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Medical Laboratory Science Education for the Online Student:

A Step into a Virtual Laboratory

By Elizabeth Brandon

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in the Department of Medical Laboratory Sciences

Idaho State University

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Committee Approval

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Elizabeth M Brandon find it satisfactory and recommend that it be accepted.

_____

Susan Galindo, Major Advisor

_____

Rachel Hulse, Committee Member

_____

Dr. Ryan Lindsay, Graduate Faculty Representative

Idaho State University IRB Approval

Idaho State
UNIVERSITY
Office for Research - Research Outreach & Compliance
921 S. 8th Avenue, Stop 8046 • Pocatello, Idaho 83209-8046

August 30, 2016

Liz Brandon
Medical Laboratory Science
1311 E Central Drive
Meridian, ID 83642

RE: regarding study number IRB-FY2016-303 : Medical Laboratory Science Education for the Online Student: A Step into a Virtual Laboratory

Ms. Brandon:

Thank you for your responses from a previous review of the study listed above. These responses are eligible for expedited review under OHRP guidelines. This is to confirm that I have approved your application.

Notify the HSC of any adverse events. Serious, unexpected adverse events must be reported in writing within 10 business days.

You may conduct your study as described in your application effective immediately. The study is subject to renewal on or before Aug 30, 2017, unless closed before that date.

Please note that any changes to the study as approved must be promptly reported and approved. Some changes may be approved by expedited review; others require full board review. Contact Tom Bailey (208-282-2179; email humsubj@isu.edu) if you have any questions or require further information.

Sincerely,

Ralph Baergen, PhD, MPH, CIP
Human Subjects Chair

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List of Abbreviations

ASCP – American Society for Clinical Pathology

CLS – Clinical Laboratory Science

DNA – Deoxyribonucleic Acid

MLS – Medical Laboratory Science

MS – Microsoft

NAACLS – National Accrediting Agency for Clinical Laboratory Science

PCR – Polymerase Chain Reaction

Abstract

Medical Laboratory Science (MLS) is a growing field within healthcare, however the number of MLS programs available in the United States is declining. As some universities close their MLS programs, others are turning towards online classes to provide education to a greater number of students and satisfy the workforce demand. MLS is a degree program heavy in laboratory classes, so these online programs will include online labs in place of traditional in-class labs. This study will determine if online lab activities are as effective for student comprehension as traditional lab activities. High school students enrolled in Advanced Biology courses were the participants in this study. Group A performed the online laboratory first, followed by the traditional laboratory, while Group B performed the traditional laboratory first, followed by the online laboratory. Both groups completed a pre-, mid-, and post-test to determine the comprehension and understanding of the topic gained from each activity. We hypothesize that online labs are just as effective as in-class labs in regards to student comprehension. Results from this study show that the participants who performed the online lab activity showed a significantly greater increase in knowledge over the participants who performed the traditional lab activity. The results of this study may provide insight into innovative methodologies for professors in the laboratory sciences who teach labs to online or distant students. Universities may then add accredited MLS programs to their online course content, provide more opportunities for students to become certified in the field, and therefore satisfy the workforce demand.

Introduction

Medical Laboratory Science (MLS) is the chosen career path of many young and aspiring individuals curious to know more about the science of clinical medicine and excited to begin work in the healthcare industry. The United States is currently suffering from a severe shortage in medical laboratory scientists, which is causing strain on many hospital and clinical laboratories across the nation (26). According to the National Accreditiong Agency for Clinical Laboratory Scientists (NAACLS) Accredited Program Search, there are only 228 accredited programs providing an MLS degree operational in the United States, which is causing the number of successful graduates of these programs entering the workforce to quickly decline throughout the United States (18). There are both short- and long-term effects to the work industry, where "in the next five years, 13% of the workforce is expected to retire, with 25% of the workforce retiring in the next 10 years" (8).

Today's typical college students, regardless of their degree program, are all "online students in some way—whether it be accessing institutional services online, taking a hybrid or fully-online course, or enrolling in an online credential" (9). Many universities that still provide an accredited MLS degree program are turning towards alternative methods of teaching, such as through video conferencing, online classes, and virtual labs in order to satisfy the workforce demand. As MLS programs adapt to methodological changes in biochemical technologies, teaching methods are being

adapted as well (25). MLS is a career that centers on problem-solving, detailed thought processes, and troubleshooting during analysis and when determining which results are accurate and correlate with the patient's symptoms and history. Although these intellectual factors include the skills necessary for a successful medical lab scientist, the hands-on skills needed in this career cannot be ignored. Adapting teaching methods is challenging in that MLS is ultimately a career where competent hands-on skills are necessary for physically performing laboratory tests, and teaching these hands-on skills may be difficult to accomplish through virtual or online methods.

Online learning labs are now being utilized by MLS teachers to help their distant students learn to the extent possible if they were physically participating in the lab. McGaghie et. al. posed the question "How shall we design an educational environment that produces maximum learning outcomes among all trainees?" which has propelled the ideology of this study forward (16). Virtual labs, such as the one we propose to develop, are a new tool for professors with distant students, but the effect of student learning within these virtual labs compared to physical labs is unknown.

The objective of this project is to improve the training of both on-campus and distant students in a molecular biology laboratory class within a Medical Laboratory Sciences degree program. We propose to design an online MLS molecular biology laboratory using various learner-centered education methods and determine if these virtual methods enhance the student understanding and comprehension. The laboratory activity we chose to virtually develop is a DNA extraction activity. We will compare competency and comprehension of MLS students participating in an online

DNA extraction laboratory with a traditional in-class DNA extraction lab. The goal is to show that online learning labs can aid professors in teaching their long-distant students laboratory methods just as effectively as if they were physically in the lab together.

Research Questions

1. Are online or virtual molecular biology labs equally effective, more effective, or less effective at teaching general understanding and comprehension of the lab activity to biology students over traditional in-class molecular biology labs?
2. Is it more effective to present both the online and the traditional labs to students, or will one activity method suffice? If both labs should be completed in succession, in which order should they be presented?
3. Are there specific areas within the topic of this molecular biology lab that either the online lab method or the traditional lab method is more effective at helping students reach understanding and comprehension?

We hypothesize that online or virtual labs are just as effective as traditional in-class labs in regards to student understanding and comprehension, such that there will be no significant difference in the student learning curve between the online and practical lab student groups, nor in the analysis of questions concerning specific topics within DNA extraction. If distant students are not inhibited by online learning, then universities can begin to adopt online methods such as this. If universities begin to see that they have the capability to add accredited MLS programs to their online programs, the hope is that the number of programs available will increase, and thus the number of

capable MLS graduates will also increase. The results of this study may provide insight into innovative methodologies for professors in the laboratory sciences who teach labs to online or distant students. In addition, the results may be used to evaluate the learning capabilities of online or distant students compared to those students physically on campus.

Personally, this study will influence my career goals as a professor in the medical laboratory sciences by allowing me to explore the way students learn in a virtual laboratory setting, especially in an atmosphere where more than half of the students are online or distant. If universities continue to gather students into online programs instead of creating more traditional programs on campuses throughout the country, then the professors have a responsibility to ensure that each student is receiving the same level of education, no matter if they are on campus or online. My goal is to be one of these professors who adapts to the change in education styles and discovers new and unique ways to teach lab science to students given the geographical restrictions.

Definitions

1. Online Laboratory Activity – An online lab includes any form of online instruction or lecture including tables, pictures, graphs, videos, and text that is presented to students solely through use of a computer, whether in the classroom or at home. In this study, the online lab activity refers to the online PowerPoint lecture about DNA Extraction that was presented to the participants.

2. Traditional Laboratory Activity – A traditional in-class lab activity is any lab experiment that is presented to students solely through use of a laboratory classroom complete with samples, safety items, equipment, reagents, and all things necessary to physically complete the lab activity. In this study, the traditional lab activity refers to the Flinn Scientific DNA Extraction Lab that participants physically completed in their classroom laboratories.
3. Pre-Test – The pre-test is used to gain a baseline value of knowledge that student participants already possess prior to agreeing to participate in this experiment. In this study, the pre-test included 15 questions concerning various topics regarding DNA Extraction and Isolation.
4. Mid-Test – The mid-test is used to determine what and how much knowledge was gained after performing the first lab activity. In this study, the mid-test contained the same 15 questions and answer choices as the pre-test, but were presented in a different order.
5. Post-Test – The post-test is used to determine what and how much additional knowledge was gained after performing the second lab activity as well. In this study, the post-test contained the same 15 questions and answer choices as the pre-test and the mid-test, but were presented again in a different order.

Assumptions and Limitations

1. There is great variety in the type of laboratory activities that Medical Lab Scientists perform in their careers daily. Participation in a DNA extraction lab activity requires similar skills and basic knowledge as many of these other MLS-

specific lab activities, so it is assumed that the results of this study may be generalized with other MLS-specific lab activities.

2. Online laboratory activities may be presented to students in various forms and via various software or internet programs, but the parallel idea across the board is that online labs are presented to primarily off-campus or distant students, or complete classes which include these students. Thus, the results of this study can be generalized to programs with these types of students, regardless of manner of presentation.
3. Due to low participation from high school biology teachers, only two classes of students were used in this study and thus made up only 28 participants in Group A and 29 participants in Group B. Due to the nature of the high school atmosphere, individual participants could not be randomly assigned to each group, however the classes as a whole were chosen based on equivalent advancement levels of each biology class between schools.

Literature Review

Demand for MLS Programs. The demand for medical laboratory science professionals is increasing due to the aging population and the demands of the healthcare system, as well as an increase in position vacancies due to the retirement of many current medical laboratory scientists. Despite this increase in demand, the number of accredited MLS programs decreased by 70% between the years 1970 and 2003 (25). The reasons for this decrease vary, but many factors include declining enrollment and increasing cost (2).

With the currently reduced number of MLS programs that are available, prospective students may need to choose programs based on availability and location, rather than program integrity or personal preference. Across the nation, some states with a large geographical footprint have a single medical laboratory science program but have the responsibility of training the MLS workforce for the entire state, forcing these programs to explore new educational models that include laboratories at distant regional campuses where instruction is provided via online classes. Idaho is one of these states (2).

The American Society of Clinical Pathologists Personnel Standards Statement states that "technologist-level personnel should possess a baccalaureate degree and successfully complete an accredited or approved training program or specified work experience," and these standards are necessary for every MLS to accomplish before entering the workforce (21). The US Bureau of Labor Statistics states that the profession

is growing “much faster than average” with a 22 percent increase in employment rate projected from 2012 to 2022 (6). Rohde found that “students typically receive one or two job offers in their final semester while doing clinical internships” which supports the claim that MLS graduates are in high demand (23).

Teaching Methods. Many academics agree that what works for one student doesn’t necessarily work for another, and one teaching style isn’t beneficial to all students. As times change with government mandates, technological advances, and an ever-increasing student population, educators need to evaluate the way they deliver content to students and determine if a shift in paradigm is necessary (4). Cleveland-Innes proposed two central themes to focus on when changing teaching paradigms: content and modularization (7). The theme of content will undoubtedly expand into various online learning methods as the need to teach a greater number of students with the same time and space increases.

Handelsman argues that “universities should place greater emphasis on awareness of new teaching methods” and should “marshal their collective will to reform science education” (11). Is it ultimately the responsibility of the student, teacher, or university to ensure the proper depth of education? Many believe a combination of university support, analysis of teaching methods, and student scholarship to be beneficial in reforming science education. For example, Beck et al. studied the effect of inquiry-based teaching in laboratory courses, and found a positive effect of inquiry-based teaching in biology laboratory courses on student learning gains (3). Norman states that “a major concern of traditional teaching methods is effectiveness” which

translates into how competent a student is at a specific task" (19). Ram studied this effectiveness in the form of a sophomore undergraduate chemistry laboratory and found that "problem-based learning has proven to be an effective way of motivating college sophomores" (22). If various methods, such as inquiry-based, problem-based, or learner-centered methods are put into place alongside online or virtual classrooms, the complex net of teacher pedagogy and student comprehension can be woven.

Online Content and Distance Education. With various technological advances creating an effect on education, we are entering a new era of reform in science education. While "both the content and pedagogy of science learning and teaching are being scrutinized…new standards intended to shape and rejuvenate science education are emerging" (13). Science educators have stated that "there are rich benefits in learning that accrue from using laboratory activities," however traditional laboratories are not available to all students (12). In order for the modern university to compete in the educational marketplace, it needs to "extend lifelong learning opportunities to its students anytime and anyplace" (24). This includes meeting the needs of online and distant students, of which the numbers are ever increasing, especially at the undergraduate level (1). The Department of Health and Human Services reported that most of the newly added curriculum content of MLS programs was online content (25).

In a study done by Lynagh, reviewing the transference of laboratory skills to 'real life' clinical measurements, there were "significant improvements in skill after receiving simulator training in comparison with standard or no training" reported (14). The results of this study show promise for student comprehension with the increasing

number of online laboratory classes for distant students. There is currently a need for "the design of distance education that focuses on the learner and on the best available knowledge about human learning" (15). Although we understand that the need for online learning modules is great, "simulation-based medical education is a complex service intervention that needs to be planned and practiced with attention to organizational contexts" (17).

Approach

Participant Selection and Grouping

The participant population was selected based on which high school principals and their respective Advanced Biology teachers were willing to lend me their Advanced Biology students for a class period. Two schools were willing to participate, given that parental and student consent was obtained. The participant population was comprised of high school students enrolled in Advanced Biology courses from two high schools within the West Ada School District in Idaho. Advanced biology students were chosen because the content included in these advanced courses is equivalent to content taught in biology courses at the college level. This college-level course is a pre-requisite for entry into any MLS program. Thus, the data from this study may be extrapolated to higher education.

The assignment of one high school to Group A and the other high school to Group B were made randomly. Group A participated in the online lab first, followed by the traditional lab. Group B participated in the traditional lab first, followed by the online lab. A parental consent form was signed by the parent or legal guardian of each student acknowledging that they have read and understood the risks and benefits, and agree to have their child participate in this study. A student assent form was signed by each student acknowledging that they have read and understood the risks and benefits, and agree to be a participant in this study. Due to the limitations of number of participants for the study, it is understood that there was a bias due to the students all

being of high school age and attending high schools in the West Ada School District in the state of Idaho. We hope that this study can be repeated at other high schools or universities to address and evaluate this possible bias.

