A HANDFUL OF BACTERIA: A SIMPLE ACTIVITY THAT ENGAGES STUDENTS TO THINK AND WRITE LIKE A SCIENTIST

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Abstract
Non-major students taking science courses to fulfill degree requirements may show low interest in traditional lecture-based classrooms. While professors may try to incorporate hands-on activities to the classroom to foster enthusiasm, the development of a low-cost, low-hazard scientific activity that engages students can be challenging. Moreover, teaching and evaluating critical thinking, scientific literacy, quantitative analysis and writing skills utilizing such activities require asking carefully designed questions. Here, I introduce a tested effective, inexpensive, 20-minute in-classroom microbiology deep-learning activity, and explain how it can be adapted to other disciplines of science. From one simple exercise, students conducted literature research, performed statistical analysis and made quantitative/causative conclusions. Students also learned to write, appreciate and critically analyze a scientific paper.

Keywords – scientific inquiry, critical thinking, quantitative reasoning, writing, engagement

1 INTRODUCTION
Scientific inquiry or similar science courses are often required for non-science majors by higher education institutions. As with other core curricula, students may show a lack of motivation due to the false impression that it is irrelevant to their careers and that it is frustratingly difficult (Glynn, Taasoobshirazi & Brickman, 2009). Consequently, the learning outcome is generally poor. Previous studies suggested that students’ engagement to an instructional episode is primarily based on the form of activity, and that “hands-on” activities with technology involvement elicit higher interest (Swarat, Ortony & Revelle, 2012). Here I report an in-classroom activity that I created to encourage deep learning core concepts of my infectious diseases course: bacteria are constantly present on human skin, and proper disinfection is essential to prevent diseases.

On average, human hands harbor more than 150 bacterial species at a density of ~10^7 cells per square centimeter (Fierer, Hamady, Lauber & Knight, 2008; Fredricks, 2001). These bacteria are divided into two communities, the long-term residental flora and the transient flora (Cogen, Nizet & Gallo, 2008). Although diversity of the residental flora varies from one individual to another, hand washing does not significantly alter its composition (Fierer et al., 2008). The transient community, a more-probable source of infectious diseases, is acquired at any given moment after individuals touch contaminated objects. While hand washing with water removes 48% of transient bacteria, and with soap 82% of them (Burton, Cobb, Donachie, Judah, Vurts & Schmidt, 2011), the remaining bacteria can still cause infections. In addition, residental bacteria can be opportunistic pathogens and enter deep tissues when the skin has a cut. Therefore, disinfecting agents must be used under circumstances to prevent the spread of potential pathogens.

In this low-budget two-classroom-period activity, my students examined the bacteria on their hands, evaluated the effectiveness of alcohol wipes on reducing bacteria, and composed a scholarly article to report their
findings. After implementing the activity for five semesters, it is proven successful to 1) foster critical thinking and creativeness; 2) demystify the process of scientific research and publication; 3) train students quantitative skills in decision-making; 4) reinforce scientific theme knowledge; and 5) promote cooperative learning and peer review.

2 METHODOLOGY

2.1 The Experiment (~ 20 min long)
This in-classroom activity required nutrient agar plates, disposable alcohol pads containing 70% isopropyl alcohol, and Sharpie permanent markers, all of which can be purchased from online stores as ready-to-use products. Nutrient agar plates were stored in a household refrigerator for up to 6 months until use. These materials were either sterile or disinfecting, therefore posing no health hazards to the students.

