

Microbes in Mascara: Hypothesis-Driven Research in a Nonmajor Biology Lab †

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In this laboratory exercise, students were taught concepts of microbiology and scientific process through an everyday activity — cosmetic use. The students' goals for the lab were to develop a hypothesis regarding microbial contamination in cosmetics, learn techniques to culture and differentiate microorganisms from cosmetics, and propose best practices in cosmetics use based on their findings. Prior to the lab, students took a pretest to assess their knowledge of scientific hypotheses, microbiology, and cosmetic safety. In the first week, students were introduced to microbiological concepts and methodologies, and cosmetic terminology and safety. Students completed a hypothesis-writing exercise before formulating and testing their own hypotheses regarding cosmetic contamination. Students provided a cosmetic of their own and, in consultation with their lab group, chose one product for testing. Samples were serially diluted and plated on a variety of selective media. In the second week, students analyzed their plates to determine the presence and diversity of microbes and if their hypotheses were supported. Students completed a worksheet of their results and were given a posttest to assess their knowledge. Average test scores improved from 5.2 (pretest) to 7.8 (posttest), with p -values < 0.0001. Seventy-nine percent (79%) of students correctly identified hypotheses that were not falsifiable or lacked variables, and 89% of students improved their scores on questions concerning safe cosmetic use. Ninety-one percent (91%) of students demonstrated increased knowledge of microbial concepts and methods. Based on our results, this lab is an easy, yet effective, way to enhance knowledge of scientific concepts for nonmajors, while maintaining relevance to everyday life.

INTRODUCTION

Undergraduate schools across the country offer introductory biology courses for nonmajors to gain understanding of scientific concepts and experimental design, and to generate basic scientific literacy. Many students enter these courses with the attitude that science is inherently difficult and doesn't apply to them; thus, it is challenging to find labs that engage students' interest. Studies have demonstrated that students retain information better when they find the course material to be personally relevant (7). As such, microbiology has become a useful tool for getting students to recognize the impact and importance of science in their daily lives (9, 13). Some examples of microbiology applications that have been explored in introductory biology courses include testing contamination of stream water, the antimicrobial properties of salsa, and culturing of swabs from skin surfaces to test for antibiotic resistance (8, 12, 15).

The Biology of Women course at Hamline University focuses on biology through the female perspective. Major

topics include the biology of sex, the reproductive cycle, the body in health and disease, and nutrition and body image. The course is intended for nonmajors to learn about the natural sciences, practice methods of scientific inquiry and analysis, assess the strengths and limitations of scientific approaches, and understand the political framework within which science is done. In Biology of Women, students learn about the role of microorganisms in each course unit. Microorganisms are introduced as an important source of human disease, and the natural flora of the body is discussed in respect to the reproductive system, the digestive system, pregnancy, and as a source of body odor. Laboratory exercises are designed to integrate the course content with lecture material and students' daily lives. Recognizing that microbiology links all the learning units in the course, we chose to focus on teaching concepts of microbiology and scientific methodology through a medium to which all students had access — their cosmetic products.

Recently, the Safe Cosmetics Act of 2011 (HR 2359) was introduced to the United States Congress to improve the safety of chemicals in cosmetics. Concerns about the risks of cosmetic use have led to investigations into the safety of natural and organic ingredients in products, allergies from antimicrobial preservatives in cosmetics, and exploration of toxic chemicals in personal care products (2, 11, 16). Additionally, it has been shown that both manufacturers and consumers may introduce bacterial contaminants into

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cosmetic products, increasing the likelihood of harm to the consumer (4, 5). In light of the debate regarding cosmetic safety and the fact that most people use cosmetic products on a daily basis, we have designed this inquiry-based laboratory exercise, which we call the Savvy Consumers Lab, to allow students to test their own cosmetic samples for microbial contamination. Studies show that teaching inquiry-based approaches to science that allow students to devise and test their own hypotheses improve student performance (1, 6). This lab is intended for students to design a hypothesis-driven experiment, learn a “real-world” application of biology, and be better prepared to make informed consumer choices.

Intended audience

This laboratory exercise was designed to complement Hamline University’s Biology of Women class, but can be modified for introductory biology or microbiology labs. This course regularly enrolls nonmajors who are juniors and seniors with little previous laboratory experience. The class size is typically 20 students, primarily female.

Learning time

Biology of Women meets for three 60-minute lecture sessions and one 2-hour laboratory session per week. This laboratory exercise is performed over a two-week period during lab time, but two 10-minute portions of lecture time are used for a pre/posttest (Appendix 1). The first week involves a background lecture, and experimental design and setup. During the second week, students analyze their plates, record data, and complete a worksheet.

Prerequisite student knowledge

Students in this course have limited familiarity with scientific concepts or techniques. In early course lectures, students learn about eukaryotic and prokaryotic cells, and are introduced to basic laboratory safety. In the first week of the Savvy Consumers Lab, students are given an introductory lecture on microbes, types and use of microbial media, culturing, and aseptic techniques. There is a discussion of serial dilutions to help students understand why it is necessary to their experiment. Cosmetic terminology and safety are also reviewed. If this is the first time students have encountered hypothesis design, an introductory session is necessary to discuss the concept.

