

# Incorporating Hands-on Flow Cytometry Into a Medical Laboratory Science Program Curriculum

RACHEL CHILDS, CRISTINA RUFFY, BARBARA KRAJ, LESLIE HOGLUND

## ABSTRACT

**INTRODUCTION:** Through grant funding, faculty provided hands-on flow cytometry experience to students who were enrolled in the Fall 2018 immunology and/or the Spring 2019 advanced hematology courses. This study assessed the effect of including hands-on flow cytometry exposure to students in a university-based medical laboratory science program.

**METHODS:** Students spent approximately 4 hours processing, staining, and analyzing samples from a nearby hospital facility. In immunology, students were tasked with identifying lymphocyte subset populations in whom comprehension was assessed through short-essay and multiple-choice questions on an online homework quiz. In advanced hematology, students were tasked with immunophenotyping leukemia/lymphoma samples, in which comprehension was assessed via interpretation of patient scattergrams and correlation of results with the corresponding patient diagnoses.

**RESULTS:** All students (100%) correctly interpreted a histogram presented to them in both the immunology and advanced hematology courses. A survey assessed students' perceptions of the effectiveness, strengths, and weaknesses of both exercises. Ninety-one percent and 100% of respondents agreed or strongly agreed that the hands-on exercise enhanced their understanding the flow cytometry principles underlying lymphocyte subset analysis and leukemia/lymphoma immunophenotyping, respectively. Respondents reported the experience as "extremely helpful" and that it allowed them to "correlate what was taught in class."

**DISCUSSION:** Results indicated successful comprehension of basic lymphocyte and leukemia/lymphoma subset analysis. It was apparent that the activity helped students apply these principles toward their understanding of the

material. Incorporating flow cytometry during didactic was beneficial, as it provided students with an experience they might not have gained during rotations.

**ABBREVIATIONS:** ASCLS - American Society for Clinical Laboratory Science, ASCP - American Society for Clinical Pathology, FC - flow cytometry, MLS - medical laboratory science.

**INDEX TERMS:** flow cytometry, education, curriculum.

**Clin Lab Sci 2025;38(1):53-58**

## INTRODUCTION

Specific, relevant verbalized objectives assigned to established learning domains and levels are provided in the entry level curriculum for medical laboratory science (MLS) published by the American Society for Clinical Laboratory Science. The immunology section of that document states "perform flow cytometry testing and report results" as a psychomotor objective in flow cytometry (FC).<sup>1</sup> Furthermore, the American Society for Clinical Pathology (ASCP) lists "Flow Cytometry Immunophenotyping" as one of the areas in the hematology laboratory testing section of the *Medical Laboratory Scientist Certification Examination Content Guidelines*.<sup>2</sup> Because of the incorporation of FC material into the certification examinations, it is "essential that educators identify elements that should be included" in the MLS curriculum.<sup>3</sup>

Previous studies have introduced active learning activities, such as using rubber balls to represent cell enumeration using forward and side scatter and providing FC analysis software for students to gate and interpret, to facilitate learning FC principles.<sup>4-5</sup> However, neither of these studies were able to provide students with the opportunity to stain and process cells or gain experience using a flow cytometer. This study assessed the effect of including hands-on FC exposure to students in a university-based MLS program. In this 2+2 program, students must complete didactic coursework before entering the clinical phase of the program. Although psychomotor objectives in FC have been deemed an essential component of the MLS curriculum, it can be difficult to provide this experience to students. Short viability of samples, lengthy processing and staining time, highly specialized training, and access to instrumentation make it difficult

*Rachel Childs, Old Dominion University*

*Cristina Ruffy, Old Dominion University*

*Barbara Kraj, Old Dominion University*

*Leslie Hogle, Old Dominion University*

**Address for Correspondence:** Rachel Childs, Old Dominion University, [rchilds@odu.edu](mailto:rchilds@odu.edu)

for students to receive hands-on FC experience.<sup>4</sup> Before Fall 2018, the immunology and hematology coursework included 1 to 2 lectures by a flow cytometer operator from a local hospital, a tour of their facility, interpretation of case studies using histograms and plots, and occasional observations of analyses during clinical rotations. Hands-on performance by all 18 to 20 students annually while in the program was not possible.