On the day of activity, students were asked to pair up with a partner, and each pair was given a set of the abovementioned materials. One student in the pair played the instructor role by labeling the plate, reading aloud the instructions, tracking the time, and ensuring accuracy of the conducts. The other student was the experimenter. I first briefly lectured the following concepts: 1) Bacteria are omnipresent; 2) Humans have a residential community that is generally harmless and cannot be removed by simple washings; 3) Bacterial can quickly multiply to form a visible mass called colony on the nutrient agar surface. I then asked students if they recalled their last vaccination experience, whether their doctors wiped their skin with an alcohol pad before giving the shots, and informed them that the activity might explain why wiping the skin before injection was necessary. Since most students had not seen an agar plate previously, I specifically pointed out location of the growth medium, explained the importance of labeling near the edge of the bottom cover, and walked students through the experimental procedure. Additionally, I warned students not to press the agar surface with more pressure than necessary to leave a fingerprint so as to avoid creating cracks on the agar surface. To minimize contamination from microbes and their spores in the air and in human mouths, I asked students to keep plates covered unless opening was necessary, and to refrain from sneezing or talking directly to the agar surface. After the short lecture, I left a recipe-type experimental procedure and a schematic cartoon on the white board while students performed the experiment.

In the experiment, students first divided each nutrient agar plate into 4 sections and labeled them with numbers. Experimenters of each group then gently pressed their left index fingers onto the agar surfaces of quadrant 1 for 2 seconds, immediately followed by a second press in quadrant 2 for 2 seconds with the same finger. The same student in each group continued to press his/her right index finger in quadrant 3 for 2 seconds, followed by a thorough cleaning (10 second wiping) of the right index finger with an alcohol pad. The wiped finger was then pressed on quadrant 4 of the plate for 2 seconds. Finally, students returned plates to the front desk and secured them by taping the covers to the bottoms of the plates. These plates were brought back to my office, and incubated at room temperature for 3 days after which growth patterns with best contrast were observed. If a class meets once a week, plates can be stored upside down in a refrigerator after the 3 day incubation. To provide students with visual records for later data analysis, I took pictures of the plates and uploaded them to the course website.

2.2 Observation And Data Recording (~ 20 minutes)
In the next class period, agar plates were returned to students for observation. I instructed students to not open the covers since bacteria from students' hands had grown into large biomass, which made them potential opportunistic pathogens. Students were asked to look through all plates from the entire class to make notice of a general growth pattern in the four quadrants before they observed their own plates. They were asked to describe colonies on their plates by comparing them with the morphologic views of common colony types (Holt, Krieg, Sneath, Staley & William, 1994), and by making notes of their colors and sizes in mm diameter. They also counted the number of colonies in each quadrant. Since most American students are not familiar with the metric system, I distributed printout paper rulers to students as a reference.

After data recording, I collected the plates and brought them back to the office for proper disposal. Plates can be autoclaved if an autoclave is available. Alternatively, they can be submerged in 10% bleach for 15 minutes before draining and discarding in regular trash.
2.3 Scientific Paper Writing Assignment

<table>
<thead>
<tr>
<th>Assignment Instruction in Bullets</th>
<th>Assessment Field</th>
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<tbody>
<tr>
<td>Correct spelling, punctuation and grammar; correct format of scientific names</td>
<td>C I P Q S T W</td>
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<tr>
<td>At least two scientific citations from the last 5 years with matching after-text references in an exampled format</td>
<td>X</td>
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<tr>
<td>Informative and concise title, affiliation and detailed contact information of the author</td>
<td>X</td>
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<tr>
<td>An abstract summarizing the entire paper including objective, brief method, major results and conclusion</td>
<td>X</td>
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<tr>
<td>Clearly marked and complete sections including Acknowledgements and References</td>
<td>X</td>
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<tr>
<td>Elements of scientific method (observation, critical question, hypothesis, prediction) logically presented in the introduction section</td>
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<tr>
<td>Importance of the study explained</td>
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<td>Detailed materials and methods in paragraphs in past tense to allow readers to precisely repeat the experiment</td>
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<tr>
<td>Identification of independent, dependent, and controlled variables</td>
<td>X</td>
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<td>Identification of the controls and explanation of their purposes</td>
<td>X</td>
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<tr>
<td>Description of colony morphology of their own plate in a table format</td>
<td>X</td>
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<tr>
<td>Speculation of microbial species found on the plate based on knowledge of common skin microbes and their colony morphologies (citations are required)</td>
<td>X X X</td>
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<tr>
<td>Reporting the trend observed from all 41 plates</td>
<td>X</td>
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<tr>
<td>Image of a representative plate with an informative figure legend</td>
<td>X</td>
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<tr>
<td>Calculation and discussion of the data outliers</td>
<td>X</td>
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<tr>
<td>A column graph to compare the effectiveness of microbial removal with and without the alcohol pad</td>
<td>X</td>
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<tr>
<td>Calculation and interpretation of error bars and the p value</td>
<td>X</td>
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<tr>
<td>Discussion of experimental design limitations</td>
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<tr>
<td>Proposal of a future experiment based on current results</td>
<td>X</td>
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<tr>
<td>A conclusion based on the statistical analysis of all plates</td>
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Note: C, critical thinking; I, information literacy; P, scientific process; Q, causative and quantitative reasoning; S, social connections; T, theme knowledge; W, written communication

