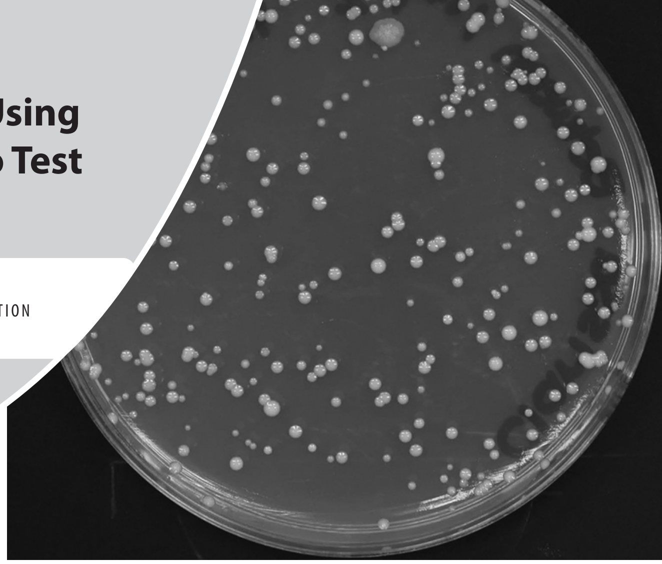
INQUIRY & INVESTIGATION

Where Are the Bacteria? Using Photos of Skin Samples to Test Hypotheses



NABT BioClub RECOMMENDATION

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Abstract

Encouraging students to engage in self-driven problem solving early in their educational career is necessary for them to be able to conduct hypothesis-driven research in the future. However, a fundamental obstacle is finding a topic and activity that is both tangible for students to understand and intelligible. Here we present a classroom activity that can be used in middle school, high school, and even undergraduate college settings to engage students in developing their hypotheses surrounding the human skin microbiome. The ability to culture skin bacteria on agar plates and extract potential environmental factors from their own everyday lives make the human skin microbiome a model example for students to develop their own hypotheses about the variability both within and between different bacterial populations. The approaches we use in our activity set the foundations for how instructors can engage their students in hypothesis-drive scientific research.

Key Words: *hypothesis testing; replicates; bacteria; skin samples.*

○ Introduction

One of the joys of studying science comes from generating and testing one's own ideas. Encouraging students early in their educational career to practice scientific problem solving is integral for their ability to conduct hypothesis-driven research in higher-level education (Chiappetta & Adams, 2004; Kao, 2016). However, the biggest obstacle preventing students from obtaining these skill sets is the lack of formal education on how to generate hypotheses, collect information, and analyze data to support their hypotheses. In order to engage students in hypothesis-driven research, new activities need to be introduced into the classroom that include some structure of inquiry, such as controls, replicates, and predictions. A fundamental step is finding topics that are both tangible and intelligible to students. Previous studies have shown that students better retain information when the taught material is more relevant to their personal lives (Davis, 1993).

Participants were able to not only engage in scientific activities but also grasp the individual steps necessary to formulate a hypothesis and use experimentation to determine whether it is supported.

Microbiology has become a common tool used to motivate students to understand the importance of scientific discovery in their lives (Handelsman, 2002; Miller, 2004). Previous examples of microbiology applications in the classroom include culturing microorganisms found in yogurt, testing cosmetics for contaminants, or isolating microbes found in stream water (Burleson & Martinez-Vaz, 2011; Gorman, 2010; Weaver et al., 2018).

Our activity engages and introduces students to hypothesis-driven inquiry using the human skin microbiome. The skin is the human body's largest organ and is home to a diverse collection of different bacteria, most of which are either harmless or beneficial to humans (Grice et al., 2009). The types of bacteria that colonize different parts of our body can vary greatly across the skin surface and depend on factors such as hairiness, sweat glands, contact with the environment, and age, to name a few (Grice & Segre, 2013). Although recent research has shown the beneficial role microbes play in our lives, many students still view bacteria as harborers of disease (Byrne, 2013; Driver et al., 2014). In reality, most bacteria are beneficial, and life as we know it would not exist without them (Byrne, 2013; Nielsen et al., 2018). Other activities have been developed for the classroom to introduce students to bacterial populations, their ecology, and the human-bacteria symbiosis (Chandana et al., 2014; Homburger et al., 2015). However, one key question that remains is how we give students some independence in hypothesis testing in a short time.