Survey Structure

The survey was created in Microsoft Word and was attached to the beginning of the pre-test. The survey was separated into three parts: Personal Information, Computer Use, and Familiarity with Computer Software. The Personal Information section contained questions regarding demographics, career interests, and history of DNA extraction lab experience. The Computer Use section contained questions regarding availability and type of computers at home, as well as home internet access. The Familiarity with Computer Software section contained questions regarding student's perceived comfort in the use of computer software.

Testing Methodology

This study focuses on the comparison of two learning methods, online and traditional in-class, and determine which, if either, is more effective at enhancing student comprehension. This comparison will be measured by percent of questions answered correctly on exams taken before either activity is completed (pre-test), between the two activities (mid-test) and after both activities are completed (post-test). A type of crossover study will be used, where each group serves as its own control. This is advantageous because it reduces between group variability, as the results of each

group's average pre-test score will be compared to that same group's average score for the mid-test and post-test.

The Pre-Test was given to the participants to gain a baseline value of prior knowledge of DNA Extraction activities for the study. There were 15 total questions on each test comprising of the exact same questions and answer options, though the question order was scrambled between tests to reduce bias from any question order effect. This provides an easily measurable result to compare between tests.

During each of these tests, students were asked to put away all notes and protocols and asked to be silent during the duration of the tests. These tests were created in Microsoft Word, then printed and distributed to the students. Each test comprised of fifteen questions regarding DNA extraction preparation, methods, and results. The test questions and multiple-choice answers on each test were contained the exact same worded questions and answers to limit experimental bias, but the order of questions and order of answers on each question were scrambled between tests to address students who may try to memorize the letter choice for each answer. The tests were graded and scored with the number and percent correct out of fifteen questions. Questions left unanswered were marked as incorrect. One question contained an ambiguous answer, and thus two answers were taken as correct. See Appendices 3-5 where correct answers are in bold.

Traditional Lab

We provided an in-class traditional DNA extraction activity that students physically completed within their classroom laboratory. We chose to use the Flinn Scientific DNA Isolation Super Value Laboratory Kit for our activity. This protocol was passed out to each student immediately preceding the traditional activity. Students were encouraged to read the entire protocol before beginning the traditional activity, take notes, and refer to it if necessary throughout the traditional activity. During the traditional activity, students were not allowed to refer to the online lab activity, nor any notes taken during the online lab.

Online Lab

Microsoft PowerPoint was used to create an online learning lab for a DNA extraction activity that contains detailed explanations and pictures of this real-life laboratory test for the students to review. The online lab was based on the Flinn Scientific DNA Isolation protocol used in the traditional lab activity. The DNA extraction method is a critical knowledge base for any molecular diagnostics laboratory and supplemental testing, which is why we have chosen it as our activity. For Group A, the online lab was uploaded to the biology teacher's web page on the high school's website immediately after the students completed the survey and pre-test. Students could then access the online lab via individual computers in the school's library. For Group B, the online lab was uploaded to the biology teacher's web page on the high school's website immediately after the students completed the mid-test. Students could then access the online lab via individual laptops within the classroom. During the online activity,

students were not allowed to refer to the traditional lab protocol nor any notes taken during the traditional lab.

Data Analysis

Data analysis from the survey and all tests was performed using Microsoft Excel as well as JMP Pro Statistical Software. The results of the pre-test, mid-test, and post-test were compared for each group, and an average difference in percent between the two groups was calculated. Nine questions regarding specific ideas or themes within the greater topic of DNA Extraction were selected and analyzed individually to determine if there is a correlation between which group does better or worse on specific questions, such as procedural or analysis questions. Any question that had greater than 80% of students answer correctly on the pre-test was not individually analyzed. Any question where multiple answers were taken as correct was not analyzed individually.

The three questions concerning sample type, collection, and storage that were individually analyzed were:

1. DNA can be isolated from which of the following?
2. Which of the following is the *best* appropriate sample type for a human DNA extraction?
3. Once extracted, how can DNA be stored for long-term later use?

The four questions concerning background information, reagent properties, and theory behind DNA Extraction that were individually analyzed were:

1. What is the best temperature at which to precipitate DNA?
2. What is the immediate danger to the DNA once it is released from the cell nucleus?
3. How are the nucleic acids precipitated and separated from the rest of the cellular impurities?
4. How is DNA separated from the cell nucleus?

The two questions concerning the procedure and final DNA product that were individually analyzed were:

1. What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?
2. What does the DNA look like once it is precipitated?

Results

Participant Demographics and Survey Results

A total of 66 high school students were recruited for participation in this study, with 57 of those students completing both labs and all three tests. The remaining 9 students either were missing a parental or student consent form, left the classroom early without completing all the activities, or requested in person or in writing that their data be eliminated from the study. Student participants were chosen based on their enrollment in the most advanced biology class available at their respective high schools. Enrollment was open to any age group or grade level, but high school curriculum requirements limited the randomization of student age and experience in previous biology labs.

Group A consisted of 25 (89%) 10^{th} graders, 2 (7%) 11^{th} graders, and 1 (4%) 12^{th} grader (Table 1). Of these students, 23 (82%) were 15 years old, 4 (14%) were 16 years old, and 1 (4%) was 17 years old, with a group mean age of 15.21 years (Table 2). Group B consisted of 2 (6.9%) 10^{th} graders, 25 (86.2%) 11^{th} graders, and 2 (6.9%) 12^{th} graders (Table 1). Of these students, 5 (17.2%) were 15 years old, 18 (62.1%) were 16 years old, and 6 (20.7%) were 17 years old, with a group mean age of 16.03 years (Table 2). Both groups reported a majority of participants who identified themselves as White, Caucasian, or European, with Group A reporting 25 (89.3%) and Group B reporting 16 (55.2%) participants who identified as such. Group A also reported 1 (3.6%) participant who identified as Hispanic, Mexican-American, or Latino and 2 (7.1%) participants who

identified as Mixed Race or Other. Group B was more diverse, with 4 (13.8%) participants who identified as Hispanic, Mexican-American, or Latino, 4 (13.8%) participants who identified as Asian or Pacific Islander, 1 (3.4%) participant who identified as Black or African American, and 1 (3.4%) participant who identified as Native American (Table 3).

Student participants were given a survey question regarding their career interests. Out of the total number of participants, only 2 (3.5%) responded with a career interest in Medical Laboratory Science, however the majority of all students (56.1%) responded with a career interest in another medical related profession (Table 4). The student participants were also given a survey question regarding their experience in performing DNA Extraction activities. Of all student participants, the majority (75.4%) have not participated in a DNA extraction lab activity before in their school experience. Group A included 4 (14.3%) participants who had performed a DNA extraction lab at least once before, over a year ago. Group B included 3 (10.3%) participants who had performed a DNA extraction lab at least once within the last year and 7 (24.1%) participants who had performed a DNA extraction lab at least once before, over a year ago (Table 5).

Information about student home computer use and availability was collected, and results show that 100% of all students owned a family or personal computer at home that is connected to the internet. Of all participants, 59.6% share their home computer with at least one other family member. The majority (45.6%) of participants own a portable laptop computer, while 42.1% own a desktop computer (Table 6). The

majority of participants in both Group A (85.71%) and Group B (86.21%) strongly agree that they are comfortable with general computer use. A strong majority of participants in both groups either agree or strongly agree that they are comfortable with use of MS Word, MS PowerPoint, and MS Excel. Out of all computer software programs inquired about, results indicate that participants are most comfortable with the use of MS Word, followed by MS PowerPoint, then MS Excel. Familiarity with the use of MS PowerPoint was the most critical area of interest, as this was the software in which the online lab was created and delivered. The majority of students in Group A (82.14%) and Group B (75.86%) strongly agreed that they are comfortable with the use of MS PowerPoint, while 14.29% of participants in Group A and 24.14% of participants in Group B indicated moderate agreement with the statement. MS Excel received the most responses in disagreement (10.71%) and in strong disagreement (7.14%) by Group A participants only (Table 7).

Online vs. Traditional Lab Activity Results

Out of the 15 total questions on the Pre-Test, Group A had an average of 5.642 (37.61%) correct answers while Group B had an average of 6.586 (43.91%) correct answers. Group A proceeded with completing the online lab while Group B proceeded with completing the traditional lab. After the completion of the first lab activity, each participant was given the Mid-Test to gain insight on how much knowledge was gained during the first activity. On the Mid-Test, Group A had an average of 9.535 (63.57%) correct answers while Group B had an average of 8.413 (56.09%) correct answers. Group A then completed the traditional lab while Group B then completed the online

lab. After the second lab activity was completed, each participant was given a Post-Test to determine if any additional knowledge was gained after the second activity. On the Post-Test, Group A had an average of 9.679 (64.53%) correct answers while Group B had an average of 10.517 (70.11%) correct answers (Table 8 and Figure A).

The difference between scores on the pre-test, mid-test, and post-test were calculated to determine average increase in knowledge after each activity. Group A participated in the online lab activity first, and gained an improvement of 3.893 points (25.95%) between the Pre- and Mid-Test. Group B participated in the traditional lab activity first, and gained an improvement of 1.827 points (12.18%) between the Pre- and Mid-Test. After Group A's participation in the traditional lab activity, there was an increase of 0.144 points (0.96%) between the Mid- and Post-Test leading to an overall increase of 4.072 points (27.15%) between the Pre- and Post-Test. After Group B's participation in the online lab activity, there was an increase of 2.104 points (14.03%) between the Mid- and Post-Test leading to an overall increase of 3.931 points (26.21%) between the Pre- and Post-Test (Table 9 and Figure B).

Students who responded either "yes, within the last year" or "yes, over a year ago" to the survey question "Have you ever performed a DNA Extraction laboratory activity before?" were temporarily eliminated from the data set to determine if inclusion of these students skewed the data at all. It was found that there was no significant change in average test scores with the inclusion of these 14 students, so all students were included in the final analysis (Table 10).

Individual Question Analysis Results

Nine questions were analyzed individually for information on student responses to various themes within DNA Extraction. The three questions concerning sample type, collection, and storage that were analyzed show that Group A had a greater increase in test scores on all three of these questions after performing the online lab than Group B had after performing the traditional lab. Group B then showed similar additional increases in correct answers after performing the online lab (Tables 11-13, Figures C-E).

The four questions concerning background information, reagent properties, and theory behind DNA Extraction that were analyzed show mixed results between the groups. For the question "What is the best temperature at which to precipitate DNA?" Group A had a three-fold greater increase in correct answers between the pre-test and mid-test over Group B. Both groups had a slight increase in correct answers after the second laboratory activity was performed (Table 14, Figure F).

For the question "What is the immediate danger to the DNA once it is released from the cell nucleus?" Group A participants had approximately a 10% increase in correct answers from the pre-test to the mid-test, while Group B had approximately a 14% decrease in correct answers from the pre-test to the mid-test. After the mid-test, both groups had a relatively equal increase in correct answers on this question (Table 15, Figure G).

For the question "How are the nucleic acids precipitated and separated from the rest of the cellular impurities?" both groups had a relatively equal percent increase in

correct answers from pre-test to mid-test. However, after the mid-test Group A had a 28% decrease in correct answers while Group B only had a 7% decrease in correct answers on the post-test (Table 16, Figure H).

For the question "How is DNA separated from the cell nucleus?" Group B had a three-fold greater increase in correct answers after performing the traditional lab than Group A had after performing the online lab. Group B had no change between the mid-test and the post-test, while Group A had a slight decrease in correct answers (Table 17, Figure I).

The two questions concerning the procedure and final DNA product that were individually analyzed showed that Group B had a significantly greater increase in test scores on both questions after performing the traditional lab than Group A had after performing the online lab (Tables 18-19, Figures J-K).

Discussion and Recommendations

Education Activities Between Groups

In comparing the percent increase in scores for each high school student group between Pre-Test and Mid-Test, it was found that Group A had a significantly greater increase in knowledge gained from the online lab activity than Group B had with the traditional lab activity. Then when the activities were switched, it was found that Group A had an additional score increase of less than 1% after completing the traditional lab, while Group B had an additional score increase of 14% after completing the online lab. This indicates that the online laboratory method can be used as the sole method of teaching students information about laboratory experiments in order to increase their *understanding and comprehension* of the subject. These results also imply that students understand and comprehend only about half as much while performing the traditional lab activity only without any online supplemental material. The data suggests that if a traditional lab is supplemented by an online lab, students will be able to understand and comprehend the material much better than with solely the traditional lab method. However, as seen in the results from Group A, if a primarily online lab activity is supplemented with a traditional lab activity, there is no significant additional increase in *understanding and comprehension* gained from the traditional lab activity after already completing the online lab activity.

Individual Question Analysis

In comparing results of individual questions across both groups, it was found that the online lab activity was better at helping student participants gain an understanding of sample type, collection, and storage. This is likely because the online lecture clearly laid out specific requirements for different sample types, procedure for sample collection, and parameters for storage in an easily readable manner. Students could take notes on the online lecture, which may have helped them to remember the correct answers. The traditional lab, on the other hand, had a brief explanation about sample type and collection in written form on the protocol that was handed out to each student to read prior to the traditional lab activity. The storage parameters were not written on the lab protocol, but instead delivered via verbal lecture from the researcher immediately prior to the traditional lab activity.

When comparing four individual questions concerning background information, reagent properties, and the theory behind a DNA Extraction activity, results show that the online activity had a greater increase in correct answers for questions concerning temperature and the danger of nucleases in DNA extraction, but Group B had a greater increase in correct answers for the question concerning separating DNA from the cell nucleus. The information for all questions was clearly presented in text in the online lab and in the verbal lecture in the traditional lab. It is surprising that the Group B participants did not have a greater percent of correct answers for the temperature question, because while physically performing the lab they had reagents on ice. Group B also had a surprising decrease in percent correct answers for the question concerning the danger of nucleases, even though the theory was presented clearly in text in the

background section of the traditional lab protocol. Instead of learning more about nucleases while performing the traditional lab, these students may have been lucky and guessed correctly on the pre-test, while then guessing incorrectly on the mid-test. Both the online and traditional lab were equally effective when participants were tested on knowledge of how the nucleic acids are isolated. However, it was unexpected to find a 28% decrease in correct answers by Group A after they had performed the traditional lab as well. This is basic background knowledge of DNA Extraction, and was presented clearly in both the online lab and the traditional lab lecture. The reason for this large decrease could have been due to student participants mixing up whether salt or the ethanol precipitated the nucleic acids, because the majority of students who did not select the correct answer selected the answer where these properties were switched.