Table 1. Instructions for the scientific paper assignment and linked assessment fields of scientific inquiry skills

Although many students had written scientific reports previously, most still had difficulty understanding the structure of scholarly articles, thereby limiting their comprehension of these valuable evidence-based information sources. In this writing assignment, students were forced to play the role of scientists, investigating a problem and communicating with other experts in the field about their findings. Students were asked to complete a publishable scientific manuscript based on the alcohol wipe activity, so that they could understand the processes of scientific research and knowledge dissemination through first-hand experiences. Prior to this assignment, a mini workshop was given to help students discern scholarly articles from popular literature. I explained to students how each section in a scientific paper could facilitate communication, collaboration and peer monitoring. I also handed out a general template of scientific paper and a few sample papers before offering some suggestions on how to write each section well. I briefly explained how scientists without being an expert in mathematics conduct statistical analyses using Microsoft Excel. I used one example dataset to show students how to calculate the averages and standard deviations, how to generate graphs to compare values of two groups, how to determine the outliers, and how to use P-value to judge whether a difference observed between two groups was statistically trustworthy. Since some students had no experience with Excel or confidence in math, I created tutorial videos and posted them on the course webpage. To some students this assignment was intimidating due to its complexity. To ease the anxiety, I provided very detailed assignment descriptions in bullets as summarized in Table 1. I assigned a point value to each bullet to ensure that students
did not omit questions. I also encouraged students to seek external help, including the tutoring center and the library specialists.

For the assignment, students were provided with pictures of 40 select agar plates from previous semesters, and an Excel spreadsheet containing the numbers of colonies counted from each quadrant of the 40 plates. Students were told that these results were from other student pairs. Students were given two weeks to complete the assignment and asked to work collaboratively with their partners.

3 EXPERIMENTAL RESULTS

After 3 days of incubation, bacterial colonies of various sizes (Ø 0.5-5 mm), shapes and colors (pink, red, orange, creamy, white, transparent, black, yellow) were observed on the plates (Figure 1). The most common morphotype showed a white color, circular and convex shape, and size of 0.5-1.5 mm in diameter, matching the description of *Staphylococcus epidermidis*, the most abundant bacteria found on human skin (Cogen et al., 2008). The second most common morphotype is yellow in color, matching the descriptions of another common skin bacterium, *Staphylococcus aureus* (Cogen et al., 2008). A limited number of plates also showed fungal growth (Figure 1D). To accurately identify the species of the colonies, if budget allows, 16s rDNA of these colonies can be sequenced and mapped to known reference genomes.

Most plates also showed no microbial growth in the quadrant containing prints from the alcohol wiped fingers. Occasionally microbial growth was found in non-fingerprint areas, likely due to exposure of the growth medium to ambient air or student’s breath. Comparison of the colony reduction rates from first press to second press yielded a p value much smaller than 0.01 between the left finger and the wiped right finger, suggesting that the observed difference was statistically trustworthy, and that it was the alcohol wipe that had made a difference rather than some random factors.