In our activity, we use photographs of plates cultured from different locations on the skin of people that differ in age and gender. The activity lets the students consider these variables, predict patterns, and then test them with these photographs. The students write a prediction, sort the photographs

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Table 1. Crosscutting concepts, science & engineering practices, and disciplinary core ideas in the Next Generation Science Standards covered by this activity.

Crosscutting Concepts	Part of Activity
Patterns: Graphs, charts, and images can be used to identify patterns in data.	Students referred to images of colonized agar plates, rather than the agar plates themselves, to qualify patterns related to the bacterial colonies pictured on them.
Cause and Effect: Cause and effect relationships are routinely identified and used to explain change.	After evaluating the results of the matching activity, we asked students to rationalize the relationships between skin microbiota of different groups.
Science & Engineering Practices	**Part of Activity**
Planning and carrying out investigations	Students are asked to form their own hypotheses and swab their own bacteria plates to describe variation between different skin microbiota.
Analyzing and interpreting data	Students must recognize, then identify, similarities and differences between bacterial plates swabbed from different skin microbiota.
Life Sciences Core Ideas	**Part of Activity**
Biodiversity and Humans: There are many different kinds of living things in any area, and they exist in different places on land and in water.	Our skin is inhabited by millions of bacteria that can be both helpful to human development and pathogenic. Skin microbiota refers to these different bacteria in aggregate.

according to that prediction, and then turn them over and see if their hypothesis was supported. The front of each card has a photograph of a bacterial plate. The back identifies the category of person (gender, age) and body location (scalp, nose, palm, foot, elbow) of the sample. There are at least two replicates of each condition.

We presented our activity at the Ferguson Farmers Market in Missouri and found that participants were not only engaged in learning about the skin microbiome but also actively formulating their own hypotheses and taking the necessary steps to collect data and determining whether their hypotheses were supported. We believe that this activity can translate to the classroom from the elementary to high school level and encourage students to readily engage in hypothesis-driven experimentation.

○ Student Objectives

After completing this activity, students should have a basic understanding of variation within and between samples from the same condition. For example, there are two bacterial plates from the palms of young men, young women, and old women. Before looking at where the bacteria samples come from, the students generate hypotheses about where they think there should be the most bacteria. They should also be able to develop hypotheses that explain that variation and how to conduct experimentation to support those claims.

○ Connection of the Activity with Next Generation Science Standards

This activity aligns with many of the Next Generation Science Standards, including the crosscutting concepts (patterns, cause and effect), science and engineering practices (planning and carrying out investigations, analyzing and interpreting data), and life sciences core ideas (biodiversity and humans) (Brown & Concannon, 2018). Parts of our activity that directly emphasize these standards can be found in Table 1.

○ Details of Exercise

All the materials for this activity and instructions on how to develop your own can be found here. To prepare the activity, we let the participant choose one of the three variables to test how the bacterial populations differed between given categories. For example, one of the cards asks how bacterial populations differ between body parts and gives us the categories of armpit, nostril, and feet. We placed the card with the question and categories on the table and then asked each participant to create their own hypothesis to answer the question and predict what they would expect to see based on their hypothesis.

It is crucial at this point that each student understand the components that make up a solid hypothesis. A hypothesis predicts that one variable changes another variable in a specific direction for a scientific reason. For example, one might predict that older people have more bacteria on their skin than younger people because the bacteria can hide in the wrinkles. Or one might predict that there are more bacteria on the scalp than on the palm of a hand because it gets washed less often. To test a hypothesis, it is necessary to have more than one replicate and to not know the answer in advance. Some key features all hypotheses must have are a possibility to support that the hypothesis is true, a possibility to support that the hypothesis is false, and reproducibility of results.

We found that it was helpful to point out specific features that differed between the categories. We discussed things that might impact bacterial populations and gave the students the opportunity to decide whether those things could affect their bacterial

