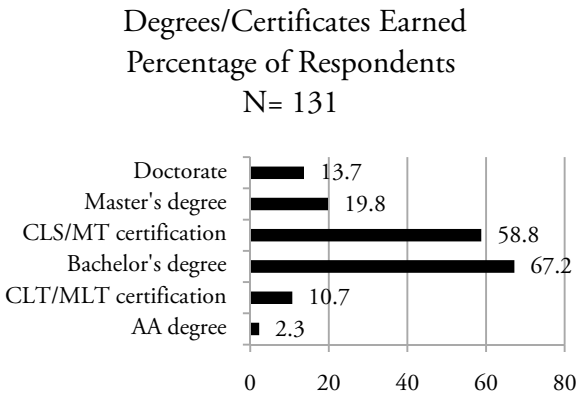

Figure 1. Respondent Demographics: Degrees/Certificates Earned by Percentage*

*Multiple answers allowed

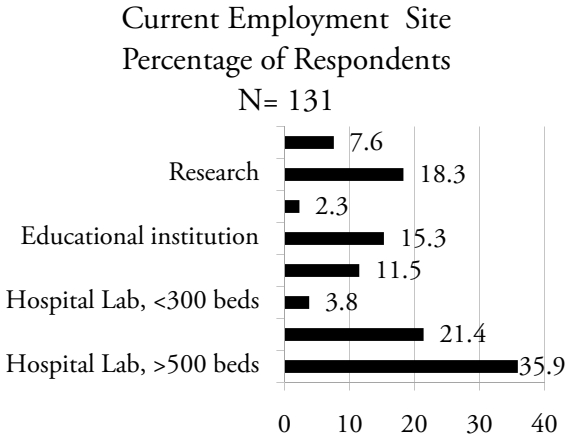

Figure 2. Respondent Demographics: Current Employment Site by Percentage*

*Multiple answers allowed

Two assay types were notable as essential to entry level. HIV monitoring using absolute CD4 counts and CD4:CD8 ratio and knowledge of general concepts in

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leukemia immunophenotyping were identified by 84.2% and 83.2% of participants, respectively. Responses to all other assays with respect to entry level competencies were less than 30%. These other assays included reticulocyte counting, fetal/maternal hemorrhage evaluation, DNA content (ploidy/S-phase) analysis, flow cross matching for transplantation, multiplex beads/flow cytometer arrays, PNH evaluation, HLA typing and cell sorting.

Basic flow cytometry instrument techniques identified as entry level were selecting cell populations from forward and side scatter data (81.2%) and use of the CD45 marker to assess lymphocyte gate (70.3%). More advanced gating techniques garnered responses in the 31-40% range, and these include identifying debris/undesirable cells in a gate, front/back gating and setting gates in evaluation of leukemias. We allowed written comments for acceptable amounts of debris and cell contamination in the gating process. Though a consensus was not observed with these values, no response exceeded 10% for monocyte contamination of CD4 gates.

Respondents felt that the following markers and their cell associations should be memorized: CD3, CD4 , CD8, CD 19/20, CD 34, CD45 and light chains. We elected to include light chains as a recommended marker since the percentage of respondents was close to our arbitrary cut-off of 50% and the next closest marker was only recommended by 35.6% of respondents. Table 1 summarizes this data. By a wide margin (74.3%), participants felt that markers for acute leukemia could be easily accessed in reference material and did not need to be memorized. We also collected written comments on recommended panels for acute leukemias of T, B and hairy cells and received responses that were so varied that we did not feel there was sufficient consensus to report them.

Respondents support entry level practitioners being able to select cell populations expressing a marker (62.1%), including the recognition of variable marker expression ("bright" versus "dim"). They also should be able to interpret basic flow data with attention to evaluating control acceptability (>61% several types) and interpreting bivariate scatterplots/contour plots (55.8%) and univariate histograms (53.7%). Familiarity with two popularly used conjugate dyes in flow cytometry, fluorescein and phycoerythrin, as well as the use of dyes for assessment of cell viability were also suggested. Performing calculations to include CD4:CD8 ratio, absolute CD4 and CD8 counts and lymphosums were deemed important entry level skills for CLS as indicated by the results of the survey. We note that to perform lymphosums, practitioners must have knowledge of T, B and NK markers, and that memorizing the NK cell markers fell below our arbitrary cut-off of 50%. It may be appropriate, therefore, to also include one or more NK cell markers in the curriculum. Psychomotor skills beyond these, however, were not considered entry level. For instance, the majority of the respondents indicated that the entry level practitioner need not have skills beyond the ability to explain or discuss the concepts of instrument set-up, quality control techniques, and simple troubleshooting techniques (detecting clogged fluidics, dye photosensitivity and debris from aged specimens).