Learning objectives

This laboratory exercise is intended to teach students hypothesis writing and experimental design using a practical application of microbiology, and to help them become informed consumers. Upon completion of the laboratory exercise, students should be able to:

1. Recognize strong hypotheses as testable statements based on prior knowledge that contain measurable variables for comparison
2. Formulate a testable hypothesis
3. Demonstrate knowledge of basic microbiology methods and microbial differentiation
4. Describe safe practices in cosmetic use, including techniques to minimize microbial and fungal contamination

PROCEDURE

Materials

Cosmetic samples and equipment

Students are asked to provide their own cosmetic samples to test for microbial contamination. Students groups then select one product for additional testing by their laboratory group. To prepare the samples, students need small weigh boats, 15 ml plastic sterile Falcon tubes, inoculating loops, sterile glass rods (“hockey sticks”), and sterile plastic dropping pipettes. A scale and 37°C incubator should be available for the lab, as well as disposal bags for bacterial plates and contaminated materials. All materials are commercially available from Fisher Scientific (Ottawa, ON Canada).

Reagents and microbial media

To determine if their individual cosmetic product was contaminated with bacteria or fungi, each student was provided a plate of nutrient agar (NA; Difco #213000) upon which they streaked a sample of their product. Cosmetic samples for group testing were serially diluted 1:10, 1:100, and 1:1000 in a Tween-Peptone solution consisting of 1.0 g peptone, 10.0 g Tween 80, and 30 g sodium chloride in 1 liter of distilled water, sterile-filtered before use. Group samples were plated on nutrient agar, mannitol salt agar (MSA; BBL Microbiology #211407) to test for *Staphylococcus*, and eosin methylene blue agar (EMB; Himedia #M022) to test for gram-negative bacteria. Sabouraud dextrose agar (SDA; Difco #210950) was used to isolate fungal contaminants. Media are commercially available from Fisher Scientific, VWR International (Radnor, PA), and Sigma-Aldrich (St. Louis, MO).

Student instructions

Students were asked to bring cosmetic products of their own to test for microbial contamination. Students were also introduced to experimental design, hypothesis writing, lab techniques, and safety. Following this introductory lecture, students processed and plated their cosmetic products as described in the handout (see Supplemental Materials, Appendix 2). In the second week of the lab, students analyzed their results.

Instructor version

Pre-assessment (15 minutes) and instructor preparation (4-5 hours)

To prepare for this lab exercise, one week prior the students were given a pretest to assess their knowledge of microbiology concepts and methodology (Appendix 1). Following the pretest, a laboratory handout was distributed to students (Appendix 2). Included in the handout was a description of the purpose of the exercise, a basic background on cosmetics terminology and safety, and a section on microbes and selective media. Students were asked to read the handout prior to the lab. At this time, students were reminded to bring a cosmetic product that they would like to test the following week.

Instructors should prepare for the lab exercise a week in advance. Instructors should designate a full afternoon to preparing media and pouring plates, and an hour the next day to store the plates and set up the lab.

Week one: introduction to the laboratory concepts (60-80 minutes)

During this first week of the lab, students were given a background lecture to introduce the lab concepts. This included a discussion of bacteria, fungi, and viruses, including where they grow and the health problems that they can cause. Following this, cosmetics terminology, expiration dates, and safety were covered (14). (For more information see www.fda.gov/Cosmetics/default.htm.) Because all students use at least one cosmetic product on a daily basis (deodorant, toothpaste, lotion, make-up, hair product), it was relatively easy to engage students in a discussion of how their products might become contaminated with microorganisms. The instructor next asked students how they would verify the presence of contaminants in their products, and explained how selective media can be used to differentiate microbes. This is an opportunity to introduce the concept of serial dilution and how it can help students determine if the source of contamination is coming from their product or is introduced during the experimental setup.

The instructor next introduced the topic of scientific hypotheses, explaining what a hypothesis is, providing examples of strong and weak hypotheses, and discussing falsifiability. One suggestion to encourage collaboration and allow students to learn from their peers is to use their own hypotheses as examples for discussion. Classmates can then select examples of strong hypotheses, as well suggest improvements for weaker hypotheses. The general process of scientific discovery was also discussed, including experimental design and refuting or revising hypotheses after data collection and analysis. To reinforce these concepts, students were provided a hypothesis-writing handout. To tie hypothesis writing into cosmetics safety, a letter from a Department of Health and Human Services (HHS) inspection of Olay LLC was supplied as an example of how a

cosmetic product could potentially become contaminated at the level of manufacturing, and the types of penalties a cosmetic manufacturer could experience if they failed to meet basic regulatory standards (Appendix 3). Students then completed a worksheet to test their understanding of scientific hypotheses and apply the concepts to their own experimental design (Appendix 4). Activities include writing a hypothesis regarding the microbial content of unopened cosmetics based on the letter from HHS, designing an experiment given a scientific hypothesis, and differentiating strong and weak hypotheses.