Figure 1. Representative results showing various microbial colony types found on student hands. Quadrants 1-4 represent left index finger first press, left index finger second press, right index finger first press and right index finger second press after applying alcohol wipe, respectively. Please note that quadrant 1 of plate D showed a penny sized fungal colony.
4 ASSESSMENT AND OUTCOMES

4.1 Student Engagement

The primary objective of this activity was to minimize student’s fear of science and inspire their curiosity (Figure 2). Based on surveys conducted at the end of each semester, this goal was successfully reached. Students considered this exercise more interesting than other engaging pedagogies offered throughout the semester including games, group discussions, movies and field trips. They were impressed by the quantity and diversity of microbes on their hands, and were excited throughout the process since it didn’t require too much previous knowledge in microbiology and they were doing research to learn about themselves. Many were eager to share pictures of their hand bacteria with friends and family via popular social media such as Facebook. Since students perceived bacteria as germs that cause diseases (although >99% of them do not cause human illnesses), this exercise also reinforced their hygienic habits and taught them effective ways to remove bacteria.

As for the writing assignment, many students considered it challenging and time consuming, yet they self-reported strong satisfactions for the many skills that they have learned from this mock writing experience. They reported that the exercise enhanced their independence of work, tolerance for obstacles, readiness for new challenges, growth of self-confidence, and sense of accomplishments. It forced them to do more literature research and delve more deeply into the theme subject. Many students also posted their scientific paper on an e-Portfolio webpage to showcase their critical thinking and writing skills to potential employers. In addition, similar to what Harrison, Dunbar, Ratmancy, Boyd and Lopatto (2010) reported, a few students revealed that they would reconsider science as a career choice.

Figure 2. Student Engagement. Top: Students performing the alcohol wipe experiment; Bottom: Students observing colony morphology during the second classroom period
4.2 Critical Thinking And Creativeness

Based on students’ submitted assignments, they have demonstrated the critical thinking skills as well as creativity in many ways. For example, some students noticed a difference between the average numbers of microbes found on their left hands and right hands during the first presses, and hypothesized that the pattern was caused by individual’s preference of hand use. Most students were able to make logical speculations of bacterial species found on the plates based on the common microflora on human skin published in recent scholarly articles and the description of these microbes in reference textbooks. Several students pointed out that the speculation needed backup from more accurate biochemical analyses. Students were also able to spot the major flaw of experimental design: since the control finger was not wiped, it was not possible to discern whether the removal of microbes was due to the wiping action or because of alcohol. Students proposed interesting and relevant future studies based on results from the experiment. Some proposed a hand-shaking experiment to study whether microbes could be easily exchanged between individuals; Some suggested to test the effectiveness of other commercial disinfecting products such as hand sanitizer gels and wipes; Some wanted to identify the bacterial species and compare the gender, handedness, race and ethnic background differences.

4.3 Scientific Process, Information Literacy And Communication

The experiment and subsequent scientific paper writing assignment was a deep-learning role-play experience for students. After conducting the experiment, collecting and analyzing data, and making a statistically relevant conclusion, they have acted as a junior scientist, and have gone through each step of the deductive scientific method. Role-play simulation is a proven highly effective tool for process understanding (Nestel & Tierney, 2007), and has become a popular approach to engage students in non-major courses, especially political science and religious studies (Kelly, 2009). At the end of the semester, students who have gone through this activity where they played the role of a scientist showed a better comprehension of scientific processes in solving scientific and non-scientific problems when they were given different scenarios to analyze elements of the scientific method.