Table 1. Entry Level Knowledge of CD Markers*

Marker	Percentage of Respondents
CD4 (T helper)	74.3
CD8 (T suppressor/cytotoxic)	71.3
CD3 (pan T cell)	70.3
CD45(pan leukocyte)	67.3
CD19/20 (B cell)	59.4
CD34 (stem cell)	50.5
Kappa/lambda light chains	47.5
CD56/57/16 (NK cell markers)	35.6
CD11b, 13, 15, 33 (myeloid markers)	30.7
CD14 (monocyte marker)	29.7
CD10 (Common Acute Lymphoblastic Leukemia Antigen or CALLA)	28.7
HIV infection markers	23.8
HLA – DR	23.8
TdT (immature cells/blasts)	21.8
CD 55, 59 (PNH markers)	20.8
Erythroleukemia marker- Glycophorin	14.9
CD 41, 61 (Platelet markers)	10.9

*Multiple answers allowed and participants were not required to respond to all items. Percentages reflect those who responded to the item.

Recognizing acceptable whole blood specimens for all flow analysis, including reticulocyte counting, was considered crucial (65.6%). This includes evaluating parameters such as proper anticoagulant, storage

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temperature, presence of clots or hemolysis, adequate sample volume and specimen age. Specimen preparation details and handling specimens other than whole blood fell under the 50% mark. Half the respondents (50%) indicated that assessing specimen viability was an entry level skill, and when we analyzed the data, responses seemed very institution and protocol dependent. For example, anyone involved in stem cell harvest always assessed specimen viability, but at big institutions with on-site flow cytometry, specimen transport was presumably not an issue and viability assessment was not performed routinely. Since viable cells are crucial for flow cytometry, this is a skill that entry level practitioners should know.

DISCUSSION

The purpose of this study was to survey flow cytometry practitioners regarding their perceptions of minimum entry level competency for CLS graduates. Though we were concerned at the onset of the study that the population of experienced practitioners in the specialty area of flow cytometry would yield a small sample size, the responses to the survey were more than ample. Thus, the target population was sampled adequately based on the number of acceptable respondents (N=131) and the demographic information they provided. Therefore, we do feel that we can make valid recommendations for CLS curricula based on our data. These recommendations are summarized in Table 2.

An important finding in the survey is that most aspects of flow cytometry can be delivered as didactic material, including the interpretation of the test results. While clinical experience reinforces information and would be desirable, programs without access to flow cytometers in clinical affiliates probably need not be concerned that their graduates are at a significant disadvantage. Though hands-on experience for students via a clinical rotation is invaluable, psychomotor proficiency at the entry level is not expected, according to the responses received on the survey. However, the fact that many CLS students are not exposed to flow cytometry in their clinical rotations poses a greater challenge for CLS educators. Many must present this material within the confines of their lecture format. Learning concepts of flow cytometry necessitates higher order cognitive skills and those psychomotor skills limited to the analysis of results involving histogram interpretation and calculation of absolute CD counts and lymphosums.

Table 2. Recommendations for Clinical Laboratory Science Flow Cytometry Curricula

Basic concepts
Concepts of forward/side light scatter and fluorescence, antibodies with fluorescent labels
Basic instrument mechanics, lasers and optics, hydrodynamic cell focusing, data handling, cell sorting, fluorescence compensation
Cell populations derived from bivariate scatter analysis; univariate and bivariate fluorescence scatterplot interpretation
Advantages, disadvantages, uses of the technique
Disease- diagnosis and monitoring with emphasis on CD4 counts for HIV and WBC malignancies
Specimen preparation - emphasis on whole blood; some mention bone marrow and tissue
Viable cells - EDTA or heparin; room temperature storage and prompt processing
Viability assessment- dyes to use, at least 80% live cells; protocols institution/procedure specific (all specimens vs. specimens > 24 hours old vs. specimens from leukemias with increased cell fragility)
Quality control and calculations
Isotype controls / positive and negative controls
Calculations- lymphosums, ratio of kappa to lambda, CD4:CD8, absolute counts
Gate analysis- CD 45, acceptable debris/contamination, CD14 monocytes in CD4 gate

Among other items related to flow cytometry, the NCA job analysis indicated that entry level practitioners should be able to select appropriate monoclonal antibody panels for diagnosis/prognosis and determine optimal dilution of monoclonal antibodies for use on various test procedure panels. This survey does not support this requirement. The demographics of the respondents did not suggest a disproportionate number of immunologists over hematologists, but to resolve the discordance further studies may be need to be conducted to collect data according to expertise. For example, tasks related to reticulocyte counting by flow cytometry (interference by platelet clumps/giant platelets, Heinz bodies/Howell-Jolly bodies/Pappenheimer bodies, malarial parasites, autofluorescence, cold agglutinins, hemolysis/cell fragments) did not meet our 50% criterion but might be very important if only

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hematologists respond to a survey or if this technique for reticulocyte counting becomes more common.

We conclude that the results of this survey do form a basis for structuring the minimum amount of flow cytometry in clinical laboratory science curriculum and that additional material may need to be added as the technology evolves and additional surveys identify skills that are becoming more commonplace.

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