Finally, students were expected to formulate a hypothesis regarding the presence of microbes in their cosmetic products and to design an experiment to test their hypothesis. It is necessary for the instructor to review these hypotheses before students proceed to the experimental setup. Students should be prepared to justify their group's product choice based on biological knowledge.

In the assignment portion of the laboratory handout (Appendix 2), students also applied what they learned from the lecture to an analysis of their cosmetic product. The questions asked students to predict which products they expected to contain microorganisms and why, as well as evaluate the product's chemical contents and their reason for purchasing the product. These questions were completed prior to students setting up their experiments.

For instructors whose labs are less than 120 minutes, the above introductory portion of the lab could be completed as a stand-alone lab, with plate setup the following week. This modification would extend the lab to three weeks. Alternately, the introduction and worksheet could be completed during the lecture portion of the course.

Week one: experimental setup (20-30 minutes)

Following the lecture and worksheet completion, students set up their experiment according to the directions in the student handout (Appendix 2), using a cosmetic product they provided. Most students brought products that they had been using regularly, although occasionally students chose to test unused or unopened products.

Each student was provided a nutrient agar plate to streak a sample of her or his product. This in particular makes the lab individually relevant as each student can choose the product that they would like to test. Working with their lab group, the students next selected one of their products for more extensive testing. This step minimizes the amount of bacterial media plates needed for the lab while still allowing each student to test a product of their own. It also allows the group to discuss their products and select one that they believe will yield the best data based on what they have learned in lecture. The student who provides the group sample should handle her/his own product exclusively to minimize risk of infection. In this study, lab groups consisted of four students, but lab groups of two are ideal to increase the chance of observed product contamination as well as engagement in the lab activity.

For group samples, 0.5 g of cosmetic product was mixed with 5 ml of sterile-filtered Tween-Peptide diluent for a 10% w/v dilution. Samples were then serially diluted to create 1% and 0.1% solutions. Fifty μ l aliquots of each sample were placed on NA, MSA, EMB, and SDA plates, yielding a total of 16 plates. It should be noted that some cosmetic products are difficult to manipulate (viscous, water-proof, small quantity, etc.). In particular, water-proof cosmetics do not easily solubilize in the diluent solution. In these cases, students are told to weigh out as much sample as they can even if it does not reach 0.5 g. It is the instructor's preference to limit the types of products students are able to test, although generally students are allowed to test whichever product they choose. Samples were spread evenly across the plates using sterile glass rods. The plates were allowed to absorb the solutions for 10 minutes, and then were wrapped in Parafilm, incubated for 48 hours at 37°C, and stored at 4°C until the following week. NA, MSA, and EMB plates were stored upside down, while SDA plates were stored right-side up to avoid spreading spores. If students do not have after-hours access to the lab room to remove their plates from the incubator and store them in the refrigerator, the instructor may need to do this.

Week Two: Analysis of results and postassessment

Upon returning to the lab for the second week, students obtained their plates and analyzed their results. Students were reminded not to open their plates to minimize transmission of any potential infection. Bacterial colonies were differentiated based on growth on the various types of media. Samples were examined for the presence of gram-negative bacteria on EMB, including nonfermenting (colorless colonies), strong lactose-fermenting (metallic green colonies), and weak lactose-fermenting bacteria (pink/purple colonies); and bacteria belonging to the *Staphylococcus* family, including nonfermenting strains (no zone surrounding colonies) and mannose-fermenting strains (yellow zone surrounding colonies) on MSA (3, 17). SDA was used to culture fungal contaminants (10). As part of the students' write-up, they determined which of their products contained microbial contaminants and what type of microbes were present based on their growth on selective media. Students filled out a table recording their data for each plate, indicating the product tested, the media type, and colony number, size, and morphology (Appendix 5). Plates were collected in biohazard bags and later autoclaved prior to disposal.

Students also explained whether their results supported their hypothesis, and provided biological explanations for their results. They were asked whether they would continue to use the product in the future, and what steps they could take to minimize contamination of their products and limit transmission of infection (Appendix 2). The instructor may choose to have student groups list their results on the board so that the class can see if there are any trends in the data. Students should be able to discuss whether they believe the source of contamination is envi-

ronmental bacteria or the user's natural flora, based on concepts learned in lecture.

Students are frequently surprised to see that many of their products do not generate any colonies. This is a good opportunity to discuss preservatives in cosmetic products, and the fact that some bacterial and fungal species cannot easily be cultured.

Following data collection and write-up, students were given a posttest to determine what they learned from the laboratory activity. The posttest was identical to the pretest (Appendix 1).