When comparing the two questions concerning the procedure and the final DNA product, results demonstrate that the traditional lab was more effective at teaching understanding and comprehension of these topics. Group B, who performed the traditional lab first, had a significantly greater increase on both of these test questions between the pre-test and mid-test than did Group A. Although this information was presented clearly and equally on both the online lab and in the traditional lab, the participants performing the traditional lab could physically perform each step in the activity, therefore helping them better understand these questions. While the online students simply read the online protocol and looked at pictures of the precipitated DNA, the traditional students were hands-on in performing each step of the analysis and had

a real, 3-dimensional DNA product to touch and observe, which likely led them to have a greater percentage of correct answers for these questions.

In conclusion, the data suggests that online lab alone is the better method of teaching students the background information, theory, sample type and collection for a DNA extraction lab. However, the results demonstrate that the traditional lab is the better method of teaching students about the use of reagents, protocol and procedure, and the final DNA product. Overall, the online lab method can stand alone as a method for teaching *understanding and comprehension* of information regarding biology labs, but it is not necessarily the best method of teaching the physical skills needed for a successful biology experiment.

Future Research Possibilities

Although the DNA Extraction lab is a necessary basic theory to know and understand in Medical Laboratory Science, a future study involving the comparison between two student groups involving different MLS-specific lab activities, such as urinalysis, blood typing, or PCR and DNA Electrophoresis, would be useful in determining if MLS-specific online labs are just as effective as traditional labs. One could also use more than one type of activity and compare the performance of these lab activities to determine if there is a correlation between the effectiveness of online labs and the type of lab activity presented. It would be useful to use either fourth-year undergraduates studying biology or first-year MLS students for these experiments instead of high school biology students to gauge the learning curve of students

specifically going into this career field. The software used for the online method could also be varied, where further studies could create an online lab via different internet websites such as Idaho State University's teaching portal, Moodle.

This study focuses primarily on the gain of knowledge and understanding about information concerning DNA Extraction the theory behind how DNA Extraction works. However, this study does not go into detail about how students who have solely participated in either online or traditional labs will do in a clinical or research lab situation. It would be interesting to conduct a study where each student group was taught a lab activity using solely either an online method or a traditional method, where the online students are physically located in different areas of the state or region and have no physical contact with the researcher or classroom lab materials. Then the student groups would enter a clinical lab situation and would be tested on whether they could successfully complete the physical lab activity as an individual in a clinical or research lab setting, and obtain a successfully isolated DNA sample for further analysis. Although the results of this research show that online methods are better at teaching understanding and comprehension of the subject, are they also just as effective at allowing the student to successfully isolate DNA that is pure enough for further biological analysis on their own within a career lab setting without help or assistance from a teacher or supervisor? Future research on laboratory education will hopefully answer these questions for us.

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Appendices

Appendix 1 – Group Analysis Tables and Figures

Table 1: Student Grade Levels – The majority (89.3%) of students in Group A were in the 10th grade while the majority (86.2%) of students in Group B were in the 11th grade.

	Group A		Group B		Total	
Grade	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
10th Grade	25	89.3%	2	6.9%	27	47.4%
11th Grade	2	7.1%	25	86.2%	27	47.4%
12th Grade	1	3.6%	2	6.9%	3	5.3%
N	28		29		57	

Table 2: Student Age – In Group A, the majority (82.1%) of students were 15 years old with a group mean of 15.21 years. In Group B, the majority (62.1%) of students were 16 years old with a group mean of 16.03 years.

	Group A		Group B		Total	
Age	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
15 Years	23	82.1%	5	17.2%	28	49.1%
16 Years	4	14.3%	18	62.1%	22	38.6%
17 Years	1	3.6%	6	20.7%	7	12.3%
N	28		29		57	
Mean Age	15.21		16.03		15.63	

Table 3: Student Race / Ethnicity – Of all student participants, the majority (71.9%) identified themselves as white, Caucasian, or European.

	Group A		Group B		Total	
Race/Ethnicity	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
White, Caucasian, or European	25	89.3%	16	55.2%	41	71.9%
Hispanic, Mexican-American, or Latino	1	3.6%	4	13.8%	5	8.8%
Asian or Pacific Islander	0	0.0%	4	13.8%	4	7.0%
Black or African American	0	0.0%	1	3.4%	1	1.8%
Other / Mixed Race	2	7.1%	0	0.0%	2	3.5%
Native American	0	0.0%	1	3.4%	1	1.8%
Prefer Not to Answer	0	0.0%	3	10.3%	3	5.3%

Table 4: Student Identified Career Interest – Of all student participants, the majority (56.1%) identified interest in a career in a medical profession other than MLS, while only 3.5% of students identified an interest in a career in MLS.

	Group A		Group B		Total	
Career Interest	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
Medical Laboratory Science	1	3.6%	1	3.4%	2	3.5%
Other Medical Profession	11	39.3%	21	72.4%	32	56.1%
Other Science Lab Profession	3	10.7%	0	0.0%	3	5.3%
Non-Science Related Profession	6	21.4%	3	10.3%	9	15.8%
Undecided	7	25.0%	4	13.8%	11	19.3%

Table 5: Student Experience with a Previous DNA Extraction Lab Activity – Of all student participants, the majority (75.4%) have not participated in a DNA Extraction Lab activity before in their school experience.

	Group A		Group B		Total	
Previous DNA Extraction Lab	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
No	24	85.7%	19	65.5%	43	75.4%
Yes, within the last year	0	0.0%	3	10.3%	3	5.3%
Yes, over a year ago	4	14.3%	7	24.1%	11	19.3%

Table 6: Student Home Computer Use – Of all student participants, 100% own a home computer that is connected to the internet. The majority (59.6%) share their home computer between other family members. The majority (45.6%) of these home computers are laptops, closely followed (42.1%) by desktop computers.

		Group A		Group B		Total	
Home Computer Use		**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
Do you own a home computer?	**Yes**	28	100.0%	29	100.0%	57	100.0%
	No	0	0.0%	0	0.0%	0	0.0%
Do you have internet access at home?	**Yes**	28	100.0%	29	100.0%	57	100.0%
	No	0	0.0%	0	0.0%	0	0.0%
Is your computer shared, or are you the only user?	**Shared**	19	67.9%	15	51.7%	34	59.6%
	Only User	9	32.1%	14	48.3%	23	40.4%
What computer device do you use at home?	**Desktop**	12	42.9%	12	41.4%	24	42.1%
	Laptop	12	42.9%	14	48.3%	26	45.6%
	Tablet	0	0.0%	2	6.9%	2	3.5%
	2+ Devices	4	14.3%	1	3.4%	5	8.8%

Table 7: Student Familiarity with Computer Software Use – The majority of participants in both Group A (85.71%) and Group B (86.21%) strongly agree that they are comfortable with general computer use. A strong majority of participants in both groups either agree or strongly agree that they are comfortable with use of MS Word, MS PowerPoint, and MS Excel. Out of all computer software programs inquired about, results indicate that participants are most comfortable with the use of MS Word, followed by MS PowerPoint, then MS Excel. MS Excel received the most responses in disagreement (10.71%) and in strong disagreement (7.14%) by Group A participants.

		Strongly Agree		Agree		Neither Agree nor Disagree		Disagree		Strongly Disagree	
		N	Percent	N	Percent	N	Percent	N	Percent	N	Percent
General Computer Use	Group A	24	85.71%	4	14.29%	0	0.00%	0	0.00%	0	0.00%
	Group B	25	86.21%	3	10.34%	1	3.45%	0	0.00%	0	0.00%
Microsoft Word	Group A	26	92.86%	1	3.57%	1	3.57%	0	0.00%	0	0.00%
	Group B	25	86.21%	4	13.79%	0	0.00%	0	0.00%	0	0.00%
Microsoft PowerPoint	Group A	23	82.14%	4	14.29%	1	3.57%	0	0.00%	0	0.00%
	Group B	22	75.86%	7	24.14%	0	0.00%	0	0.00%	0	0.00%
Microsoft Excel	Group A	9	32.14%	13	46.43%	1	3.57%	3	10.71%	2	7.14%
	Group B	21	72.41%	7	24.14%	1	3.45%	0	0.00%	0	0.00%

Table 8 and Figure A: Average Group Percent on Each Test: Out of 15 total questions on the Pre-Test, Group A had an average of 5.642 (37.61%) correct answers while Group B had an average of 6.586 (43.91%) correct answers. Group A proceeded with completing the online lab while Group B proceeded with completing the traditional lab. On the Mid-Test, Group A had an average of 9.535 (63.57%) correct answers while Group B had an average of 8.413 (56.09%) correct answers. Group A then completed the traditional lab while Group B then completed the online lab. On the Post-Test, Group A had an average of 9.679 (64.53%) correct answers while Group B had an average of 10.517 (70.11%) correct answers.

	Group A		Group B	
	Raw Score	Percent	Raw Score	Percent
Pre-Test	5.642	37.61%	6.586	43.91%
	Online Lab		Traditional Lab	
Mid-Test	9.535	63.57%	8.413	56.09%
	Traditional Lab		Online Lab	
Post-Test	9.679	64.53%	10.517	70.11%

Figure A.

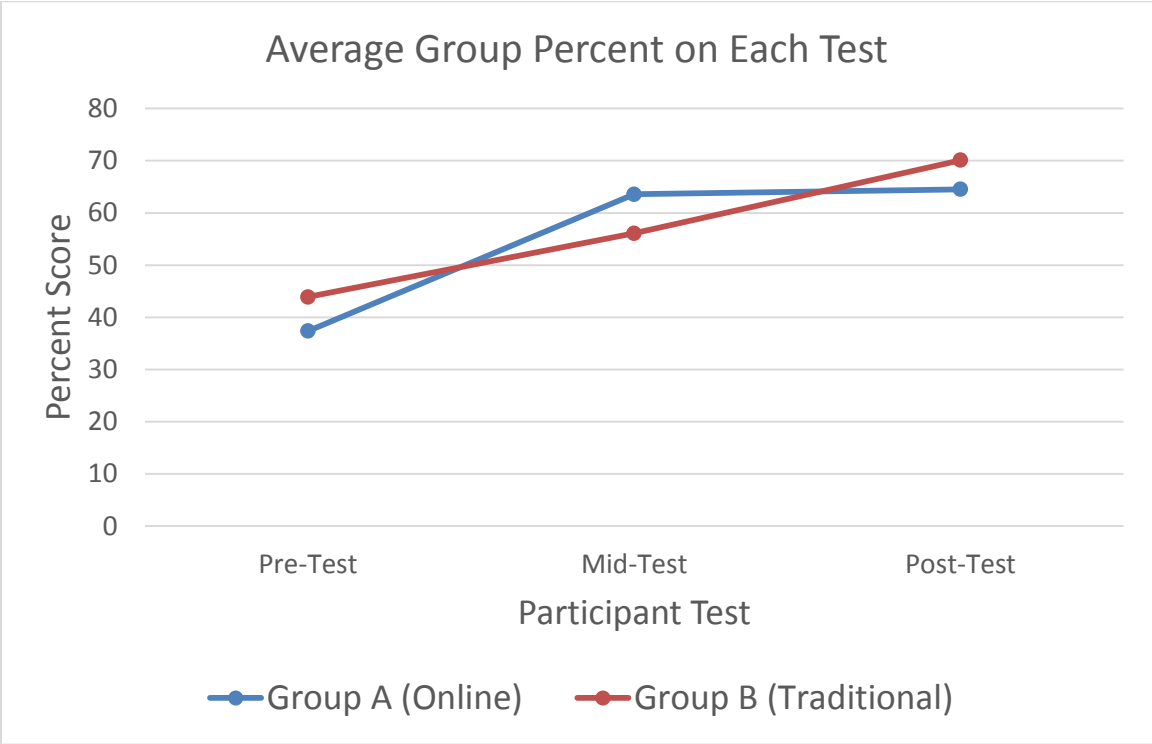


Table 9 and Figure B: Average Group Improvement for Each Test – The difference between scores on the pre-test, mid-test, and post-test were calculated to determine average increase in knowledge after each activity. Group A participated in the online lab activity first, and gained an improvement of 3.893 points (25.95%) between the Pre- and Mid-Test. Group B participated in the traditional lab activity first, and gained an improvement of 1.827 points (12.18%) between the Pre- and Mid-Test. After Group A's participation in the traditional lab activity, there was an increase of 0.144 points (0.96%) between the Mid- and Post-Test leading to an overall increase of 4.072 points (27.15%) between the Pre- and Post-Test. After Group B's participation in the online lab activity, there was an increase of 2.104 points (14.03%) between the Mid- and Post-Test leading to an overall increase of 3.391 points (26.21%) between the Pre- and Post-Test.

	Group A		**Group B**	
Improvement	**Points**	**Percent**	**Points**	**Percent**
Pre-Test to Mid-Test	3.893	25.95%	1.827	12.18%
Mid-Test to Post-Test	0.144	0.96%	2.104	14.03%
Pre-Test to Post-Test	4.072	27.15%	3.931	26.21%

Figure B.

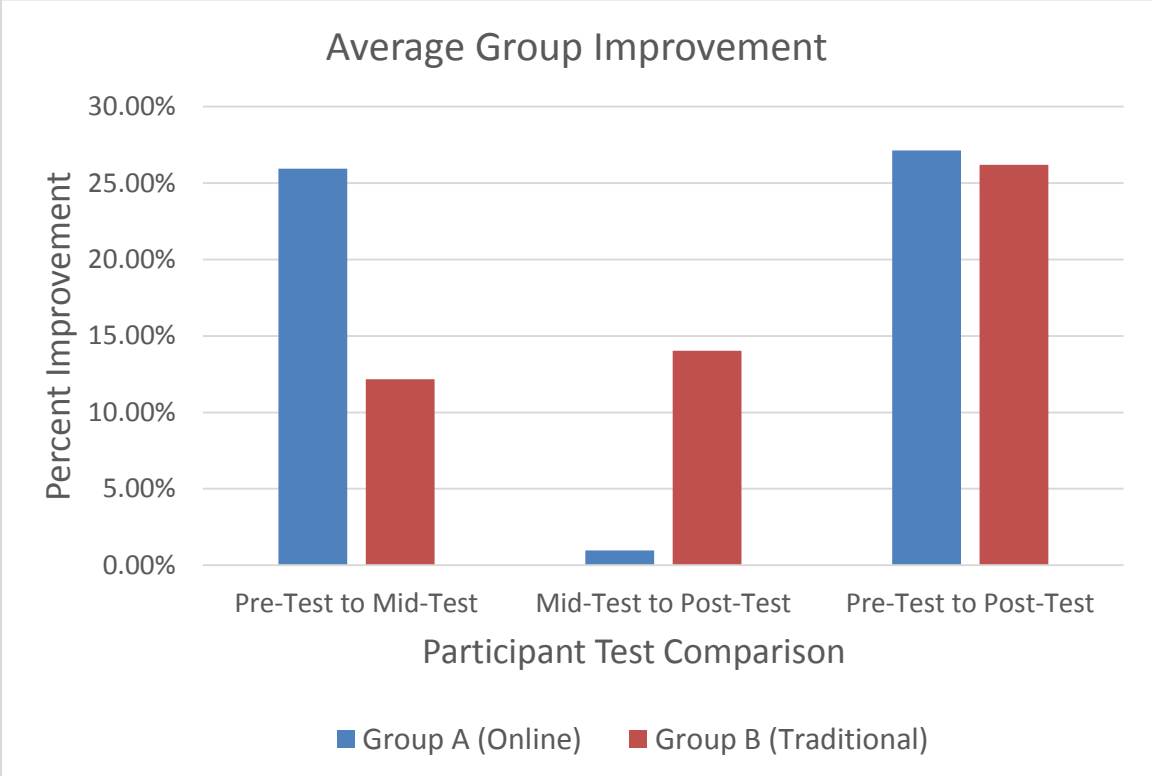


Table 10: Data Excluding Students with Prior DNA Extraction Experience – Data excluding students who have previously performed a DNA Extraction Lab in their school experience. There was no significant change in average test scores when excluding these 14 students, so all students were included in the final analysis.