The FC core facility on the university campus provided permission to use their flow cytometer (MACSQuant Analyzer 10, Miltenyi Biotec) with supervision at a discounted rate during the immunology course. Preliminary results from this experience indicate that after inclusion of a hands-on exercise, students were able to correctly interpret histograms of varying percentages of lymphocyte subset populations.<sup>6</sup> To include psychomotor objectives as recommended, an ASCP Foundation Laboratory Science Program Educational Grant was obtained in Fall 2018. Grant funds were used to purchase student reagents and train faculty on the school's research flow cytometer (BD Accuri C6 Plus, Becton Dickinson, Franklin Lakes, NJ) for use during the advanced hematology course. The purpose of this manuscript is to present the findings from both exercises as one cohesive product.

## METHODS

Hands-on FC experience was provided to students who were enrolled in the program's immunology course in the fall semester and/or the advanced hematology coursework in the spring semester. Seventeen out of the 20 students were enrolled in both courses during Fall 2018 and Spring 2019 and were exposed to the hands-on activity in both courses (3 students had completed the immunology course prior to Fall 2018). MLS students were placed into groups of 3 to 5. Each group spent 2 to 4 hours at the facility, including instruction and direct observation by the manager, processing and staining time, hands-on contact running the instrument by each student (Institutional Biosafety Committee approval #1301949-3), and observation of other classmates performing the same analysis using different samples. The protocols were adapted from one of the clinical sites affiliated with the MLS program.

### Immunology Course Experience

Students analyzed lymphocyte subset populations for the assessment of HIV infection status. The goal for this exercise was to facilitate understanding of HIV disease monitoring using the CD4/CD8 T-cell ratio. The activity included whole-blood antibody staining before entry to the facility and CD45/CD8/CD4/CD3 lymphocyte subset analysis using the MACSQuant Analyzer 10 (Miltenyi Biotec). Prior to student analysis, the supervisor of the core facility performed internal quality control and evaluated instrument settings and compensation necessary for

student use and sample analysis. Seventeen students participated in the activity and were assigned a control sample (Immunotrol Normal or Immunotrol Low Cells, Beckman-Coulter Inc., Brea, CA) and deidentified patient samples with prior HIV-1 infection. Students stained each single-tube sample with CD45-PC7, CD8-FITC, CD4-PE, and CD3-PC5.5 (Beckman-Coulter Inc., Brea, CA). Students followed guidelines established by the Centers for Disease Control and Prevention for the detection and enumeration of CD4+ T cells for persons infected with HIV.<sup>7</sup> Students analyzed both samples, distinguished between normal vs abnormal control, and inferred the HIV status of the assigned patient sample. The protocol for the lymphocyte subset analysis is found in Supplemental 1.

Following the experience, students completed an online homework quiz including 7 multiple choice and open-ended questions relevant to FC. These included 4 items requiring interpretation of images recorded during the exercise by each student and a newly introduced psychomotor question to assess this domain. Students were also asked to calculate the CD4/CD8 ratio and conclude whether the sample represented a patient with active HIV infection, a control, or a normal result, indicating the effectiveness of treatment. Comprehension of the concepts was assessed using Blackboard test-item analysis.

### Advanced Hematology Course Experience

Following the immunology course experience, faculty were trained on BD Accuri C6 Plus (Becton Dickinson, Franklin Lakes, NJ) in the school's research laboratory. For the advanced hematology course, students analyzed leukemia/lymphoma immunophenotyping using this analyzer. The purpose of this exercise was to strengthen the students' understanding of FC applications in the assessment of leukemia and lymphoma using B-cell, T-cell, and stem-cell CD markers. Prior to student analysis, the instructor ran similar samples on the analyzer to ensure the instrument settings and compensation were adjusted for student use and sample analysis. Minor gating adjustments were made after analysis of each sample.

Twenty students were tasked with immunophenotyping patients with leukemia/lymphoma based on B-cell, T-cell, and stem-cell CD markers. Students prepared bone marrow or peripheral blood specimens for analysis on the BD Accuri C6 Plus (Becton Dickinson, Franklin Lakes, NJ) using a 4-fluorochrome panel with 3 tubes containing various combinations of conjugated antibodies (Beckman-Coulter Inc., Brea, CA). Tube 1 contained CD8-FITC, CD4-PE, CD3-PC5.5, and CD45-APC. Tube 2 contained kappa light chains-FITC, lambda light chains-PE, CD19-PC5.5, and CD45-APC. Tube 3 contained CD8-FITC, CD33-PE, CD34-PC5.5, and CD45-APC. Samples represented patients with suspected leukemia/lymphoma conditions presenting with biphenotypic acute lymphocytic leukemia, mediastinal mass, sudden weight loss, and pancytopenia. Comprehension of FC principles was determined via an

open-ended essay question asking students to interpret patient scattergrams to identify T- and B-cell subsets, stem cells (if applicable) subsets, and correlate results with the corresponding patient diagnoses representing various hematological conditions. The protocol for leukemia/lymphoma immunophenotyping and the student worksheet used for this exercise are found in Supplementals 2 and 3, respectively.