Suggestions for determining student learning

Students were given a pretest and a posttest (Appendix 1) to assess their learning. The pretest was not announced ahead of time. Students were told that the test would be used to assess their learning outcomes from the lab exercise. The pretest consisted of 13 multiple-choice questions, some of which had more than one correct answer. Two weeks later, following data collection and experimental write-ups, students were given a posttest. Only data from those students who took both the pretest and posttest were included in the analysis. The posttest was identical to the pretest. The grading rubric for the assessment test can be found in the Supplementary Materials (Appendix 7). Questions 1, 2, 5, 6, 7, 9, 11, and 12 assess students' understanding of microbial identification and methodology.

We suggest two modifications to this assessment tool for faculty adopting the exercise. The first would be to change the pre/posttest questions so that each only has one correct answer. This makes scoring the quiz simpler. Additionally, it may be clearer to see students' improvements if the posttest has different questions than the pretest. Therefore, we suggest adopting faculty offer an alternate posttest to better assess student learning.

To assess their understanding of safe use of cosmetics, students were asked how they would minimize infections from cosmetics as part of their lab write-up during the second week (Appendix 2). Knowledge of these concepts is also assessed in the pre/posttest by questions 4, 8, 10, and 13.

Additionally, students were given a worksheet to assess their understanding of hypotheses and experimental design (Appendix 4; answer key Appendix 7). On the worksheet, students were asked to write a hypothesis regarding the contamination of unopened products based on a letter from the HHS (Appendix 3). Students were also presented with a hypothesis and asked to design an experiment to test it. Students were next given three hypotheses, and asked to justify whether they were strong or weak. If the hypothesis was weak, students were asked to rewrite it. Finally, students were asked to formulate their own scientific question and hypothesis regarding microbial contamination in the cosmetic product they brought to test. For most of the students, this was their first experience in crafting a scientific hypothesis. We scored the quality of their hypotheses using

a grading rubric (Appendix 6), comparing scores from the first hypothesis (question #1 on the hypothesis worksheet) to the second hypothesis (question #4 on the hypothesis worksheet). Question 3 in the pre/posttest can also be used to assess this knowledge.

The hypothesis grading rubric (Appendix 6) suggests that a strong hypothesis includes an “if ... then” or “because ... we predict” format. Ideally, the format is less important than students providing justification for their hypothesized results. We suggest that adopting faculty modify the rubric criteria so this concept is clear.

A qualitative way to assess students' perception of their learning is to provide a survey. In summer of 2011, we sent a survey out to three semesters of former students to gauge their response regarding the Savvy Consumer Lab. The survey questions and responses can be found in the Supplementary Materials (Appendix 9).

Sample data

Sample responses to the students' lab and hypothesis exercise worksheets are provided in Appendix 8. Students generally found that microbial contaminants, both bacterial and fungal, were present only in those products that had been previously used. Based on the results of five field tests, students were able to successfully isolate bacteria from products used in regions of the body conducive to their growth, such as the mouth or underarms (Fig. 1). Out of seven mascaras tested, only two had cultivable colonies, and 1/7 lotions had bacterial contaminants. On the other hand, 3/3 deodorants showed significant microbial contamination, and 6/7 lip gloss products grew large numbers of colonies. As an example, one lip gloss tested showed the presence of both bacterial and fungal contaminants (Fig. 2). The lip gloss generated colonies on NA, EMB, MSA, and SDA for all three dilutions. Serial dilutions showed a consistent lessening of the number of colonies present on the plates. No evidence

of lactose-fermenting colonies was present on EMB (Fig. 2). Culturing of a used deodorant generated colonies on NA, EMB, MSA, and SDA. The colonies on EMB showed both strong lactose-fermenting properties evidenced by a green sheen, and weak lactose-fermenting strains evidenced by pink-purple colonies (photo unavailable). This was the only student product tested over five semesters that showed any evidence of *E. coli*.

Students sometimes find that colonies grow on their dilute plates but are not present on concentrated plates. Nonmajors, due to inexperience or lack of practice with aseptic technique, may introduce bacterial or fungal contaminants to the plate during the experimental setup. When this occurs, these plates serve as good examples to the class about serial dilution and aseptic technique, and the instructor can use them to ask students whether they believe their product is contaminated or if the source of the contaminant might have arisen elsewhere. Students should justify their responses with biological explanations.

Safety issues

Students are asked to handle their own cosmetic product to minimize transmission of bacterial, fungal, or viral contaminants to others. There is a small risk of infection from contaminated cosmetic products, so students should wear gloves at all times when handling the plates. If gloves are not available or are cost-prohibitive, students should be provided with hand sanitizers and be encouraged to wash their hands following the lab activity.

Plates are sealed with Parafilm to prevent students from opening the dishes during their analysis of the results in the second week. This minimizes the transmission of any potential contaminant to the student after the plates have been cultured. Additionally, SDA plates should be incubated and stored upright to prevent the spread of spores. All used plates should be disposed of in biohazard bags and autoclaved.

