Student Molecular Laboratory Performance Outcomes in a Baccalaureate CLS Program

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ABSTRACT: As new molecular assays are developed in research laboratories and approved by the Food and Drug Administration (FDA) for clinical use, molecular diagnostics becomes an integral discipline of clinical laboratory science. Since 2001, guidelines of the National Accreditation Agency for Clinical Laboratory Science (NAACLS) have required that CLS Educational Programs incorporate molecular diagnostics into the curriculum.

SETTING: In fall of 2005, CLS faculty/researchers, affiliated with a baccalaureate program in an academic medical university, incorporated molecular diagnostic lecture content with online virtual laboratories into the Clinical Chemistry course. Then beginning in fall of 2006, manual performance of molecular laboratory exercises was introduced.

OBJECTIVE: The aim of this study was to assess whether inclusion of hands-on molecular laboratories improved student outcomes on molecular questions during the final course examination.

METHOD: CLS faculty evaluated student learning by written examination of lecture and laboratory content. Researchers performed two-sample *t*-tests to establish if significant differences existed in molecular questions scores achieved by students exposed to virtual and hands-on exercises.

RESULTS: The researchers found a statistically significant difference in examination performance between the students that had a hands-on experience and students with virtual laboratory experience only. Further data analysis suggested that hands-on experiential laboratories had the greatest effect on students who performed in the middle percentiles.

CONCLUSION: The researchers proposed that in order to improve examination scores of the weakly performing students other interventions may be necessary such as more lecture or laboratory time. This prompted development of a full time clinical molecular methods course, separate from Clinical Chemistry.

INDEX TERMS: molecular diagnostics, polymerase chain reaction, DNA typing.

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INTRODUCTION

With the emergence of molecular diagnostic assays, the proliferation of Food and Drug Administration approved assays, and the introduction of these assays into the clinical setting, there is an increased need for clinical laboratory scientists to understand and perform molecular-based techniques. In addition, the National Accreditation Agency for Clinical Laboratory Science (NAACLS) modified the Clinical Laboratory Science (CLS) curriculum standards to require the inclusion of molecular diagnostics.[1] The standards state:

> "The curriculum shall include …components of laboratory services such as hematology, hemostasis, chemistry, microbiology, urinalysis, microscopy, molecular diagnostics, immunology and immunohematology. This includes … performance of assays…"

Introduction of molecular content was the most frequently occurring curricular change among the programs surveyed by the American Society for Clinical Pathology in 2002.[2] A brief informal electronic survey e-mailed in June 2005 to over 220 accredited CLS/MT Program Directors or Faculty listed on the NAACLS website, revealed that one-third of the programs that responded had offered molecular diagnostics as a separate subject. In the remaining programs molecular content had been incorporated into existing microbiology, hematology or chemistry courses. Less than one-third of the surveyed programs stated that they included some laboratory instruction.[3] In the CLS program at an academic medical university described here, faculty first added a molecular diagnostic module with virtual student laboratories to the curriculum at the end of a Clinical Chemistry course in fall 2005 because the purchase of a thermal cycler for testing was cost-prohibitive the first year the content was taught. However, CLS administration allocated funds to purchase equipment and supplies beginning in 2006 and traditional "hands-on" manual molecular student laboratories were added to the Clinical Chemistry course content at that time.

With the change from virtual to hands-on, the question arose as to which type of laboratory experience would result in better student learning outcomes. Based on an extensive literature review of outcomes from laboratories in educational programs, Ma and Nickerson pointed out that, even though virtual or computer simulated laboratories were a cost efficient alternative to hands-on exercises that allowed for reaching geographically distant students and promoting conceptual understanding, the psychology of presence was important in the development of other aspects of learning, especially in the area of design skills.[4] Many researchers observed equivalence between the two types of instruction but some provided evidence for more cognitive gains and better satisfaction with hands-on exercises.[5,6,7,8] According to Ma and Nickerson, the effectiveness of computer based laboratories compared to traditional laboratories was seldom explored and the debate on the superiority of one type of laboratory instruction over the other remained unresolved.[4] Therefore, the purpose of this research study was to determine if there was a significant difference in exam question scores among students who had virtual molecular laboratories as compared to those who had hands-on molecular laboratories.

METHODS

In 2005, the molecular module incorporated into the senior clinical chemistry course lasted for a week and consisted of a sequence of four lectures supplemented with virtual exercises: the Bacterial Identification Lab developed at the Howard Hughes Medical Institute and the DNA Forensic Problem Set designed by the University of Arizona.[9,10] The purpose of the Bacterial Identification Lab was to familiarize the student with the science and techniques used to identify different types of bacteria based on their DNA sequence. The laboratory consisted of the following steps in which students pointed and clicked their way through the procedure: sample preparation, polymerase chain reaction (PCR) amplification, purification of the PCR product, preparing the PCR product for sequencing, and DNA sequence analysis. Upon completion of the procedure, students were able to describe sample requirements, explain how PCR worked and its utilization in the clinical setting, describe a technique for separating DNA fragments, explain how a DNA sequencer worked, and how to identify bacteria by its DNA sequence.