## Qualtrics Survey

Approximately 4 months after graduation, students were asked to voluntarily participate in a 15-minute anonymous Qualtrics<sup>XM</sup> survey approved by the institutional review board (protocol number 1532870-3). The 19-item survey was designed to assess their perceptions of the effectiveness, strengths, and weaknesses of both hands-on FC exercises and how they affected their experience during didactic, clinical rotations, and employment. Lumivero NVivo software was used to analyze qualitative themes from students' open-ended responses.

## RESULTS

### Immunology Course Experience

To assess comprehension, questions relating to FC content were included in the final clinical immunology examination. Blackboard online test-item analysis revealed that the median percentage of students correctly answering each homework quiz question relevant to FC was 88.24% (Table 1). A newly introduced psychomotor

question was added to assess this domain. All students ( $n = 17$ ) correctly interpreted a histogram representing either an active HIV infection or a normal sample.

### Advanced Hematology Course Experience

After the advanced hematology FC experience, comprehension was assessed based on the student's ability to correlate the patient diagnosis with the scattergrams of the samples used during the experience. For each tube, students were required to interpret the type of cells present and identify abnormal findings. All students ( $n = 20$ ) correctly interpreted their assigned scattergrams by identifying lymphocyte subsets and/or stem cells.

### Survey Results

The survey response rate was 70% (14/20 students). Three students who submitted responses to the survey only answered descriptive questions relating to the performance or observance of FC during their clinicals. Therefore, the remainder of the survey questions received 11 responses. Only 5 out of 14 participants reported performing and observing FC during clinical rotations, and 1 reported observation only. However, 91% of respondents agreed or strongly agreed that the hands-on exercise enhanced their understanding of FC principles underlying lymphocyte subset analysis. All (100%) of respondents agreed or strongly agreed that the hands-on exercise enhanced their understanding of FC principles underlying leukemia/lymphoma immunophenotyping. In addition,

**Table 1.** Blackboard online test-item analysis

Question	Question Type	% Students Answering Correctly	Graded Attempts	Average Score	SD	Standard Error
Email the 2 photos taken at the end of lymphocyte subset analysis by flow cytometry [ . . . ]. In the space below state whether your assigned immunocontrol was . . .	Essay	100.00	17	5.00	0.00	0.00
CD4CD8 ratio for patient: Enter the calculated CD4/CD8 ratio for your assigned patient.	Essay	64.71	17	3.24	2.13	0.52
CD4 population: Your assigned patient's CD4 population is (depleted/ not depleted):	Essay	88.24	17	4.42	1.41	0.35
Your assigned patient's results indicate (type below 1 of the following):	Essay	82.36	17	4.12	1.76	0.43
CD45: What type of cells will be positive for CD45?	Multiple choice	86.77	17	4.34	1.48	0.36
Side scatter: Which of these white blood cell populations would have the least side scatter . . .	Multiple choice	88.24	17	4.42	1.67	0.41
Overlapping signal: What procedure is required to correct the overlapping signal shown:	Multiple choice	88.24	17	4.42	1.67	0.41
<b>Median % correct answers:</b>				<b>88.24</b>		

Analysis last run July 25, 2019, 8:43 a.m. Blackboard online test-item analysis for flow cytometry homework quiz questions. Only questions relevant to FC shown.

**Table 2.** Qualtrics quantitative survey results

Question	Strongly Disagree, %	Disagree, % (n)	Somewhat Disagree, % (n)	Neither Agree nor Disagree, % (n)	Somewhat Agree, % (n)	Agree, % (n)	Strongly Agree, % (n)
The flow cytometry hands-on exercise in Clinical Immunology lab (MLS 331) enhanced my understanding of flow cytometry theoretical principles underlying lymphocyte subset analysis.	0	0	0	0	9.09 (1)	27.27 (3)	63.64 (7)
The flow cytometry hands-on exercise in Advanced Hematology (MLS 337) enhanced my understanding of flow cytometry theoretical principles underlying leukemia/lymphoma immunophenotyping.	0	0	0	0	0	45.45 (5)	54.55 (6)
I believe future students would benefit from performing the flow cytometry in immunology and advanced hematology.	0	0	0	9.09 (1)	27.27 (3)	36.36 (4)	27.27 (3)
I believe the hands-on experience in flow cytometry during the didactic part of the program was relevant to my clinical experience during hospital rotations.	0	9.09 (1)	18.18 (2)	36.36 (4)	9.09 (1)	18.18 (2)	9.09 (1)
I believe I am better prepared for practice in the laboratory workforce because of practical experience with flow cytometry.	0	18.18 (2)	9.09 (1)	18.18 (2)	27.27 (3)	18.18 (2)	9.09 (1)
I believe I am more competitive on the MLS job market because of practical experience with flow cytometry.	0	18.18 (2)	9.09 (1)	36.36 (4)	9.09 (1)	18.18 (2)	9.09 (1)