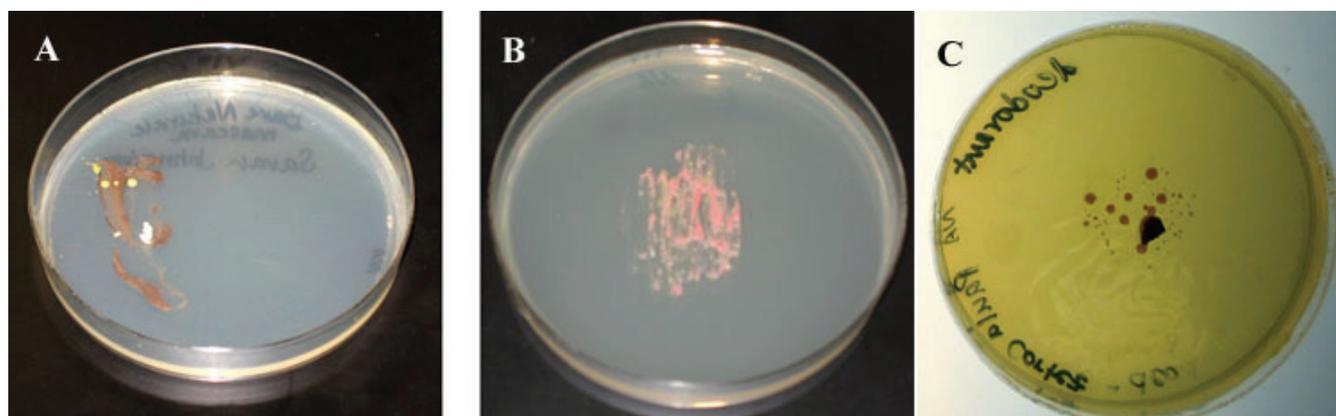


FIG. 1. Bacterial contamination of cosmetic products. Students dry-streaked cosmetic samples on nutrient agar and cultured them for 48 hrs. Plates are representative of student data: (A) mascara, (B) lip gloss, and (C) deodorant. Lip gloss and deodorant are the products that are most consistently contaminated.