	Group A		Group B	
	Raw Score	**Percent**	**Raw Score**	**Percent**
Pre-Test	5.500	36.67%	6.57	43.85%
Mid-Test	9.458	63.05%	8.36	55.79%
Post-Test	9.416	62.77%	11.00	73.33%

Appendix 2 – Individual Question Analysis Tables and Figures

Table 11a and Figure C: Participant Correct Answers for the Question "DNA can be isolated from which of the following?" – Group A had a significantly greater percent of participants choose the correct answer after the online lab than Group B had with the traditional lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	13	46.43%	11	37.93%
Mid-Test	27	96.43%	7	24.14%
Post-Test	26	92.86%	25	86.21%

Table 11b: Change in Number of Correct Answers for the Question "DNA can be isolated from which of the following?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	50.00%	-13.79%
Mid-Test to Post-Test	-3.57%	62.07%
Pre-Test to Post-Test	46.43%	48.28%

Figure C.

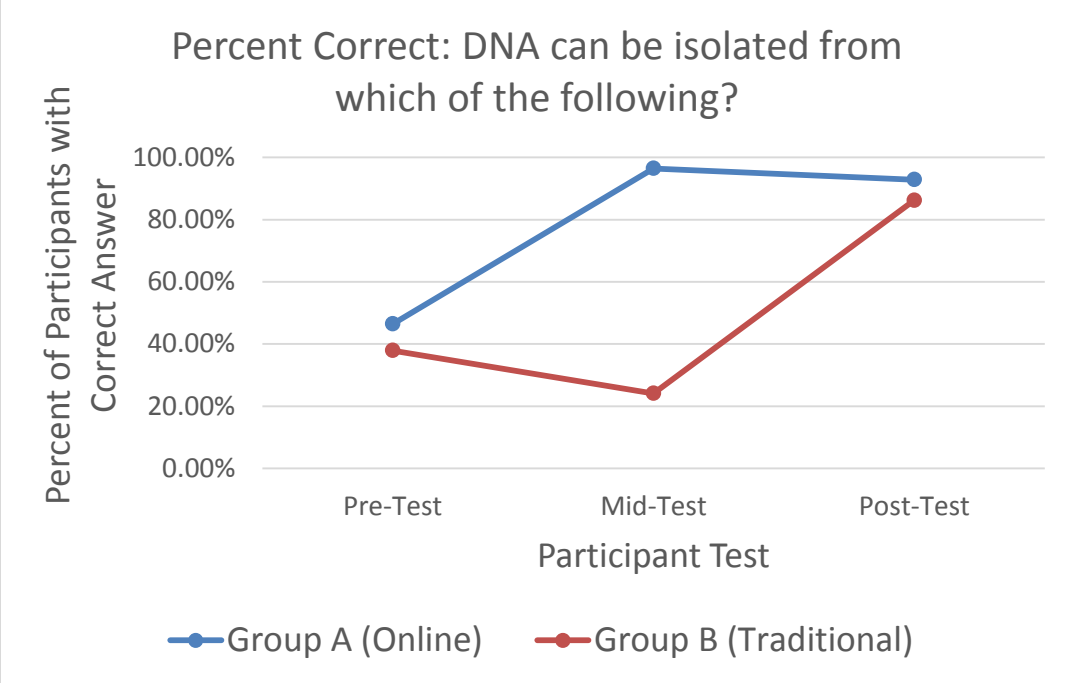


Table 12a and Figure D: Participant Correct Answers for the Question "Which of the following is the best appropriate sample type for a human DNA extraction?" – Group A had a significantly greater percent of participants choose the correct answer after the online lab than Group B had after the traditional lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	12	41.38%	6	20.69%
Mid-Test	19	65.52%	7	24.14%
Post-Test	19	65.52%	22	75.86%

Table 12b: Change in Percent of Correct Answers for the Question "Which of the following is the best appropriate sample type for a human DNA extraction?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	24.14%	3.45%
Mid-Test to Post-Test	0.00%	51.72%
Pre-Test to Post-Test	24.14%	55.17%

Figure D.

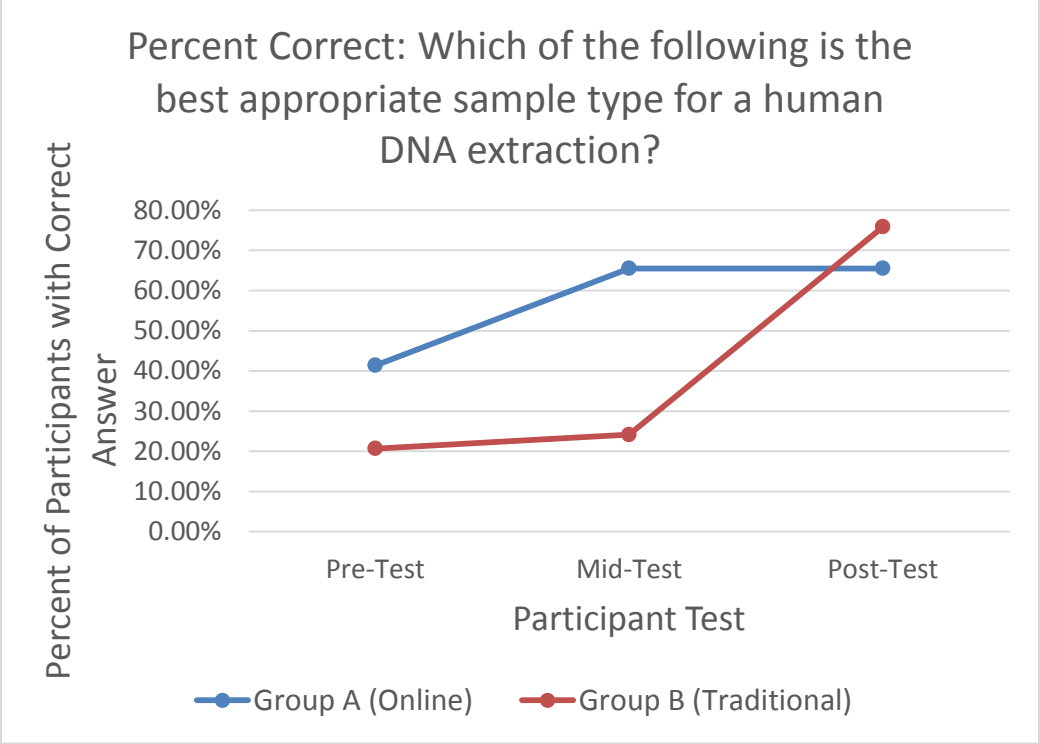


Table 13a and Figure E: Participant Correct Answers for the Question "Once extracted, how can DNA be stored for long-term later use?" – Group A had a significantly greater percent of participants choose the correct answer after the online lab than Group B had after the traditional lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	8	28.57%	15	51.72%
Mid-Test	21	75.00%	19	65.52%
Post-Test	18	64.29%	23	79.31%

Table 13b: Change in Percent of Correct Answers for the Question "Once extracted, how can DNA be stored for long-term later use?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	46.43%	13.79%
Mid-Test to Post-Test	-10.71%	13.79%
Pre-Test to Post-Test	35.71%	27.59%

Figure E.

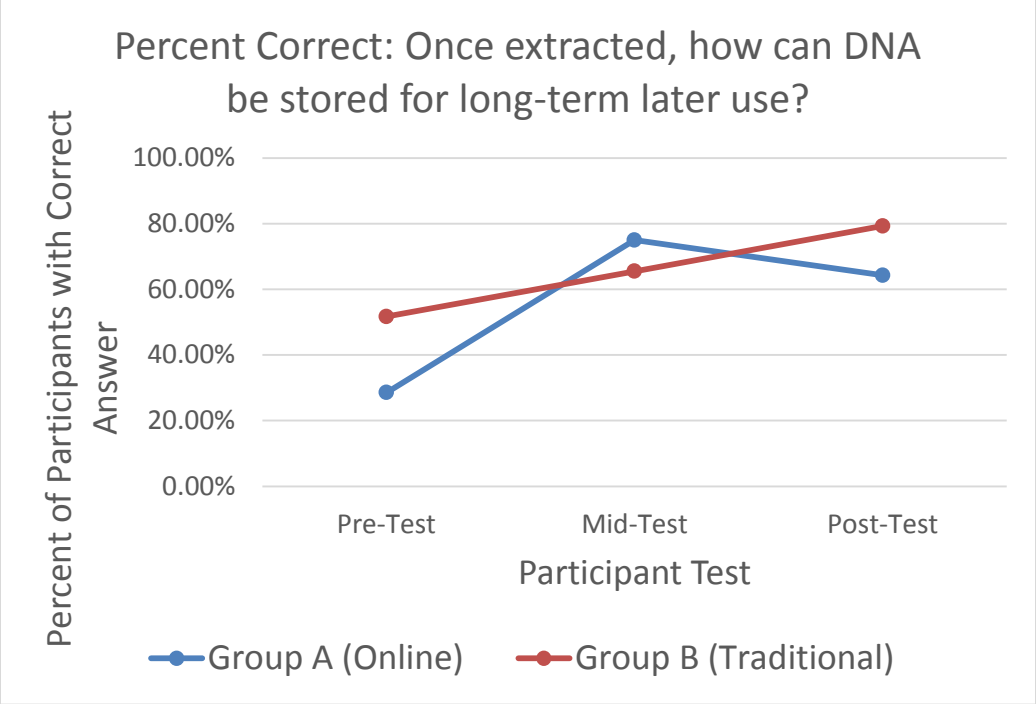


Table 14a and Figure F: Participant Correct Answers for the Question "What is the best temperature at which to precipitate DNA?" – Group A had a three-fold increase in correct answers between the pre-test and the mid-test when compared to Group B.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	2	7.14%	8	27.59%
Mid-Test	13	46.43%	12	41.38%
Post-Test	16	57.14%	13	44.83%

Table 14b: Change in Percent of Correct Answers for the Question "What is the best temperature at which to precipitate DNA?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	39.29%	13.79%
Mid-Test to Post-Test	10.71%	3.45%
Pre-Test to Post-Test	50.00%	17.24%

Figure F.

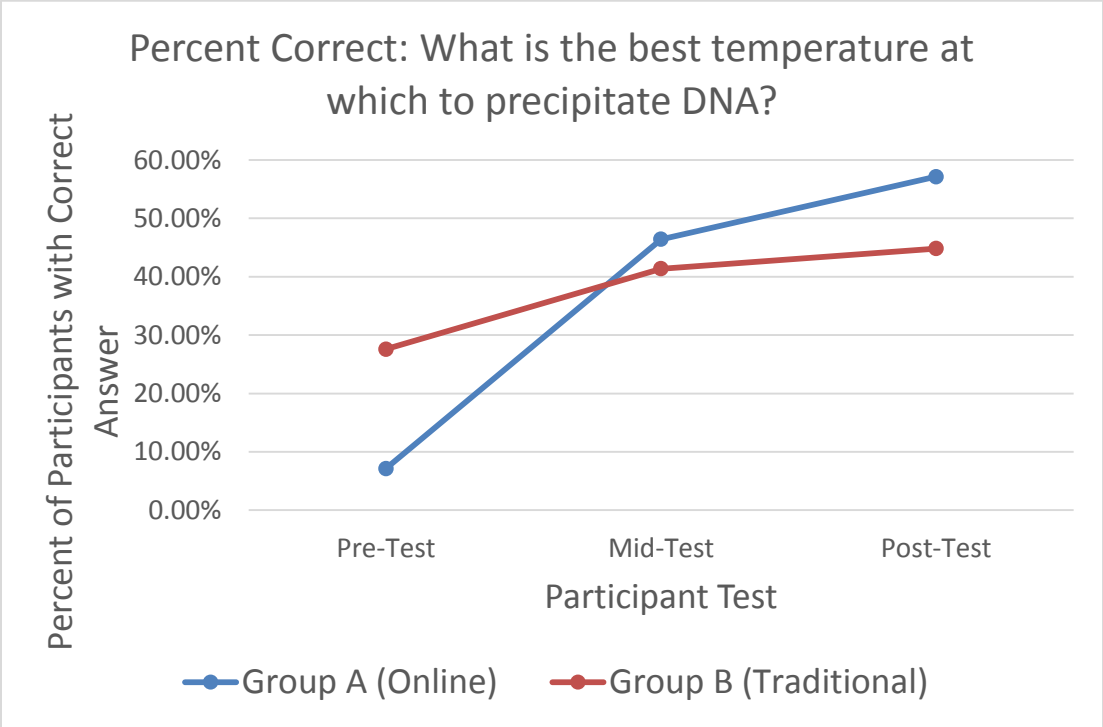


Table 15a and Figure G: Participant Correct Answers for the Question "What is the immediate danger to the DNA once it is released from the cell nucleus?" – Group A had a 10.71% increase in correct answers between the pre-test and mid-test, however Group B had a 13.79% decrease in correct answers. After the mid-test, both groups had a relatively equal increase in correct answers on the post-test.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	4	14.29%	8	27.59%
Mid-Test	7	25.00%	4	13.79%
Post-Test	13	46.43%	9	31.03%

Table 15b: Change in Percent of Correct Answers for the Question "What is the immediate danger to the DNA once it is released from the cell nucleus?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	10.71%	-13.79%
Mid-Test to Post-Test	21.43%	17.24%
Pre-Test to Post-Test	32.14%	3.45%

Figure G.