The purpose of the DNA Forensic Problem Set was the introduction of the student to the use of the Restriction Fragment Length Polymorphism (RFLP) method to characterize human DNA samples as applied in paternity analysis and sex crimes investigations. The problem set consisted of reading assignments after which student learning was enforced by exercises involving the interpretation of DNA electrophoretic patterns. Students had the opportunity to interpret actual case results as might be produced by the FBI laboratory or a commercial, paternity-testing facility. At the end of this problem set, students were able to discuss the technique of Southern Hybridization, describe hypervariable regions of DNA, and determine biological offspring, paternity, and possible rape suspects given DNA fingerprinting data. Each student performed all virtual laboratories in class at a separate computer station with the instructor's assistance to

assure adequate completion of all exercises prior to the submission of answers included in each problem set.

In 2006, CLS faculty introduced manual rapid DNA isolation from fingerstick blood and subsequent Polymerase Chain Reaction - based exercises. These hands-on laboratories were modified from experiments designed by Edvotek: PCR - based DNA typing of a human chromosome 1 fragment D1S80, one of many DNA variable number of tandem repeat (VNTR) sequences used by the forensic database for "DNA fingerprinting" of subjects of kinship or crime investigations; and PCR of human chromosome 16 polymorphic locus PV92 with and without an insertion of a 300 bp transposon fragment containing Alu restriction enzyme recognition site (Edvotek, Inc., Bethesda, MD, cat # 334 and 333). The faculty chose these exercises to illustrate principles underlying laboratory identification of various gene mutations involved in naturally occurring polymorphisms or in disease development and to introduce students to automatic micropipettes and manual handling of microliter volumes typical of molecular procedures. The students isolated their own DNA from dried blood spots (DBS) deposited on FTA^R Elute MicroCards (Whatman Ltd., cat# WB120401). Then students amplified their own D1S80 and PV92 fragments following Edvotek's recommendations.[11] The faculty demonstrated programming of the thermal cycler in class and also demonstrated a session on computer-based primer design using GeneFisher2 on-line software.[12] During the primer design session, the students accessed GenBank National Center for Biotechnology Information (NCBI) database through PubMed in the university library electronic resources.[13] The students performed horizontal DNA electrophoresis in 1% agarose gel to separate the PCR reaction products. Upon staining, students viewed the separated products in ultraviolet light and, following documentation using digital photography, interpreted the results. All controls worked as expected. The protocol outline for this exercise is provided in Table 1.

Exam performance of the students exposed to virtual versus hands-on laboratories was compared to assess whether the inclusion of hands-on laboratory exercises had resulted in significantly improved molecular questions scores, indicating a better understanding of the material taught in the module. Ten multiple choice questions derived from the molecular module content were included in the clinical chemistry course written final examination. The average score for each question was obtained for the years 2005, 2006, and 2007. The year 2005 only included virtual laboratories. The years 2006 and 2007 incorporated the hands-on laboratories, so scores for these two years were combined. All questions, except for one, were the same in 2005, 2006, and 2007 exams. One question from the 2006 and 2007 exam related directly to the hands-on laboratory and therefore did not appear in the 2005 exam. The content of the remaining questions germane to both virtual and hands-on designs included the history and

Table 1. Student protocol outline for hands-on molecular laboratories.

Day 1		
	1.	Wash your hands, warm up the fingers, and, after wiping with an alcohol swab, apply a few drops of blood from a fingerstick on the circular area on the **FTA^R Elute MicroCard**. Do not rub in. Let air dry in the biosafety hood overnight. Record the number of the card assigned to you.
	2.	Watch primer design session and choose primers for a human DNA sequence of choice.
Day 2		
	3.	Using the **Harris Uni-Core device** cut a 3 mm disc from the card and place in a 1.5 ml screw cap tube with 500µl sterile H_2O – pulse vortex 3 times
	4.	Replace wash H_2O with 30 µl of fresh H_2O and incubate for 30 min. at 95°C.
	5.	Pulse vortex 60 times and centrifuge for 30 sec. – the supernatant contains DNA ready for PCR.
	6.	Observe thermal cycler programming performed by the instructor.
	7.	Add 5 µl of DNA solution to a 0.2 ml tube containing a PCR bead (Edvotek) and D1S80 or PV92 primers (provided by the instructor). Mark the PCR tube with a number corresponding to the number on the FTA card.
	8.	Insert the tube into the thermal cycler and record the PCR parameters displayed on the instrument.
Day 3		
	9.	Perform agarose gel electrophoresis and staining according to instructions. Observe results on the transilluminator. Interpret the results recorded on digital photograph provided by the instructor.

discovery of DNA, the structure of DNA, DNA melting and annealing, DNA amplification by PCR, separation and detection of the PCR products, and other specific DNA analytical methods and instrumentation for analyzing DNA products. Of the ten test questions, five covered the lecture components, and five covered the experimental methods taught in the course. Students took exams on their own computers, off campus, during a designated and restricted time. Exams were administered by computer software in WebCT CE 4.1 and WebCT VISTA 3.x with test questions presented one at a time. Students could go back and review questions (again, one at a time) and change answers while the browser was opened. However, students could not review the exam questions or their answers once the test was submitted. Students also were not informed which question they missed or how they scored on the entire molecular section of the exam. They were only provided with one final examination score.