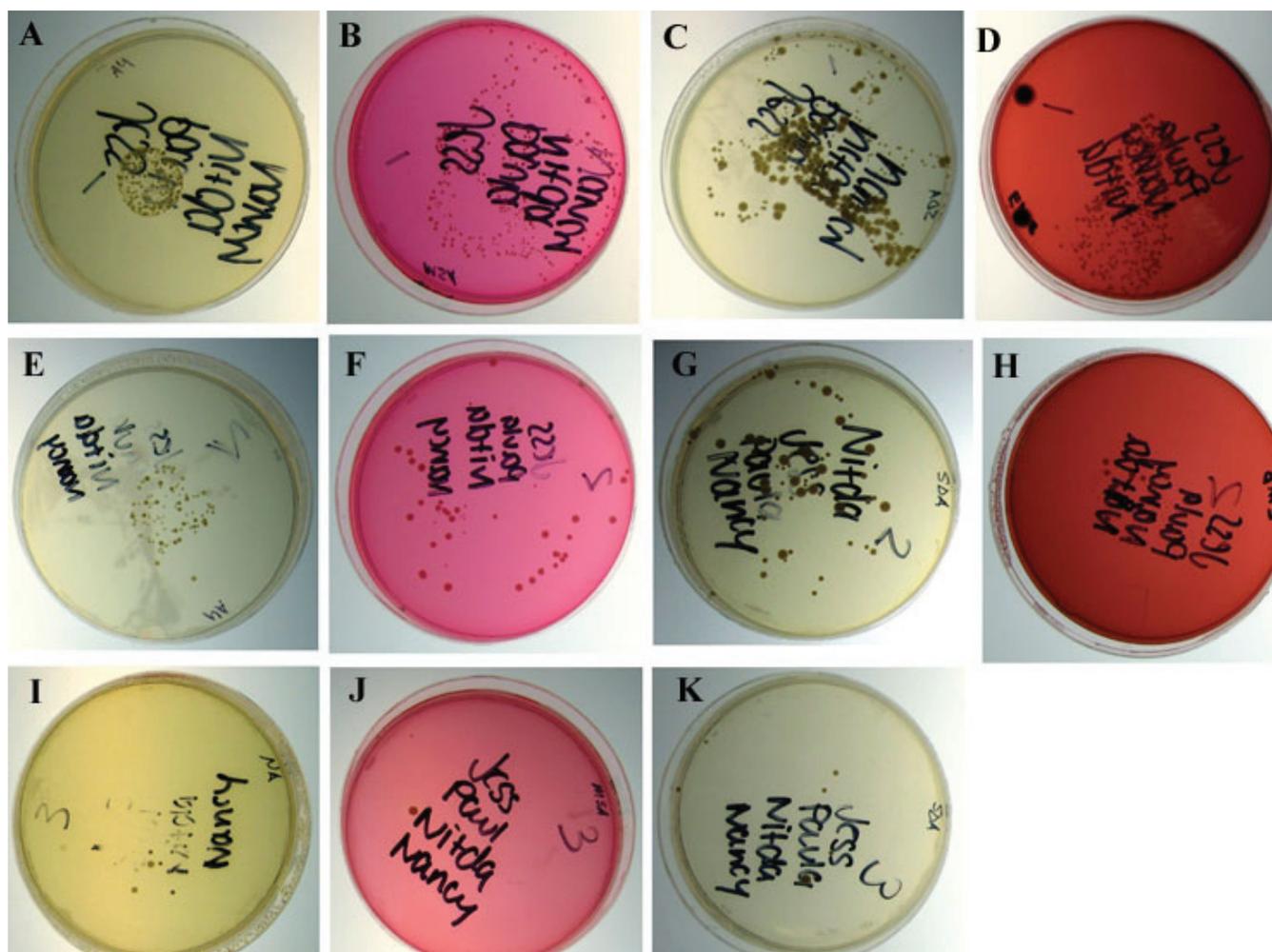


FIG. 2. Growth on selective media. Students serially diluted used lip gloss to a 10% dilution (row 1), 1% dilution (row 2), and 0.1% dilution (row 3) in Tween-Peptone and cultured them for 48 hrs on selective media as follows: NA plates (A, E, I), MSA (B, F, J), SDA (C, G, K), and EMB (D, H). Colonies consistently decreased in number with serial dilutions.

DISCUSSION

Field testing

This laboratory activity has been field-tested five times between spring of 2009 and fall 2011 in the Biology of Women course at Hamline University. The course focuses on biology through the female perspective. Major topics include the biology of sex, the reproductive cycle, the body in health and disease, and nutrition and body image. The data for this particular study were collected over three semesters, with a total of 54 students participating in the laboratory project and assessment data.

Evidence of student learning

Students were given a pretest to evaluate prior knowledge, followed by a posttest to assess what was learned (Table 1). The assessment test was 13 multiple-choice questions; half of the questions had more than one correct

answer. Students were given partial credit when more than one answer applied. Over the course of three semesters ($N = 54$), students performed an average of 2.6 points better on the posttest compared to the pretest, and this improvement was statistically significant, with a p -value of < 0.0001 (Table 2). It is to be noted that in every semester, some students misread the instructions and choose only one answer for each question; this is likely to be a source of error that lowers the overall scores. Additionally, while the pre- and posttest used in this study were identical, students were not given their graded test back. Thus, their improved scores likely reflect their understanding of the concepts rather than their familiarity with the test.

Learning objective 1 asked for students to be able to recognize strong hypotheses, defined as testable statements based on prior knowledge that contain measurable variables for comparison. To test students' ability to do this, a hypothesis-writing worksheet was provided (Appendix 4; answer key Appendix 7). Students were asked to read three hypotheses, determine if they were strong

TABLE 1.

Student responses to individual questions on the pre- and posttests (n = 54). The test had a multiple-choice format, with some having more than one correct answer. The full assessment is provided in the supplementary materials (Appendix 1). Some of the questions below have been slightly modified to fit into the table. Questions indicated with a (*) have multiple correct answers.

Question	Percent Correct Answers	
	Pretest Fall 09/ Summer 10/Fall 10	Posttest Fall 09/ Summer 10/Fall 10
*1. You have received a sample contaminated with <i>E. coli</i> . How can the presence of these organisms be confirmed?	41/50/43 Average: 44%	100/81/95 Average: 93%
*2. Microorganisms are identified based on what features?	94/100/90 Average: 94%	88/94/100 Average: 94%
*3. Which of the following is characteristic of a sound scientific hypothesis?	94/94/90 Average: 93%	100/100/95 Average: 98%
*4. Are “natural” products safer to use than comparable types made from synthetic materials?	76/88/86 Average: 81%	100/100/100 Average: 100%
5. Some microbial media contain dyes that change color upon acid production. Why is this type of test important?	53/69/57 Average: 59%	65/69/81 Average: 72%
6. Circle the set of steps that best describe the treatment of a cosmetic sample for microbial analysis.	29/50/48 Average: 43%	88/50/67 Average: 69%
7. Which of the following is an example of a non- selective, general isolation medium?	24/19/38 Average: 28%	88/88/76 Average: 83%
*8. Why do cosmetic products provide a favorable environment for microbes to grow?	35/44/33 Average: 39%	76/56/67 Average: 67%
9. Which microbial media would be effective at detecting fungal contamination of a cosmetic product?	41/44/24 Average: 35%	71/63/33 Average: 54%
*10. Which of the following is a “best practice” when using cosmetics?	41/50/48 Average: 46%	53/75/86 Average: 72%
11. The salt content of mannitol salt agar (MSA) allows microbiologists to detect contamination with what?	24/25/14 Average: 22%	53/38/38 Average: 43%
12. When grown on eosin methylene blue (EMB) medium, some bacteria appear to have green coloration. What does the color change indicate?	29/50/33 Average: 37%	65/56/33 Average: 50%
13. What is the purpose of expiration dates on cosmetics?	82/75/76 Average: 78%	88/75/86 Average: 83%