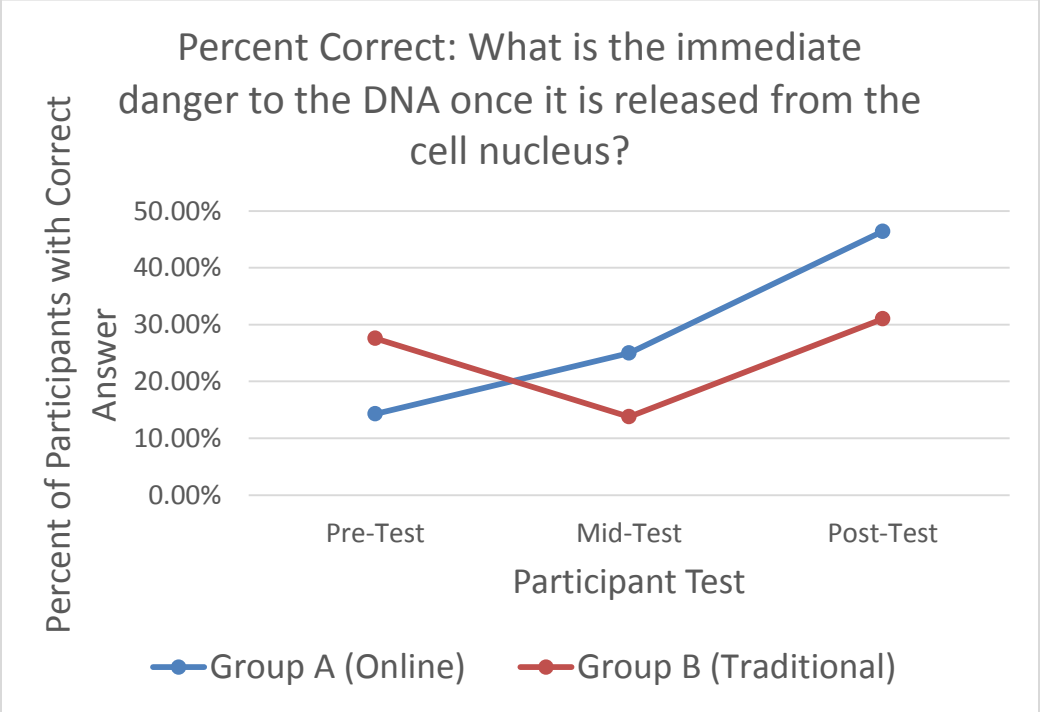


Table 16a and Figure H: Participant Correct Answers for the Question "How are the nucleic acids precipitated and separated from the rest of the cellular impurities?" – Both groups had an approximately equal increase in correct answers between the pre-test and mid-test. However, Group A had a decrease of 28.57% correct answers between the mid-test and the post-test, while Group B only had a decrease of 6.90% correct answers.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	7	25.00%	9	31.03%
Mid-Test	22	78.57%	25	86.21%
Post-Test	14	50.00%	23	79.31%

Table 16b: Change in Percent of Correct Answers for the Question "How are the nucleic acids precipitated and separated from the rest of the cellular impurities?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	53.57%	55.17%
Mid-Test to Post-Test	-28.57%	-6.90%
Pre-Test to Post-Test	25.00%	48.28%

Figure H.

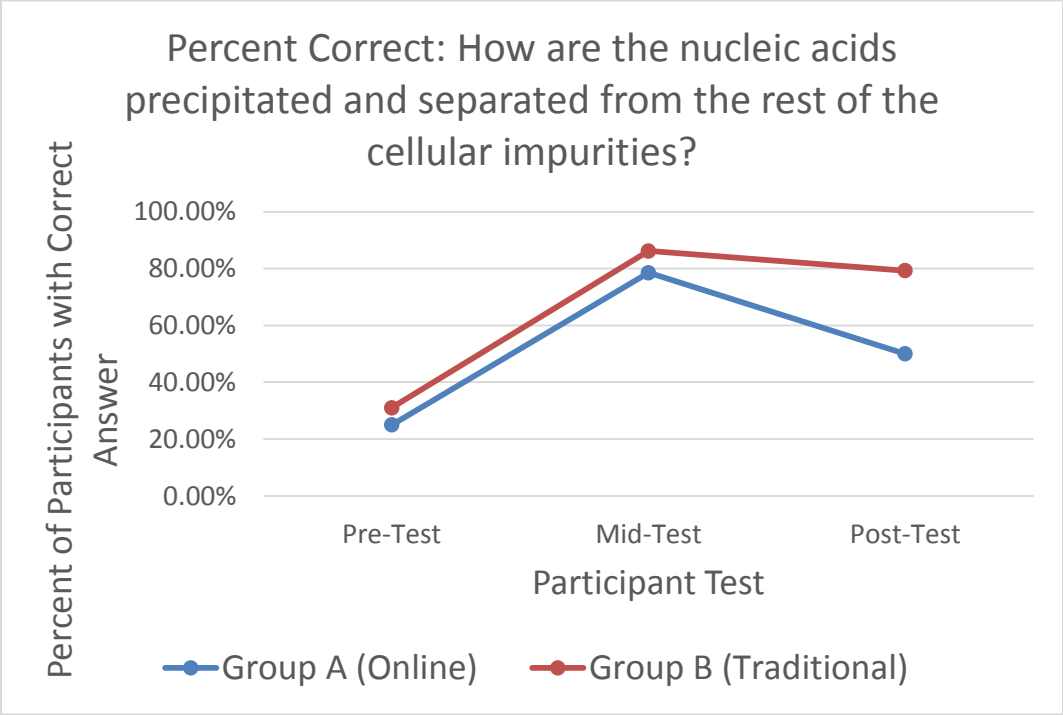


Table 17a and Figure I: Participant Correct Answers for the Question "How is DNA separated from the cell nucleus?" – Group B had a significantly greater percent of participants choose the correct answer after the traditional lab than Group A had with the online lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	6	21.43%	12	41.38%
Mid-Test	9	32.14%	21	72.41%
Post-Test	7	25.00%	21	72.41%

Table 17b: Change in Percent of Correct Answers for the Question "How is DNA separated from the cell nucleus?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	10.71%	31.03%
Mid-Test to Post-Test	-7.14%	0.00%
Pre-Test to Post-Test	3.57%	31.03%

Figure I.

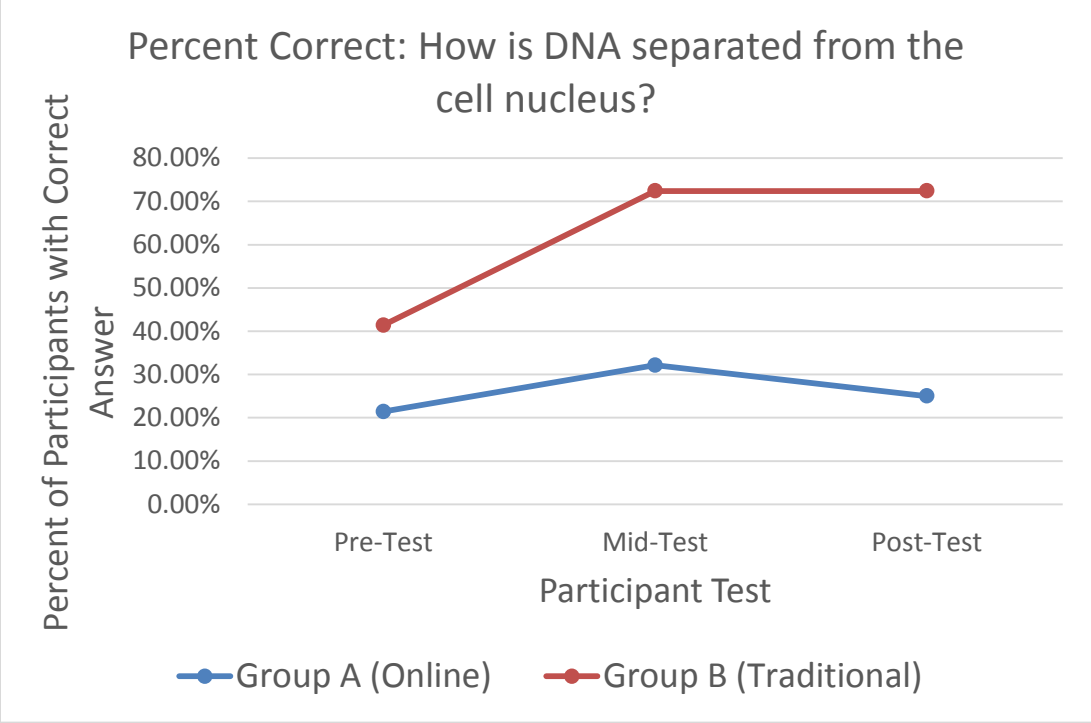


Table 18a and Figure J: Participant Correct Answers for the Question "What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?" – Group B had a significantly greater percent of participants choose the correct answer after the traditional lab than Group A had with the online lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	3	10.71%	8	27.59%
Mid-Test	11	39.29%	22	75.86%
Post-Test	13	46.43%	21	72.41%

Table 18b: Change in Percent of Correct Answers for the Question "What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	28.57%	48.28%
Mid-Test to Post-Test	7.14%	-3.45%
Pre-Test to Post-Test	35.71%	44.83%

Figure J.

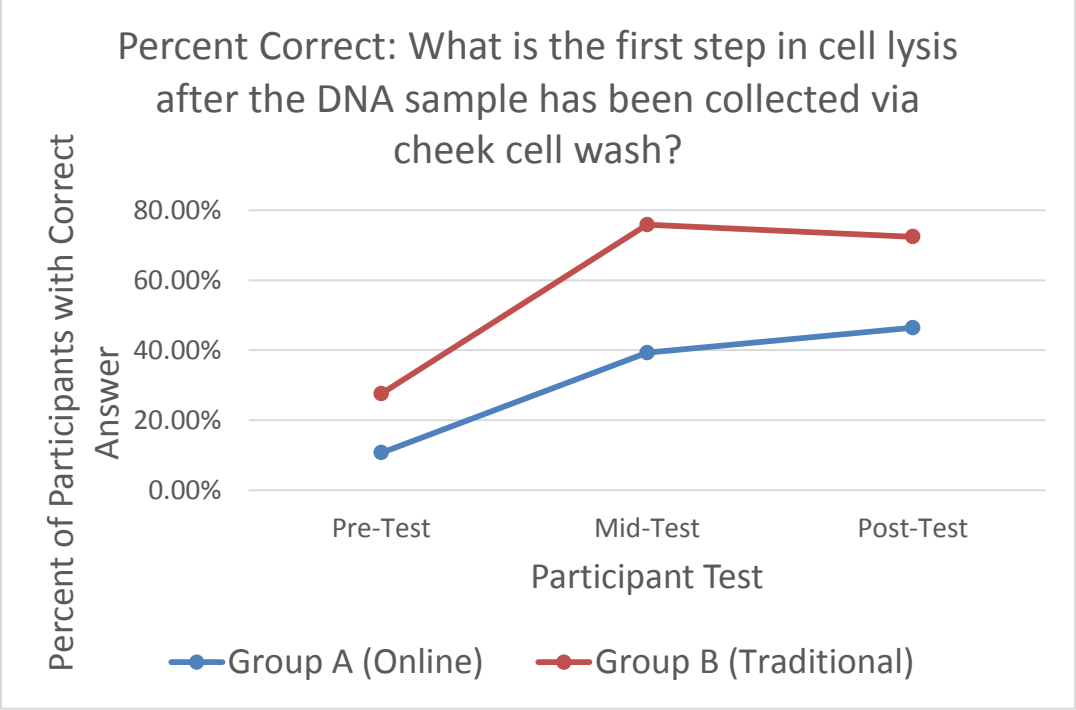


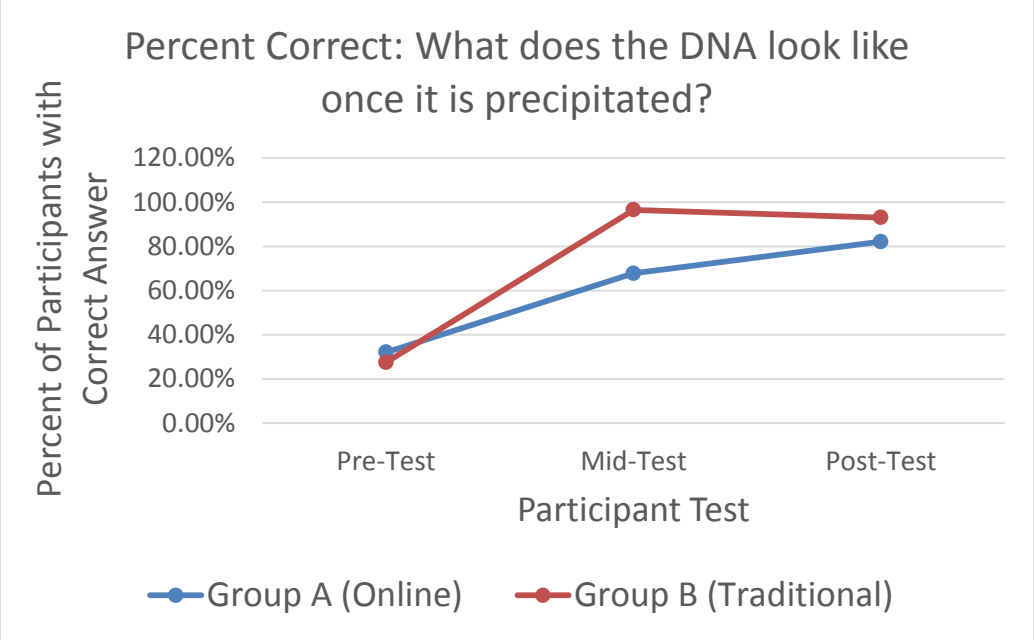
Table 19a and Figure K: Participant Correct Answers for the Question "What does DNA look like once it is precipitated?" – Group B had a significantly greater percent of participants choose the correct answer after the traditional lab than Group A had with the online lab.