The research question for this study addressed whether there was a significant difference in percentages of students correctly answering molecular exam questions among students who had virtual molecular laboratories as compared to those that had hands-on molecular laboratories. A two-sample *t*-test was used to determine if there was a significant difference between the two groups, virtual versus hands-on laboratories, for each molecular diagnostic question. A two-sample *t*-test was then performed to determine if there were significant differences in the number of students correctly answering molecular questions for the students performing in the upper 25th quartile on the exam. A third two-sample *t*-test was preformed comparing the difference between the two groups, virtual versus hands-on, for those students performing in the lower 25th quartile on the exam.

RESULTS

In 2005, there were a total of 27 baccalaureate clinical laboratory science students. In 2006 there were 17, and in 2007 there were 15. Since the 2006 and 2007 students received the hands-on laboratories these two samples were combined for a total of 32 students. The percentage of students correctly answering ten multiple choice questions (n=10) were compared between the two groups. Graphic representation of these outcomes is shown on Figure 1.

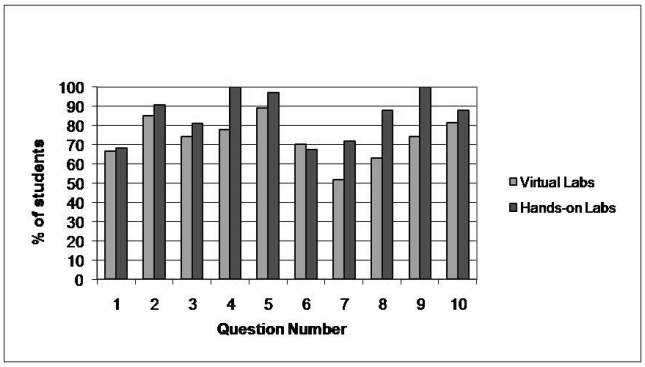

Figure 1. Percentages of students correctly answering examination questions for virtual vs. hands-on laboratory groups. Students exposed to hands-on laboratories scored significantly higher than the virtual lab group.

The two-sample *t*-test for group comparison revealed that the students exposed to the hands-on laboratories scored significantly higher than the virtual lab group on the molecular questions incorporated into the clinical chemistry final written exam. However, statistical analysis of the scores achieved by students performing in either the upper 25th percentile or the lower 25th percentile showed no significant difference in student performance between the two groups. This meant that treatment of these higher performing and lower performing groups had no effect on their ability to answer questions correctly. Results of the *t*-tests are presented in Table 2. Since there was a statistically significant difference in student performance for the whole group (upper, middle, and lower percentiles combined), the researchers proposed that the scores achieved by students performing in the middle percentiles were likely responsible for the statistically significant differences.

CONCLUSIONS

Data analysis supported our prediction that inclusion of hands-on laboratory instruction should improve students' performance on final course examinations. However, closer data inspection indicated that in order to improve scores of the weakly performing students other interventions may be necessary such as more lecture or more laboratory time with emphasis on repetition. Also, since the data have implied that the inclusion of hands-on exercises did not significantly improve performance of those who already were in the upper 25th percentile, one may consider more challenging assignments for this group.

Table 2. Results of the t-tests for the Comparison of Examination Scores for Virtual vs. Hands-on Molecular Laboratories. Total number of students taking examination: Virtual labs = 27, Hands-on Labs = 32. Levene's Test for Equality of Variances: F = 0.59, p = 0.45 (Equal variances assumed).

	Virtual Labs			Hands-on Labs			95% CI For Mean		
	M	SD	n†	M	SD	n†	Difference	t	df
All Students	73.4	11	10	85.2	12.6	10	-23.0,0.8	2.25*	18
Students in upper 25%	92.7	12.4	10	96.5	7.7	10	-13.5, 5.9	0.82	18
Students in lower 25%	55.2	24.6	10	68.8	28.4	10	-38.5, 11.4	1.14	18

* $p < 0.05$
† Number of multiple choice molecular questions on final examination

In fall 2008, the program introduced a revised BS-CLS and MHS-CLS curriculum which included a 3 credit hour clinical molecular methods lecture course and 2 credit hour clinical molecular methods laboratory during the last year of each program. An instructional laboratory manual for use in the course was developed with guidelines for 12 molecular diagnostics laboratories[14] and a supplement with an advanced assignment for the graduate students.

Some limitations of the study should be acknowledged. The study included only a small number of students. The electronic testing software used by the school at that time captured only limited statistical data from student testing.

In order to generalize these findings, further studies need to be done that compare the academic outcomes of students who perform virtual laboratories versus hands-on laboratories in all aspects of the clinical laboratory science curriculum. In addition, as molecular content is added to the curriculum, studies need to be performed to determine the knowledge and skills that entry-level practitioners need in order to practice in this area so that preservice education adequately prepares students for career opportunities.

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