or weak, and rewrite those that were weak. Eighty-three percent (83%) of students correctly identified hypothesis 1 as weak and were able to satisfactorily rewrite it (Table 3). An additional 15% of students recognized the hypothesis as weak but were not able to adequately rewrite it. Seventy-four percent (74%) of students correctly identified and rewrote hypothesis 2, which was also weak, while a remaining 13% identified a problem but were unable to rewrite the hypothesis correctly (Table 3). Hypothesis 3

was strong; 83% of students correctly identified this, while 17% chose to reword the hypothesis (Table 3). In total, 93% of students were able to differentiate strong from weak hypotheses following the exercise. Question 3 in the pre/posttest was also used to assess students’ knowledge of the critical components of a scientific hypothesis. This question had two possible answers. On the pretest, 93% of students were able list one correct answer, with seven students receiving full points. On the posttest, 98% of

TABLE 2.

Results from pre/posttests to evaluate student learning. Students were provided a pretest prior to the lab to assess their knowledge of microbiology concepts and methodology, scientific hypotheses, and cosmetic safety. A posttest was given following the second week of the lab. The results were analyzed by a two-tailed t-test, and all data were found to be significant.

Semester	Average Pretest Score	Average Posttest Score	Difference	p-value
Fall 2009	4.99	8.21	3.32	<0.0001
Summer 2010	5.76	7.56	1.81	0.00014
Fall 2010	5.04	7.58	2.54	<0.0001
Average (n=54)	5.23	7.79	2.57	<0.0001

TABLE 3.

Hypothesis-writing worksheet questions. Students read hypotheses and determined if they were strong or weak. If weak, students were asked to rewrite the hypothesis such that it was strong. (A detailed copy of the handout is available in Appendix 4.)

Hypothesis	Summary of Correct Answers
Hand soap is bad for bacteria. (<i>not falsifiable, vague</i>)	39/47 correctly identified the hypothesis as not falsifiable and rewrote it; an additional 7 recognized the problem but were unable to properly re-write the hypothesis
Use of antibacterial hand sanitizer is better at reducing the spread of germs. (<i>no variable for comparison</i>)	35/47 correctly identified the hypothesis had no variable for comparison and rewrote it; an additional 6 recognized the problem but were unable to properly reword the hypothesis
<i>E. coli</i> are present in raw hamburger, and will be evidenced by a green sheen when cultured on EMB agar. (<i>strong hypothesis</i>)	39/47 determined the hypothesis to be strong, while an additional 8 reworded the hypothesis

students provided one correct answer, with 19 students receiving full points.

To assess learning objective 2 (“formulate a testable hypothesis”), students again used the hypothesis-writing worksheet to write two separate hypotheses. Using a grading rubric (Appendix 6), student hypotheses were scored out of a total of 5 points. The first hypothesis was based on students reading the letter to Olay LLC from the HHS, and formulating a hypothesis regarding contamination in unopened cosmetics. This was the first time most students had written a hypothesis, and the average score across three semesters was 2.87/5.00. Students then completed several exercises designed to help them differentiate strong from weak hypotheses before writing a second hypothesis regarding the microbial contamination of their own cosmetic product. The average score across three semesters on the second hypothesis was 3.80/5.00 (Table 4). The ability of the students to write a strong hypothesis thus improved by 1 point, or nearly 20%, following the hypothesis worksheet, with the data statistically significant (p-value less than 0.0001). The most common error was that students failed to provide justification for their hypothesis.

In learning objective 3, students were expected to demonstrate knowledge of microbial methodology and differentiation. On the assessment test, eight questions related to microbial differentiation and knowledge of selective growth and methodology (questions 1, 2, 5, 6, 7, 9, 11, and 12). Table 1 shows the breakdown of correct answers for individual questions. Overall, student scores on the assessment test improved from 2.76 (pretest) to 4.59, with a p-value < 0.0001. The greatest improvements were seen for questions 1 (44% correct on pretest versus 93% on posttest) and 7 (28% correct on pretest versus 83% on posttest). In total, 49/54 students received the same or higher scores on these questions on the posttest compared with the pretest. Students’ ability to apply this knowledge was also tested qualitatively through the hypothesis-writing worksheet (Appendices 4 and 7). Students were also asked to design an experiment to test the effects of communal use of a lipstick, using knowledge of microbiology concepts and methodology.

Learning objective 4 was to describe safe practices in cosmetic use, including techniques to minimize microbial and fungal contamination. This was assessed quantitatively through the pre/posttest questions 4, 8, 10, and 13, and

TABLE 4.

Hypothesis scores before and after a writing exercise. Students were asked to formulate two hypotheses: one following a lecture on hypotheses, and the second after completing a hypothesis worksheet (Appendix 4). Both hypotheses were scored using a rubric (Appendix 6). The results were analyzed by a two-tailed t-test to determine statistical significance.