	Group A		Group B	
	Number	Percent	Number	Percent
Pre-Test	9	32.14%	8	27.59%
Mid-Test	19	67.86%	28	96.55%
Post-Test	23	82.14%	27	93.10%

Table 19b: Change in Percent of Correct Answers for the Question "What does the DNA look like once it is precipitated?"

	Group A (Online)	Group B (Traditional)
Pre-Test to Mid-Test	35.71%	68.97%
Mid-Test to Post-Test	14.29%	-3.45%
Pre-Test to Post-Test	50.00%	65.52%

Figure K.



Appendix 3 – Survey and Pre-Test

Participation Survey and Pre-Test

If you are taking this survey and pre-test, you acknowledge that you have understood and signed the Student Assent Form provided to you, which states the purpose, risks, benefits, and confidentiality of this study.

The results of this survey and pre-test are anonymous and your responses will only be linked to your results in the study, not to your individual person. Please provide a single, honest answer to each question.

Part 1: Personal Information

Please choose a single answer.

1. What is your age?
    a. 14 years or younger
    b. 15 years
    c. 16 years
    d. 17 years
    e. 18 years or older
    f. Prefer not to answer
2. What is your grade level?
    a. Freshman
    b. Sophomore
    c. Junior
    d. Senior
    e. Prefer not to answer
3. What race or ethnicity do you consider yourself?
    a. White, Caucasian, or European
    b. Hispanic, Mexican-American, or Latino
    c. Asian or Pacific Islander
    d. Black or African American
    e. Other: (fill in) _____
    f. Prefer not to answer
4. I am interested in a career in:
    a. Medical Laboratory Science
    b. Other medical profession
    c. Other science laboratory profession
    d. Non-science related career
    e. Undecided
    f. Prefer not to answer

5. Have you ever performed a DNA Extraction laboratory activity before?
    a. Yes, within the last year
    b. Yes, over a year ago
    c. No
    d. Prefer not to answer

Part 2: Computer Use

Please answer the following questions about your personal computer use.

6. Do you own a home computer?
    a. Yes
    b. No (If no, please skip to question 10)
    c. Prefer not to answer
7. Is your home computer shared with one or more people, or are you the only user?
    a. My home computer is shared
    b. I am the only user
    c. Prefer not to answer
8. What type of computer do you own?
    a. Desktop computer
    b. Laptop computer
    c. iPad, Tablet or other handheld computer device
    d. Prefer not to answer
9. Is your computer connected to the internet?
    a. Yes
    b. No
    c. Prefer not to answer

Part 3: Familiarity with Computer Software

For the following questions, please indicate whether you: Strongly Agree (a); Somewhat Agree (b); Neither Agree nor Disagree (c); Somewhat Disagree (d); Strongly Disagree (e); or Prefer not to answer (f).

10. I am comfortable with general computer use, including accessing and using the internet, email, apps, or computer software programs.
    a. Strongly Agree
    b. Somewhat Agree
    c. Neither Agree nor Disagree
    d. Somewhat Disagree
    e. Strongly Disagree

f. Prefer not to answer

11. I am comfortable with the general use of Microsoft Word, including accessing, creating, saving, and retrieving Word documents.
    a. Strongly Agree
    b. Somewhat Agree
    c. Neither Agree nor Disagree
    d. Somewhat Disagree
    e. Strongly Disagree
    f. Prefer not to answer
12. I am comfortable with the general use of Microsoft Powerpoint, including accessing, creating, saving, and retrieving Powerpoint documents.
    a. Strongly Agree
    b. Somewhat Agree
    c. Neither Agree nor Disagree
    d. Somewhat Disagree
    e. Strongly Disagree
    f. Prefer not to answer
13. I am comfortable with the general use of Microsoft Excel, including accessing, creating, saving, and retrieving Excel documents.
    a. Strongly Agree
    b. Somewhat Agree
    c. Neither Agree nor Disagree
    d. Somewhat Disagree
    e. Strongly Disagree
    f. Prefer not to answer

Part 4: Pre-Test

1. What is the general purpose of a DNA (nucleic acid) extraction?
    a. To extract DNA for use in paternity testing
    b. To extract DNA for use in disease identification
    c. To extract DNA for use in forensic identification
    d. To extract DNA for use in genetic engineering
    e. All of the above are purposes of a DNA extraction
2. DNA can be isolated from which of the following?
    a. Mammals
    b. Plants
    c. Viruses
    d. Bacteria
    e. All of the above
    f. All of the above except viruses

3. Where in the human cell is DNA located?
    a. Cell centromere
    b. Cell nucleus
    c. Ribosomes
    d. Endoplasmic Reticulum
    e. Transcription factors
4. What is the actual end product of a nucleic acid extraction procedure?
    a. Isolated cell nucleus containing DNA
    b. Isolated RNA, used to transcribe DNA
    c. Isolated single-stranded DNA
    d. Isolated double-stranded DNA
    e. An isolated mixture of DNA and a little bit of RNA
5. Which of the following is the **best** appropriate sample type for a human DNA extraction?
    a. Hair sample without the root end attached
    b. Freshly collected urine sample
    c. Freshly collected blood sample in EDTA anti-coagulant
    d. Freshly collected serum from a blood sample that has been allowed to clot and centrifuged
    e. All of the above are appropriate sample types for a DNA extraction
6. What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?
    a. Put the cheek wash in an acid solution, such as 0.1M HCl, to break down the cell wall
    b. Put the cheek wash in a detergent solution to break apart the lipids of the cell membrane
    c. Put the cheek wash in an ice water solution to crystallize the lipids of the cell membrane to more easily break them apart
    d. Put the cheek wash in an alcohol solution, such as methanol, to dissolve the fatty acids in the cell membrane
    e. Put the cheek wash in sterile water and boil it for 10 minutes to break down the proteins in the cell membrane
7. How is the DNA separated from the cell nucleus?
    a. The same solution used to break down the cellular membrane is also used to break down the nuclear membrane
    b. An ice-cold solution of ethanol is used to precipitate the cell nucleus, which is then boiled to break down the nuclear membrane to access the DNA
    c. DNA is not separated from the cell nucleus, because the cell nucleus containing the DNA is the end product of a DNA extraction
    d. DNA is not separated from the cell nucleus, because the RNA is isolated instead and transcription factors are used to transcribe the DNA from the RNA
    e. DNA is not separated from the cell nucleus, because the isolated DNA-associated proteins are used to replicate DNA

8. What is the immediate danger to the DNA once it is released from the cell nucleus?
    a. Proteases located in the ribosomes can attack the proteins surrounding the DNA, rendering it useless for extraction
    b. Nucleases located in the cell nucleus can attack the nucleotides, rendering the DNA useless for extraction
    c. Nucleases located in the cytoplasm can attack and degrade the DNA molecules, rendering it useless for extraction
    d. RNases located in the endoplasmic reticulum can attack RNA molecules, rendering the RNA useless for extraction
    e. There is no immediate danger to the DNA once it is released from the cell nucleus
9. How are the nucleic acids precipitated and separated from the rest of the cellular impurities?
    a. DNase is added to the solution and acts as a magnet to attract and precipitate the nucleic acids. Salt is added to attract and separate the impurities.
    b. Protease is added to the solution and breaks down the cellular proteins, leaving the nucleic acids behind in an aqueous solution. This solution is then put in the freezer to precipitate DNA.
    c. The solution is poured through filter paper. The nucleic acids are small enough to leak through into the filtrate while the proteins and carbohydrates are too large to filter, and are thus separated. Salt is then added to precipitate the DNA from the filtrate.
    d. Ethanol and salt are added to the solution. While the ethanol attracts the impurities of the cell materials, such as proteins and carbohydrates, the salt precipitates and separates the DNA
    e. Ethanol and salt are added to the solution. While the salt attracts the impurities of the cell materials, such as proteins and carbohydrates, the ice-cold ethanol precipitates and separates the DNA
10. Sodium chloride is a salt commonly used in DNA extraction. Molecularly speaking, how does the addition of sodium chloride aid in separating the DNA from cellular impurities?
    a. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it less soluble in water
    b. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it less soluble in water
    c. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it more soluble in water
    d. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it more soluble in water
    e. It is not the sodium chloride that aids in separating the DNA from cellular impurities, but rather the addition of detergent
11. What is the best temperature at which to precipitate DNA, and why?

a. Reagents kept at body temperature (37˚C) are best used, because this is the temperature at which DNA is found in living things
b. Reagents kept at room temperature (20˚C to 25˚C) are best used, because this is the temperature at which the DNA will be analyzed after extraction and purification
c. Reagents kept in the refrigerator (2˚C to 8˚C) are best used, because the chemicals in the reagents would begin to degenerate at temperatures any greater than this
d. Ice-cold reagents (0˚C to -20˚C) are best used, because the extremely low temperature slows down enzymes that would otherwise further break down the DNA
e. Reagents can be kept at any temperature, as temperature does not affect the yield of DNA

12. What does the DNA look like once it is precipitated?
    a. White "fluff balls" that look like they are mixed evenly in the solution. This is removed from the solution via filter paper.
    b. A white oil-like layer that floats on top of the solution. This is removed from the solution with a glass stir rod.
    c. White, stringy, mucous-like material clumped together in the solution. This is removed from the solution with a glass stir rod.
    d. A white crystalline layer that sinks to the bottom of the solution. This is removed by decanting the top layer of the solution off, leaving the DNA behind in the test tube.
    e. DNA is not visual to the naked eye once precipitated. Further steps are necessary to view the DNA.
13. What extra steps can be taken to increase the yield of DNA?
    a. Increase the original concentration of DNA (i.e. use 5mL instead of 10mL of water when rinsing cheeks)
    b. Increase the incubation time with the solution used to break up the cell membrane
    c. Increase the incubation time with the solution used to precipitate the DNA
    d. Ensure that the reagent temperature is correct, and incubate the DNA and salt solution at this temperature
    e. All of the above are various way to increase the yield of DNA
14. How can you confirm that you have actually isolated nucleic acids, and not something else in the cell?
    a. If you have isolated anything other than nucleic acids, the final precipitation step will not work, leaving you with a clear solution. Hence, if you see your DNA, it worked!
    b. Add lipase to the precipitated DNA solution. If the solution turns clear, then you have isolated lipids as well as DNA, and will have to repeat the procedure.

c. Perform a Polymerase Chain Reaction (PCR) procedure to amplify a portion of the DNA, followed by a DNA electrophoresis procedure with staining to visualize the specific DNA sequence you amplified

d. Add protease to the precipitated DNA solution. If the solution turns cloudy, then you have isolated proteins as well as DNA, and will have to repeat the procedure.

e. There is no way to confirm that you have isolated only nucleic acids.

15. Once extracted, how can DNA be stored for long-term later use?

a. Precipitated DNA is stored in formaldehyde, and can be used indefinitely

b. Precipitated DNA is stored in ice-cold ethanol at -20˚C, and can be used for up to four years, depending on the purification of the DNA

c. Precipitated DNA is dissolved into sterile water and is stored at -20˚C for up to one year, depending on the purification of the DNA

d. Precipitated DNA is stored in Tris-EDTA buffer at room temperature, and can be used for up to six months, depending on the purification of the DNA

e. Precipitated DNA is dried in an oven on special filter paper, and that filter paper is stored at -20˚C for up to one year, depending on the purification of the DNA

Appendix 4 – Mid-Test

Mid-Test

1. Where in the human cell is DNA located?
    a. Ribosomes
    b. Cell centromere
    c. Transcription Factors
    d. Endoplasmic Reticulum
    e. Cell nucleus
2. What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?
    a. Put the cheek cell wash in sterile water and boil it for 10 minutes to break down the proteins in the cell membrane
    b. Put the cheek cell wash in an ice water solution to crystallize the lipids of the cell membrane to more easily break them apart
    c. Put the cheek cell wash in an acid solution, such as 0.1M HCl, to break down the cell wall
    d. Put the cheek cell wash in a detergent solution to break apart the lipids of the cell membrane
    e. Put the cheek cell wash in an alcohol solution, such as methanol, to dissolve the fatty acids in the cell membrane
3. What does the DNA look like once it is precipitated?
    a. White, stringy, mucous-like material clumped together in the solution. This is removed from the solution with a glass stir rod.
    b. DNA is not visual to the naked eye once precipitated. Further steps are necessary to view the DNA.
    c. White "fluff balls" that look like they are mixed evenly in the solution. This is removed from the solution via filter paper.
    d. A white crystalline layer that sinks to the bottom of the solution. This is removed by decanting the top layer of the solution off, leaving the DNA behind in the test tube.
    e. A white oil-like layer that floats on top of the solution. This is removed from the solution with a glass stir rod.
4. What is the actual end product of a nucleic acid extraction procedure?
    a. Isolated RNA, used to transcribe DNA
    b. An isolated mixture of DNA and a little bit of RNA
    c. Isolated double-stranded DNA
    d. Isolated cell nucleus containing DNA
    e. Isolated single-stranded DNA

5. How can you confirm that you have actually isolated nucleic acids, and not something else in the cell?
    a. There is no way to confirm that you have isolated only nucleic acids.
    b. Add protease to the precipitated DNA solution. If the solution turns cloudy, then you have isolated proteins as well as DNA, and will have to repeat the procedure.
    c. If you have isolated anything other than nucleic acids, the final precipitation step will not work, leaving you with a clear solution. Hence, if you see your DNA, it worked!
    d. Perform a Polymerase Chain Reaction (PCR) procedure to amplify a portion of the DNA, followed by a DNA electrophoresis procedure with staining to visualize the specific DNA sequence you amplified
    e. Add lipase to the precipitated DNA solution. If the solution turns clear, then you have isolated lipids as well as DNA, and will have to repeat the procedure.
6. How is the DNA separated from the cell nucleus?
    a. DNA is not separated from the cell nucleus, because the RNA is isolated instead and transcription factors are used to transcribe the DNA from the RNA
    b. The same solution used to break down the cellular membrane is also used to break down the nuclear membrane
    c. DNA is not separated from the cell nucleus, because the cell nucleus containing the DNA is the end product of a DNA extraction
    d. DNA is not separated from the cell nucleus, because the isolated DNA-associated proteins are used to replicate DNA
    e. An ice-cold solution of ethanol is used to precipitate the cell nucleus, which is then boiled to break down the nuclear membrane to access the DNA
7. What is the general purpose of a DNA (nucleic acid) extraction?
    a. To extract DNA for use in forensic identification
    b. To extract DNA for use in genetic engineering
    c. To extract DNA for use in disease identification
    d. To extract DNA for use in paternity testing
    e. All of the above are purposes of a DNA extraction
8. What is the best temperature at which to precipitate DNA, and why?
    a. Reagents can be kept at any temperature, as temperature does not affect the yield of DNA
    b. Reagents kept at room temperature (20˚C to 25˚C) are best used, because this is the temperature at which the DNA will be analyzed after extraction and purification
    c. Ice-cold reagents (0˚C to -20˚C) are best used, because the extremely low temperature slows down enzymes that would otherwise further break down the DNA

d. Reagents kept in the refrigerator (2˚C to 8˚C) are best used, because the chemicals in the reagents would begin to degenerate at temperatures any greater than this
e. Reagents kept at body temperature (37˚C) are best used, because this is the temperature at which DNA is found in living things