By acting as a scientist writer, students also developed a better understanding of the journal article structure, and subsequently a set of skills required by information literacy. Students realized the following: 1) The fixed format of scientific papers helps readers to quickly locate information and understand the otherwise difficult-to-read jargon-filled article; 2) Authors must provide complete and detailed contact information to allow readers seeking collaboration or answers to their questions; 3) The funding source listed in the acknowledgements section may reveal a conflict of interest that downgrades the value of a paper; 4) Only original, authoritative, objective and valid data are allowed in scientific scholarly articles; 5) Conclusion of a study are usually drawn upon quantitative reasoning; 6) Scientific research doesn’t always happen in the perfect sequence of deductive scientific method, but scientists present their data in that order to avoid confusion and enhance readability. In the writing assignment, students were required to cite scholarly references. Google Scholar was the search engine most students used. Their understanding of the anatomy of a scholarly paper helped them to find and filter information from relevant reference articles. They also followed certain citation styles and paraphrased major findings from these articles to support their own statements. Later assignments of the semester all required citations from scholarly articles, and this exercise prepared students to become comfortable in searching and citing from scientific literature, and even critiquing some studies as what peer scientists would do.

In addition, this assignment enhanced students’ communication skills through writing and visually presenting information in images, graphs and tables.

4.4 Quantitative Reasoning

In science, a conclusion is valid only when it is supported by statistics. One individual agar plate may show no reduction of bacterial colonies between two presses, but it’s the overall trend from all of the replicates that allows scientists to make a meaningful conclusion. From the writing exercise, students learned to identify outliers and conduct simple statistical analyses. They were able to use the P-value to determine whether a difference observed between the control and treatment groups was from random effects or the treatment. They also learned the difference between correlation and causation (e.g., the presence of alcohol in the pad is correlated with reduction of bacteria on the wiped finger, but it may not be the cause of the reduction).
4.5 Theme Knowledge
Theme of this scientific inquiry class was infectious diseases. This activity helped students to understand topics including bacterial growth, human normal flora, food poisoning, and the control of microbial growth with antimicrobials.

4.6 Informal Cooperative Learning
Students who took this course were mostly lower grade college undergraduate students, and most of them came from different fields of study. It was easily recognizable that these students have different strengths and weaknesses. To foster peer learning, students were assigned to random pairs for writing the scientific paper. Compared with the few students who opted to work on the assignment alone, students who worked collaboratively completed the writing with noticeably higher quality. Working with a partner allowed students to better grasp related course materials and follow the assignment instructions. For example, when one partner overlooked a problem or made a careless mistake, the other can catch it during proofreading; the student better at math and creating charts may compensate well for the partner who is better at writing and searching supporting information but weak in math. To avoid unfairness in work share and schedule conflicts, I set aside one classroom period to allow collaborative work and supervised their participations. This small group informal collaboration in fact mimics the collaborative nature of scientific research in the real world: scientists with different expertise work together for complex projects and publish together as a group. This is also the preferred working model for productive businesses at a time when work has become more interdisciplinary and complicated (Barron, 2000). Therefore, the teamwork skill that students learned from this activity will eventually benefit them when they transition from school to workplace.

4.7 Cost Of Activity
The cost per student for the entire activity was less than $1 when students worked in pairs and bleach was the chosen method of disposal. If the professor wants to make the activity more hands-on and more classroom time is available, students can prepare their own nutrient agar plate with agar powder and a microwave. Either way, this activity is safe for college-aged students and easy for the professor to set up. Its cost effectiveness may be especially desirable for institutions that are restricted by funding, and/or laboratory spaces.

5 CONCLUSIONS
In conclusion, this inexpensive, simple in-classroom experiment and its associated assignment proved to be an effective method in teaching key concepts of scientific inquiry. Students were actively engaged, and gained specific skills in research design, hypothesis formulation, data analysis, presentation, and quantitative reasoning.

The reported experiment and its associated teaching-evaluation methods can be easily adapted to other science disciplines. For example, in physics, students may throw eggs connected to different shaped parachutes off the balcony of a building to test which shape has the highest drag force; in chemistry, students may titrate to determine whether the concentration of an active ingredient is higher in one commercial detergent than a competitor product.

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