Extract of responses from the Qualtrics survey.

36% neither agreed nor disagreed, and 27% disagreed or somewhat disagreed that the didactic was relevant to their clinical rotation experience. Students were undecided on whether FC immunophenotyping better prepared them for practice in the laboratory workforce or made them more competitive in the MLS job market. Table 2 depicts all quantitative responses to the survey.

Students’ narrative comments on their perceptions of their experience and suggestions for design changes of the exercises were subjected to qualitative analysis via NVivo software. Four major themes were apparent upon analysis: time and structure, practical skills, benefits, and outcomes.

**Time and Structure**

Students enjoyed the step-by-step approach to the “preparation, testing, and analysis” and aligning “the process in tandem with learning the concepts behind it.” Visiting the specialty lab set the expectation “to know the function and structure of each blood cell in order to understand an interpret the graph and how that related to a patient’s disease.” Future students are encouraged to have “the chance to participate in this too.” A few students reported that there needed to be “more time” and “structure with the lecture

portion,” whereas others found that it “was too much” and “only very basic information is needed.” One student suggested that they would have “preferred more molecular diagnostic hands-on experience.”

**Practical Skills**

The key theme of these results was the “hands-on aspect with practice” and “practical hands-on experience.” Students found the experience “extremely helpful” and that the “trip to the Navy Medical Center Portsmouth was great.” Being able to “correlate what was taught in class to the instruments in the lab” by learning how to set up specimens, read graphs, and go through the whole process is “what helped make everything . . . read actually stick.” “It was accumulation of many concepts learned in the first semester of the program,” as one student stated. Two students shared that this was “only relevant if you can specialize in flow” and that doing it “more than twice would’ve helped to further improve knowledge and experience.”

**Benefits**

Perceived benefits from this experience enabled students to “see the results” and helped them “to think critically to

learn patience and how to follow directions thoroughly." More practical benefits include "how to read the scatter plot," improved understanding of "the principles behind the testing methodology," and learning how to "manually analyze and characterize the flow cytometry data" to determine results. Overall, the learning experience was described as excellent, fun, and interesting. One student suggested a "greater emphasis on the different flow cytometry data patterns."

## Outcomes

Students noted that this experience "puts things into perspective," especially for patients with cancer and HIV. For one student, it was "the most constructive in regards to my learning." "Seeing how it benefited the healthcare field and the patients that treatment depended on it" is a strong sentiment of the students who had this FC experience.

## DISCUSSION

This article describes the MLS program's attempt to align the curriculum with American Society for Clinical Laboratory Science (ASCLS) entry-level curriculum guidelines relevant to FC by providing hands-on experience in this area.<sup>1</sup> Results were used to assess and evaluate the experience.

The results indicated good comprehension of basic lymphocyte subset analysis concepts following the hands-on FC exercise in the MLS immunology course. Specifically, students learned how to identify the relevant populations of cells stained with the labeled antibodies directed against the corresponding CD markers used in the assessment of HIV infection. The survey results indicated that a majority of the students thought that the immunology course FC experience enhanced their understanding of the principles underlying lymphocyte subset analysis.

For the advanced hematology course, the results indicated successful interpretation of leukemia/lymphoma scattergrams. After the hands-on immunophenotyping exercise, students were able to correlate the disease with the abnormal findings seen on the scattergrams. All students stated that the hands-on exercise further enhanced their understanding of FC principles.