9. What is the immediate danger to the DNA once it is released from the cell nucleus?
    a. There is no immediate danger to the DNA once it is released from the cell nucleus
    b. Nucleases located in the cell nucleus can attack the nucleotides, rendering the DNA useless for extraction
    c. Proteases located in the ribosomes can attack the proteins surrounding the DNA, rendering it useless for extraction
    d. RNases located in the endoplasmic reticulum can attack RNA molecules, rendering the RNA useless for extraction
    e. Nucleases located in the cytoplasm can attack and degrade the DNA molecules, rendering it useless for extraction
10. Which of the following is the **best** appropriate sample type for a human DNA extraction?
    a. Freshly collected urine sample
    b. Freshly collected blood sample in EDTA anti-coagulant
    c. Freshly collected serum from a blood sample that has been allowed to clot and centrifuged
    d. Hair sample without the root end attached
    e. All of the above are appropriate sample types for a DNA extraction
11. Sodium chloride is a salt commonly used in DNA extraction. Molecularly speaking, how does the addition of sodium chloride aid in separating the DNA from cellular impurities?
    a. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it more soluble in water
    b. It is not the sodium chloride that aids in separating the DNA from cellular impurities, but rather the addition of detergent
    c. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it less soluble in water
    d. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it more soluble in water
    e. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it less soluble in water
12. Once extracted, how can DNA be stored for long-term later use?
    a. Precipitated DNA is stored in Tris-EDTA buffer at room temperature, and can be used for up to six months, depending on the purification of the DNA
    b. Precipitated DNA is dried in an oven on special filter paper, and that filter paper is stored at -20˚C for up to one year, depending on the purification of the DNA
    c. Precipitated DNA is dissolved into sterile water and is stored at -20˚C for up to one year, depending on the purification of the DNA

d. Precipitated DNA is stored in formaldehyde, and can be used indefinitely
e. Precipitated DNA is stored in ice-cold ethanol at -20˚C, and can be used for up to four years, depending on the purification of the DNA

13. DNA can be isolated from which of the following?
    a. Viruses
    b. Mammals
    c. Bacteria
    d. Plants
    e. All of the above
    f. All of the above except viruses
14. What extra steps can be taken to increase the yield of DNA?
    a. Ensure that the reagent temperature is correct, and incubate the DNA and salt solution at this temperature
    b. Increase the incubation time with the solution used to break up the cell membrane
    c. Increase the original concentration of DNA (i.e. use 5mL instead of 10mL of water when rinsing cheeks)
    d. Increase the incubation time with the solution used to precipitate the DNA
    e. All of the above are various way to increase the yield of DNA
15. How are the nucleic acids precipitated and separated from the rest of the cellular impurities?
    a. Protease is added to the solution and breaks down the cellular proteins, leaving the nucleic acids behind in an aqueous solution. This solution is then put in the freezer to precipitate DNA.
    b. Ethanol and salt are added to the solution. While the salt attracts the impurities of the cell materials, such as proteins and carbohydrates, the ice-cold ethanol precipitates and separates the DNA
    c. Ethanol and salt are added to the solution. While the ethanol attracts the impurities of the cell materials, such as proteins and carbohydrates, the salt precipitates and separates the DNA
    d. The solution is poured through filter paper. The nucleic acids are small enough to leak through into the filtrate while the proteins and carbohydrates are too large to filter, and are thus separated. Salt is then added to precipitate the DNA from the filtrate.
    e. DNase is added to the solution and acts as a magnet to attract and precipitate the nucleic acids. Salt is added to attract and separate the impurities.

Appendix 5 – Post-Test

Post-Test

1. Sodium chloride is a salt commonly used in DNA extraction. Molecularly speaking, how does the addition of sodium chloride aid in separating the DNA from cellular impurities?
    a. It is not the sodium chloride that aids in separating the DNA from cellular impurities, but rather the addition of detergent
    b. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it less soluble in water
    c. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it less soluble in water
    d. The positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA, making it more soluble in water
    e. The negatively charged chloride ions neutralize the positively charged phosphate group on the backbone of DNA, making it more soluble in water
2. What does the DNA look like once it is precipitated?
    f. A white oil-like layer that floats on top of the solution. This is removed from the solution with a glass stir rod.
    g. White "fluff balls" that look like they are mixed evenly in the solution. This is removed from the solution via filter paper.
    h. DNA is not visual to the naked eye once precipitated. Further steps are necessary to view the DNA.
    i. A white crystalline layer that sinks to the bottom of the solution. This is removed by decanting the top layer of the solution off, leaving the DNA behind in the test tube.
    j. White, stringy, mucous-like material clumped together in the solution. This is removed from the solution with a glass stir rod.
3. What is the best temperature at which to precipitate DNA, and why?
    a. Reagents can be kept at any temperature, as temperature does not affect the yield of DNA
    b. Ice-cold reagents (0˚C to -20˚C) are best used, because the extremely low temperature slows down enzymes that would otherwise further break down the DNA
    c. Reagents kept at body temperature (37˚C) are best used, because this is the temperature at which DNA is found in living things
    d. Reagents kept in the refrigerator (2˚C to 8˚C) are best used, because the chemicals in the reagents would begin to degenerate at temperatures any greater than this
    e. Reagents kept at room temperature (20˚C to 25˚C) are best used, because this is the temperature at which the DNA will be analyzed after extraction and purification

4. What is the general purpose of a DNA (nucleic acid) extraction?
    a. To extract DNA for use in disease identification
    b. To extract DNA for use in genetic engineering
    c. To extract DNA for use in forensic identification
    d. To extract DNA for use in paternity testing
    e. All of the above are purposes of a DNA extraction
5. How can you confirm that you have actually isolated nucleic acids, and not something else in the cell?
    f. Add protease to the precipitated DNA solution. If the solution turns cloudy, then you have isolated proteins as well as DNA, and will have to repeat the procedure.
    g. Perform a Polymerase Chain Reaction (PCR) procedure to amplify a portion of the DNA, followed by a DNA electrophoresis procedure with staining to visualize the specific DNA sequence you amplified
    h. There is no way to confirm that you have isolated only nucleic acids.
    i. If you have isolated anything other than nucleic acids, the final precipitation step will not work, leaving you with a clear solution. Hence, if you see your DNA, it worked!
    j. Add lipase to the precipitated DNA solution. If the solution turns clear, then you have isolated lipids as well as DNA, and will have to repeat the procedure.
6. Where in the human cell is DNA located?
    a. Endoplasmic Reticulum
    b. Transcription Factors
    c. Cell nucleus
    d. Ribosomes
    e. Cell centromere
7. What extra steps can be taken to increase the yield of DNA?
    a. Increase the incubation time with the solution used to precipitate the DNA
    b. Increase the incubation time with the solution used to break up the cell membrane
    c. Ensure that the reagent temperature is correct, and incubate the DNA and salt solution at this temperature
    d. Increase the original concentration of DNA (i.e. use 5mL instead of 10mL of water when rinsing cheeks)
    e. All of the above are various way to increase the yield of DNA
8. Which of the following is the **best** appropriate sample type for a human DNA extraction?
    a. Freshly collected serum from a blood sample that has been allowed to clot and centrifuged
    b. Hair sample without the root end attached
    c. Freshly collected urine sample
    d. Freshly collected blood sample in EDTA anti-coagulant
    e. All of the above are appropriate sample types for a DNA extraction

9. How are the nucleic acids precipitated and separated from the rest of the cellular impurities?
    a. The solution is poured through filter paper. The nucleic acids are small enough to leak through into the filtrate while the proteins and carbohydrates are too large to filter, and are thus separated. Salt is then added to precipitate the DNA from the filtrate.
    b. Ethanol and salt are added to the solution. While the ethanol attracts the impurities of the cell materials, such as proteins and carbohydrates, the salt precipitates and separates the DNA
    c. DNase is added to the solution and acts as a magnet to attract and precipitate the nucleic acids. Salt is added to attract and separate the impurities.
    d. Protease is added to the solution and breaks down the cellular proteins, leaving the nucleic acids behind in an aqueous solution. This solution is then put in the freezer to precipitate DNA.
    e. Ethanol and salt are added to the solution. While the salt attracts the impurities of the cell materials, such as proteins and carbohydrates, the ice-cold ethanol precipitates and separates the DNA
10. What is the actual end product of a nucleic acid extraction procedure?
    a. An isolated mixture of DNA and a little bit of RNA
    b. Isolated single-stranded DNA
    c. Isolated RNA, used to transcribe DNA
    d. Isolated double-stranded DNA
    e. Isolated cell nucleus containing DNA
11. What is the immediate danger to the DNA once it is released from the cell nucleus?
    a. RNases located in the endoplasmic reticulum can attack RNA molecules, rendering the RNA useless for extraction
    b. Nucleases located in the cytoplasm can attack and degrade the DNA molecules, rendering it useless for extraction
    c. Proteases located in the ribosomes can attack the proteins surrounding the DNA, rendering it useless for extraction
    d. There is no immediate danger to the DNA once it is released from the cell nucleus
    e. Nucleases located in the cell nucleus can attack the nucleotides, rendering the DNA useless for extraction
12. What is the first step in cell lysis after the DNA sample has been collected via cheek cell wash?
    a. Put the cheek cell wash in a detergent solution to break apart the lipids of the cell membrane
    b. Put the cheek cell wash in an alcohol solution, such as methanol, to dissolve the fatty acids in the cell membrane

c. Put the cheek cell wash in sterile water and boil it for 10 minutes to break down the proteins in the cell membrane
d. Put the cheek cell wash in an acid solution, such as 0.1M HCl, to break down the cell wall
e. Put the cheek cell wash in an ice water solution to crystallize the lipids of the cell membrane to more easily break them apart

13. Once extracted, how can DNA be stored for long-term later use?
    a. Precipitated DNA is dried in an oven on special filter paper, and that filter paper is stored at -20˚C for up to one year, depending on the purification of the DNA
    b. Precipitated DNA is stored in Tris-EDTA buffer at room temperature, and can be used for up to six months, depending on the purification of the DNA
    c. Precipitated DNA is stored in ice-cold ethanol at -20˚C, and can be used for up to four years, depending on the purification of the DNA
    d. Precipitated DNA is dissolved into sterile water and is stored at -20˚C for up to one year, depending on the purification of the DNA
    e. Precipitated DNA is stored in formaldehyde, and can be used indefinitely
14. DNA can be isolated from which of the following?
    a. Bacteria
    b. Mammals
    c. Plants
    d. Viruses
    e. All of the above
    f. All of the above except viruses
15. How is the DNA separated from the cell nucleus?
    a. DNA is not separated from the cell nucleus, because the RNA is isolated instead and transcription factors are used to transcribe the DNA from the RNA
    b. The same solution used to break down the cellular membrane is also used to break down the nuclear membrane
    c. DNA is not separated from the cell nucleus, because the cell nucleus containing the DNA is the end product of a DNA extraction
    d. DNA is not separated from the cell nucleus, because the isolated DNA-associated proteins are used to replicate DNA
    e. An ice-cold solution of ethanol is used to precipitate the cell nucleus, which is then boiled to break down the nuclear membrane to access the DNA

Appendix 6 – Traditional Lab Activity Protocol

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Catalog No. FB2065
Publication No. 11171

DNA Isolation Kit

Super Value Laboratory Kit

Introduction

Learn how to isolate DNA from human cells. Yours!

Concepts

- DNA spooling/isolation
- Cell lysis
- Solubility

Background

Less than fifty years ago the nature of the genetic code still eluded scientists. Since then, the structure of DNA was first unraveled, it has become the most significant biological topic of the century. Understanding the structure of DNA helps to explain many life processes and leads to greater knowledge of why we are the way we are.

The process of DNA extraction is of primary importance in many fields of biotechnology. It is critical for genetic research, DNA fingerprinting, and creating recombinant organisms to produce beneficial products in the field of medicine.

The process of DNA extraction, regardless of the tissue used, usually involves the same key steps:

1. A detergent/salt solution is used to break down and emulsify the fat and proteins that make up the cell membrane. The salt causes the phosphate ends of the DNA to come closer together making it easier to precipitate the DNA out of solution.
2. Shaking or blending then breaks down the cell and nuclear membranes, releasing the DNA.
3. The solution is treated (with heat or chemically) to break down any DNase enzymes present, which can digest the long DNA strands into smaller pieces making spooling more difficult.
4. DNA is soluble in water and insoluble in ethanol. The addition of ethanol causes the DNA to precipitate and come out of solution. The DNA precipitates at the water/alcohol interface allowing it to be "spooled" onto a spooling device.

Materials

Ethyl alcohol, 95% denatured, 6 mL, ice cold	Dropping bottles, 3
Ethylenediaminetetraacetic acid solution (EDTA), 0.1 M, 20 drops	Stirring rod, glass
Sodium chloride solution, 8%, NaCl, 20 drops	Stopper, #2
Sodium dodecyl sulfate solution, (SDS), 10%, 20 drops	Test tube rack
Water, tap, 10 mL	Test tube, 16 × 100 mm
Drinking cup, plastic, 30-mL	Test tube, 12 × 75 mm

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk; keep from flame and sources of ignition. Use only clean drinking cups for this procedure. Never ingest anything from a container that has previously been in the lab. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron.

Procedure

1. Add 1 mL (20 drops) of the 8% sodium chloride solution to the larger test tube. Set the tube aside in a test tube rack.
2. Pour 10 mL of fresh tap water or bottled water into a clean 30-mL plastic drinking cup.