Semester	Average Score Hypothesis #1	Average Score Hypothesis #2	p-value
Fall 2009	2.84	3.88	0.001
Summer 2010	2.73	3.40	0.01
Fall 2010	3.06	4.12	0.002
Average (n=45)	2.87	3.80	<0.0001

qualitatively through answers on the lab worksheet (Appendices 2 and 8). Student scores on these questions improved from 1.93 (pretest) to 2.55 (posttest) with a p-value < 0.0001. Percentages for individual questions are listed in Table 1, with the most noticeable gains for questions 4, 8, and 10. In total, 48/54 students performed equal to or better on these posttest questions compared to the pretest. Learning objective 4 was also assessed in the worksheet. Sample responses to lab worksheet questions are included in the Supplementary Materials (Appendix 8). All students were able to correctly list three “best practices” for cosmetic use following the lab.

Student perceptions

Our intention with the Savvy Consumer Lab is for students to learn applicable science while having fun. Former students frequently recall the Savvy Consumer Lab and comment that it was a memorable experience, their favorite lab, and a fun way to learn science. To gain access to student perceptions, in summer of 2011 an informal e-mail survey was sent to students from the three semesters represented in this study, and 13 students responded. The purpose of the survey was to solicit student perceptions of what they learned, and attempt to assess whether students believed the lab was relevant to their daily lives. Because the survey was sent 1-2 years after the students completed the course, it demonstrated the longitudinal impact of the lab on their understanding of microbiology and cosmetic safety. The full set of questions to the students and their responses are included in the Supplementary Materials (Appendix 9).

Eleven students responded that their experiences in the lab altered the way that they think about or use cosmetic products. “I actually think often about this lab while using

my cosmetics. Sometimes I’ll pick up some eye shadow or eyeliner and wonder how long I’ve had it. It has definitely changed my cosmetic use. I now find myself throwing old makeup without hesitation. I would often swap makeup with a friend if I liked something of hers or vice-versa. I didn’t realize how gross that is until this lab!”

Twelve of the students found the exercise memorable and relevant to their lives, some even two years later. “I do remember the lab ... using materials that we use every day, even those that we possibly used that morning, was a really neat addition to the course. That is what made it memorable; it ‘personalized’ the lab and my understanding of the material.” “I think the reason I remember it is because it felt relevant to me, like ‘Hey, this directly affects me because I use makeup and my friends use makeup.’ Sometimes I can’t see how they relate to me at all, but this lab obviously did.”

Eleven students stated that the lab helped them learn about bacteria and fungi, although they do not recall details. “From what I remember from the lab, I learned that bacteria can be everywhere and not visible. Being cognizant how you keep things like makeup is important to staying healthy, especially since you’re putting it on your face and close to areas bacteria can enter the body (eyes, mouth, nose, and skin).”

Possible modifications

Variations of this laboratory exercise include investigative projects for nonmajors or introductory microbiology courses. Examples of such projects include experiments to test used versus unused cosmetics, investigations on the effect of storage conditions on product contamination, and examining cosmetic counter samples to test for microbial contamination. Additionally, a quantitative component could be added to the lab if students calculate the CFU/g of the bacteria in their serial dilutions.

In a majors-level microbiology course, students could extend the laboratory exercise by designing investigative experiments to identify the type of bacteria or fungi that grows on their plates, using selective media, rRNA gene sequencing, and bioinformatics tools. Alternatively, plates from a nonmajors course could be provided as sample plates for an advanced microbiology course, and students from the upper-level course could present their results to the nonmajors. For either option, safety precautions should be taken to minimize infection from exposed plates. Students have commented that they would be interested in learning about the specific type of microbes present on their plates. Thus, in this arrangement the nonmajors would learn about their plates, while the majors gain experience identifying microbes and presenting data to a general audience.

Students often find that the NA plates on which they tested their individual product show contamination while their group product does not. Some students are disappointed that the lab leaves them unable to test their own product extensively. An extension of this lab could be to

allow students to further test their own product during a third week using all the media types available, and performing serial dilutions to determine whether the product is contaminated. This would require the students to bring their cosmetic product to the second week of the lab, when they get their results, as well as time to set up during week 2 and collect data in week 3. A second round of testing would also require twice the number of bacterial plates, diluents, and supplies.

SUPPLEMENTAL MATERIALS

- Appendix 1: Lab pre/post assessment
- Appendix 2: Student handout and instructions
- Appendix 3: Warning letter to Olay LLC
- Appendix 4: Hypothesis handout and worksheet
- Appendix 5: Student data table
- Appendix 6: Hypothesis grading rubric
- Appendix 7: Answer key to assessment test and hypothesis worksheet
- Appendix 8: Sample student responses to Savvy Consumer Lab worksheets
- Appendix 9: Savvy Consumers Lab survey questions and responses

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