The survey results were also expected. Only 2 out of 13 clinical sites that offered hematology rotations to our students performed FC. Our goal for this project was to assess the outcome of incorporating psychomotor objectives into the MLS curriculum as recommended by ASCLS. Although a few students believed the experience might not be necessary for job placement or preparation for the workforce, it was apparent that the activity helped students apply FC principles toward their didactic understanding. Similar studies have shown that simulation exercises in which hands-on exposure was used improved

students' confidence and understanding of content for dental hygiene and medical students.<sup>8-10</sup> In addition, students who performed hands-on molecular lab exercises showed significant improvements in the examination performance of the content.<sup>11</sup> Incorporating FC during the didactic curriculum was beneficial, as it provided students with experience they might not have gained during rotations. The faculty plan is to continue providing this experience to future MLS students. The inclusion of FC psychomotor objectives in the curriculum, as recommended in the MLS entry level curriculum, will improve the students' preparation for practice. As indicated by the survey, the participants agreed that future students would also benefit from performing FC in both immunology and advanced hematology courses. The faculty continue to provide this opportunity to future MLS students through external grant funding.

## FUNDING

This work was supported by the ASCP Foundation Laboratory Science Program Director Educational Grant.

## ACKNOWLEDGEMENTS

We wish to thank Dr Yu (Ellen) Jing at the Frank Riedy Research Center for Bioelectrics Flow Cytometry Core Facility for sharing her expertise with our students and their oversight while operating the instrument. We also wish to thank Mittal Patel, MLS (ASCP), FC/polymerase chain reaction lab manager at Portsmouth Naval Medical Center, for her annual guest lectures in FC. We wish to thank Gordon Ward for purchasing the necessary reagents and preparing the laboratory for student use during these sessions. We also wish to thank Innovative Research Park for allowing us to access their facility for student use.

## REFERENCES

1. American Society for Clinical Laboratory Science. *Entry Level Curriculum for Medical Laboratory Science*. ASCLS; 2016.
2. American Society for Clinical Pathology. *Medical Laboratory Scientist Certification Examination Content Guidelines*. ASCLS; 2021. Accessed May 23, 2022. [https://www.ascp.org/content/docs/default-source/boc-pdfs/boc-us-guidelines/mls\\_content\\_guideline.pdf?sfvrsn=6](https://www.ascp.org/content/docs/default-source/boc-pdfs/boc-us-guidelines/mls_content_guideline.pdf?sfvrsn=6).
3. Davis D, Nadder T. CLS entry level competencies in flow cytometry. *Clin Lab Sci*. 2011;24(1):29-34. doi: 10.29074/ascls.24.1.29
4. Baker JC, Walton KLW. Illustrating flow cytometry in an immunology lab by using a hands-on, low-tech simulation. *J Microbiol Biol Educ*. 2020;21(2):21.2.51. doi: 10.1128/jmbe.v21i2.2063
5. Fuller K, Linden MD, Lee-Pullen T, Fragall C, Erber WN, Röhrig KJ. An active, collaborative approach to learning skills in flow cytometry. *Adv Physiol Educ*. 2016;40(2):176-185. doi: 10.1152/advan.00002.2015

6. Kraj BJ, Ruffy C. Flow cytometry psychomotor exercise in medical laboratory science curriculum facilitates correct interpretation of lymphocyte subset analysis. *Am J Clin Pathol*. 2019;152(1):S101. doi: [10.1093/ajcp/aqz119.006](https://doi.org/10.1093/ajcp/aqz119.006)
7. Mandy FF, Nicholson JKA, McDougal JS; CDC. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. *MMWR Recomm Rep*. 2003;52(RR-2):1–13.
8. Choi MI, Noh HJ, Han SY, Bae SS, Kim G, Mun SJ. Development and evaluation of simulation-based scenario education program for professional oral hygiene care in intubated patient. *J Dent Educ*. 2023;87(11):1512–1522. doi: [10.1002/jdd.13345](https://doi.org/10.1002/jdd.13345)
9. Ladurner A, Nijman T, Gill TK, Smitham PJ. The impact of a hands-on arthrocentesis workshop in undergraduate medical education. *BMC Med Educ*. 2020;20(1):260. doi: [10.1186/s12909-020-02174-6](https://doi.org/10.1186/s12909-020-02174-6)
10. Pearson J, Greminger A, Onello E, Stover S. Teaching the evaluation of female pelvic pain: a hands-on simulation to reinforce exam skills and introduce transvaginal ultrasound. *MedEdPORTAL*. 2021;17;11080. doi: [10.15766/mep\\_2374-8265.11080](https://doi.org/10.15766/mep_2374-8265.11080)
11. Kraj BJ, Lester P, Russell B. Student molecular laboratory performance outcomes in a baccalaureate CLS program. *Clin Lab Sci*. 2011;24(4 Suppl):4–36. doi: [10.29074/ascls.24.4\\_supplement.31](https://doi.org/10.29074/ascls.24.4_supplement.31)