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3. Put the 10 mL of water in your mouth and "swish" the water around between your cheek and gums for at least 30 seconds. Spit the water back into the plastic cup. (The swishing of the water washes cells from inside your cheeks into the water.)
4. Pour several mL of the "cheek cell" water into the test tube containing the salt solution from Step 1.
5. Add 1 mL (20 drops) of the 10% SDS solution and 1 mL (20 drops) of the 0.1 M EDTA solution to the "cheek" mixture in the test tube.
6. Stopper the test tube and mix the contents of the tube by gently inverting the test tube several times. **Do not shake the test tube.** (The SDS breaks down the cell membrane from the cheek cells, releasing the DNA into the salt solution. The EDTA solution inactivates the DNA digesting enzymes.)
7. Holding the test tube at a slight angle, carefully add 5 mL of 95% ethyl alcohol down the side of the test tube so that it forms a layer over the "cheek" mixture in the test tube (see Figure 1). Do not mix the water and ethyl alcohol layers.
8. Hold the test tube upright for one minute and observe what happens at the interface between the ethyl alcohol and the "cheek" solution. (The clouds of white strands are the DNA. The DNA is not soluble in ethyl alcohol, so it precipitates where the two liquids meet. Soap bubbles from the "cheek" solution may get trapped in the DNA strands.)

Figure 1.

9. Add about 1 mL (20 drops) of 95% ethyl alcohol to the smaller, empty test tube.
10. Place a clean glass stirring rod in the test tube containing the DNA. Collect the DNA by turning the rod in one direction and thus winding the DNA strands around the rod.
11. Carefully remove the rod and DNA from the solution and transfer it to the smaller test tube containing 1 mL of 95% ethyl alcohol. Observe the DNA floating in the alcohol.

Disposal

Consult your instructor for appropriate disposal procedures.

Teacher's Notes

DNA Isolation Super Value Laboratory Kit

Materials Included in Kit

Ethyl alcohol, 95% denatured, CH_3CH_2OH, 1-L

Ethylenediaminetetraacetic acid (EDTA), 0.1 M, 150 mL

Sodium chloride solution, 8%, 150 mL

Sodium dodecyl sulfate solution, (SDS), 10%, 150 mL

Drinking cups, plastic, 30-mL, 150

Stirring rods, glass, 30

Additional Materials Needed (for each lab group)

Water, distilled

Water, tap, 10 mL

Dropping bottles, 3

Ice bath (shared)

Stopper, #00

Test tube, 12 × 75 mm

Test tube, 16 × 100 mm

Test tube rack

Preparation

Place ethyl alcohol into an ice bath.

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk; keep from flame and all sources of ignition. Ethyl alcohol is denatured and is toxic by ingestion. Provide students new clean drinking cups. Never allow students to drink or eat from an apparatus in the lab. Wear chemical splash goggles. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The resulting mixtures can be flushed down the drain according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation

Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems, diversity and adaptations of organisms

Content Standards: Grades 9–12
Content Standard C: Life Science, matter, energy, and organization in living systems

Lab Hints

- Enough materials are provided in this Super Value Kit for 5 classes of 30 students each (150 total students) all spooling their own DNA. The laboratory can be completed in one laboratory period.
- If your tap water has any unusual properties, use store-bought bottled water for this laboratory.

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Teacher's Notes *continued*

- If the DNA yield is not sufficient for spooling, try the following:

  1—Rinse your mouth more vigorously and for a longer period of time.

  2—The action of the detergent in step 5 can be enhanced by placing the test tube in a water bath at 55 °C. This enhances the action of the detergent and also helps the EDTA denature the enzymes that might digest the DNA.

  3—The alcohol used in Step 7 might be more effective if it is made ice-cold in an ice bath.

- The collection of cheek cells from inside the mouth highlights the nature of body tissue. Dead cells are continually being sloughed off on both the inside and outside of the body. Recently-sloughed cells still contain their nucleus and their DNA genetic material. This DNA can be collected and, if in a forensics situation, analyzed and traced to a specific individual. Many tissues are potentially good sources for crude DNA extraction following the same basic steps utilized in this lab. Many variations of the same basic procedure have been developed and proposed. All produce varying results. The idea of extracting one's own DNA has a certain appeal to students. If you choose to experiment with other tissues or not to do human DNA at all, other common tissues can be used following the same basic procedure.

- Banana, Kiwi fruit, onion, liver and beef thymus are all excellent tissue sources for DNA. The procedure is usually varied during steps 2–4 in this lab. The tissues are not "washed" like the cheek cells in this lab. Instead, the tissues are usually ground with a mortar and pestle or in a high speed blender. After the tissue has been macerated, it is treated with the solutions used in Steps 1 and 5. The slurry of tissue is then centrifuged or filtered to "clean out" the cell debris. The supernatant solutions are then treated with alcohol, etc. as in Step 7. The hot and cold treatments of the solutions are usually more critical with these other tissues.

The *DNA Isolation Kit—Super Value Laboratory Kit* is available from Flinn Scientific, Inc.

Catalog No.	Description
FB2065	DNA Isolation Kit—Super Value Laboratory Kit

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

DNA Extraction Lab Activity

ONLINE VERSION

BY: LIZ BRANDON

What is DNA?

Deoxyribonucleic Acid (DNA) is a macromolecule of carbon, oxygen, phosphorous, and hydrogen atoms

Comprised of nucleotides containing a phosphorylated ribose sugar and a nitrogen base

- Adenine, cytosine, guanine, thymine
- Phosphate group provides an overall negative charge

Double helix structure of DNA results from the physiochemical affects of the specific sequence of nucleotides in the strand

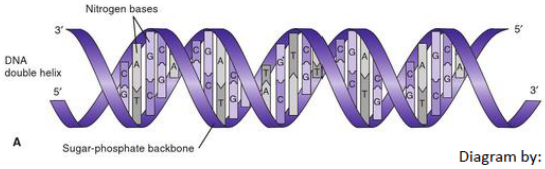


Diagram by: Buckingham (2012)

Where is DNA found?

DNA may be isolated from:

- Animals, plants, fungi, bacteria, parasites, viruses

DNA is found in the nucleus of eukaryotic (animal, plant, fungal, parasitic) cells and in the nucleus of prokaryotic (bacterial) cells

DNA is found in the nucleocapsid of some viral cells

- Other viruses are RNA-based only

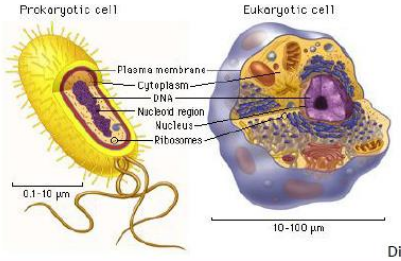


Diagram by: Pearson Education

Purpose of DNA Extraction

To extract deoxyribonucleic acid (DNA) from cells for further testing

Further testing may include genetic research, genotyping, disease identification, paternity testing, and forensic identification

Extracted DNA can be used for PCR, sequence analysis, or other laboratory tests, however we will not do other testing in this lab activity

Sample Type and Storage

Different DNA Isolation kits are specific for different sample types

In human cells, DNA can be isolated from:

- Squamous epithelial cells (skin, cheek swab, saliva)
- Hair sample with the root end (root end contains epithelial cells, while hair shaft contains degraded DNA)
- EDTA Whole Blood
  - Fresh
  - Stored at 4 deg C
  - Stored at -70 deg C

The best sample is EDTA whole blood, because it has the most stable storage capacity and contains the most molecules of DNA per microliter of sample

In this lab, we will use a simple mouth wash containing buccal (cheek) cells and extract our DNA from squamous epithelial cells

Supplies and Reagents

SUPPLIES	REAGENTS
Clean plastic cup	95% Ethyl Alcohol, ice cold
Distilled water	EDTA solution
Disposable pipettes	8% Sodium Chloride
Glass stirring rod	10% Sodium Dodecyl Sulfate (SDS)
Large glass test tube	
Small glass test tube	
Test tube rack	

Safety

Personal Protective Equipment is Required

- Gloves and goggles
- Tie back long hair

Safety items located around the lab

- Emergency Eye Wash Station
- Sharps container with Biohazard label for disposal of glass test tubes
- Reagent Safety Data Sheets provided at instructor's table
- **No open flames allowed in the laboratory when working with alcohol**

Keep your DNA collection cup and samples away from others

- Properly label your specimen

Preparation

In any lab, sample identification is extremely important.

- Label your DNA collection cup, small test tube, and large test tube with your name, the date, and the time of collection

Ensure your reagents are intact and stored at the right temperature

- 95% Ethyl Alcohol is ice cold

Read through the entire protocol before beginning

- Do you have the necessary supplies and reagents?
- Pay attention to steps stating "**Do not shake, mix gently**" or "**Do not mix**"
- Pay attention to incubation temperatures and times
  - None in this lab, but incubation steps at various temperatures are common in other methods

Part 1: DNA Collection

Proper PPE is required

Do not eat or drink 15 minutes before sample collection.

Confirm the subject's mouth is empty of any contaminating substances.

Perform DNA Collection:

- Pour 5-10mL of water into a clean plastic drinking cup.
- Pour the 5-10mL of water into your mouth and "swish" the water around between your cheeks and gums for 60 seconds.
- You may "scrape" the inside of your cheeks with your teeth to help slough off cheek cells to increase DNA yield.
- Spit all of the water back into the plastic cup.

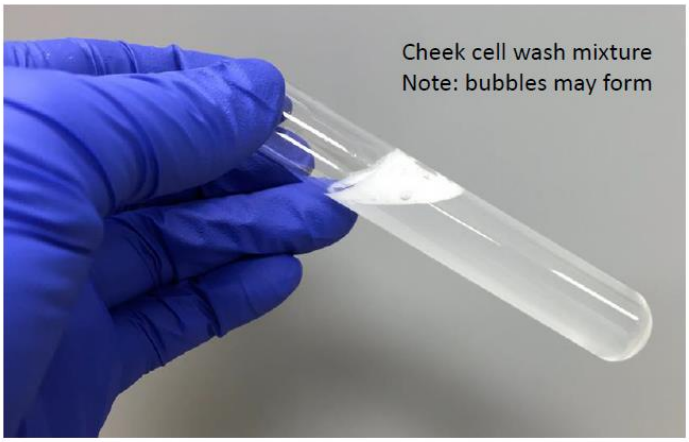
Your plastic cup now contains cells from your cheeks, called buccal cells. These cells still contain an intact nucleus, where the DNA is found.

Parts 2 and 3: Cell Lysis and Acidification

These steps are done in a quick succession to preserve integrity of DNA.

Prepare your DNA sample for extraction. Lyse your buccal cells and acidify your DNA.

- Add 20 drops of 8% sodium chloride solution to the large test tube.
- Pour several mL of the DNA sample (cheek cell water) into the large test tube containing the salt solution.
- Add 20 drops of the SDS solution and 20 drops of the EDTA solution to the cheek mixture in the test tube.
- Stopper the test tube and mix by inverting gently.
- **Do not shake the test tube.** Excess shaking can break the long strands of DNA, making it harder to see and collect when precipitated.



Why is salt so important?

Sodium chloride neutralizes the charge of the DNA sugar phosphate backbone, making it less hydrophilic in water.

- Positively charged sodium ions neutralize the negatively charged phosphate group on the backbone of DNA

Essentially, this causes the phosphate ends of the DNA to come closer together, making it easier to precipitate the DNA in the ethyl alcohol solution.

The salt also attracts impurities, such as proteins and carbohydrates, to render the DNA more pure.

What is happening to the cells and DNA in the test tube?

SDS is a detergent that breaks down the lipids and proteins that make up the cell membrane, thus releasing the DNA into the salt solution.

- Soap and other detergents act in the same way by creating rifts between the lipid bilayer of the cell membrane, causing it to rupture

EDTA destroys any nuclease enzymes present in the cells. Once the DNA is released from cells, it is free to come in contact with any cellular materials, including nucleases. These enzymes, normally present in the cellular cytoplasm, can digest the DNA and break it down into smaller segments, making extraction more difficult. Thus we destroy these enzymes so we can easily spool our long DNA strands.

- Using ice-cold reagents also helps slow down the enzymes

Part 4: Precipitation

Precipitate your DNA out of the cheek cell mixture with the alcohol.

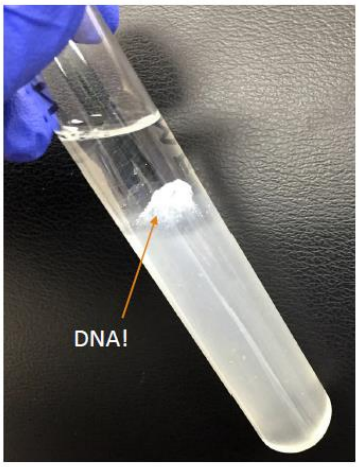
- Holding the test tube at a slight angle, **carefully** add 5mL of the ice-cold 95% ethyl alcohol down the side of the test tube so it forms a layer over the cheek cell mixture. **Do not mix the water and ethanol layers.**
- Hold the test tube upright for 60 seconds and observe what happens at the interface between the ethyl alcohol and the cheek cell solution.
- The clouds of white strands are the DNA. While the DNA is soluble in water, it is not soluble in the ethyl alcohol solution and thus precipitates where the two liquids meet.

Observe the DNA form at the interface between the two liquids.

Part 5: Extraction

Remove your DNA strands from the cheek cell solution and ethyl alcohol mixture and isolate it from the other cellular materials.

- Add 20 drops of ice-cold 95% ethyl alcohol to the smaller, empty test tube.
- Place a clean glass stirring rod in the large test tube containing the DNA. Collect the DNA by turning the rod in one direction and thus winding the DNA strands around the rod.
- Carefully remove the rod and DNA from the solution and transfer it to the smaller test tube containing the 95% ethyl alcohol. Observe the DNA floating in the alcohol.



Part 6: DNA Storage and Further Testing

Expected yield from a buccal cell wash on average is 1-10ug DNA from an adult

- DNA yield contains a small bit of RNA

Yield can be increased by increasing the original sample concentration, increase incubation times, and evaluate the optimal temperature at each step.

DNA sample is now ready for use in further testing such as amplification, sequencing, PCR, and other laboratory methods.

- PCR followed by gel electrophoresis is a useful confirmatory method to ensure you have extracted DNA, as you can visualize the DNA

Extracted DNA sample may be stored at 4 deg C for short term storage (6 months) or -20 deg C for long term storage (4 years)

References

Buckingham, L. (2012). DNA, DNA extraction methods. In *Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications* (2nd ed., pp. 2-5, 70-77). Philadelphia, PA: F.A. Davis Company.

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Pearson Education. (n.d.). The Biology Place. In *Pearson Education*. Retrieved September 13 2016, from http://www.phschool.com/science/biology_place/biocoach/cells/common.html