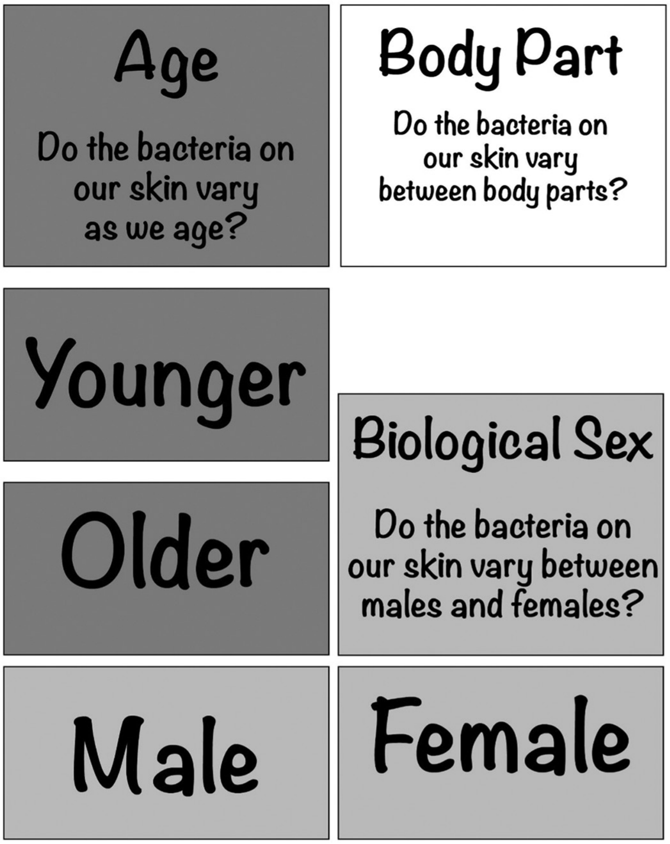

Figure 1. One example of the table setup for the card activity. What questions the students pose will determine which cards they are given.

populations (Figure 1). For the body parts example, we pointed out features such as which body parts are cleaned more often, or which come in contact with surfaces. Some other possible questions can be found in Table 2.

It can also help to think about the dependent and independent variables of the experiment. Because our project is aimed at students or the general public, we explain what independent and dependent variables are. An independent variable is a measurement you are making that stands alone and isn't changed by the other variables you are trying to measure. Dependent variables have measurements that depend on other factors. We can also think of changing the independent variable as a "cause" and the subsequent change in the dependent variables as the "effect." After an overview of the different types of variables, we gave each participant the chance to determine for themselves what the independent and dependent variables are for the activity. Our independent variable is the category we are using to differentiate our bacterial populations and the dependent variables are the features we are measuring to quantify those differences, such as number of bacterial colonies, morphology, or color.

Once the participant has formed their hypothesis, we then provided pictures of various bacterial samples swabbed from human skin. An example of our activity setup can be found in **Figure 2.** We then asked them to use their hypothesis to organize the cards under the category they think they belong to. As the students are analyzing the plates, we found it helpful to point out characteristics of the bacterial populations that exhibit variation between and within groups. The size, shape, and number of bacterial colonies on each plate are important factors, of many, to take into account when comparing different plates.

Table 2. Questions about bacteria to guide hypothesis-forming.

Question: Does the amount of bacteria on our skin increase as we age?	
Is there a difference between the personal hygiene of younger and older people?	Lots of bacteria grow outdoors. Do younger people spend more time in nature than older people?
Do you think the bacteria on our skin adapt to us as we age?	
Question: Does the male skin microbiome have increased bacterial diversity compared to females?	
Is there a difference between the personal hygiene of females and males?	Do men sweat more than women?
Do men or women interact with "dirty" surfaces more often?	
Question: Do different body parts have increased bacterial diversity?	
What body parts do you clean more often?	Bacteria grow well in humid environments. Which body parts are exposure to more moisture?
How often does each body part interact with surfaces that could have bacteria?	Think about the shape of each body part. Is the body part more flat, or does it have more "nooks and crannies" for bacteria to live in?

Once the students have finished sorting the plates, they then flipped them over to see if the answers on the back of the card matched their guesses. For the bacterial samples we used in our activity, the participants would find no major differences in bacterial populations on subjects of different ages or genders. However, between body parts on the same person, we found that bacteria taken from hair or feet showed the most variation in shape, number, and size of bacterial colonies. In all cases we observed evidence of variation within the replicates of each grouping. If their answers did not support the hypothesis, we then asked students to think about potential reasons. Possible questions can be found in Table 2.

At this point, we felt it was important to emphasize the ideas of variation between and within groups. While students were formulating their own hypotheses, we asked them to focus on features between the categories that could affect their bacterial populations. This pushed students to think about the variation of bacterial populations between groups. However, we also want students to recognize that there are differences in the replicates of bacterial samples. For example, in our two bacterial samples taken from the mouth of

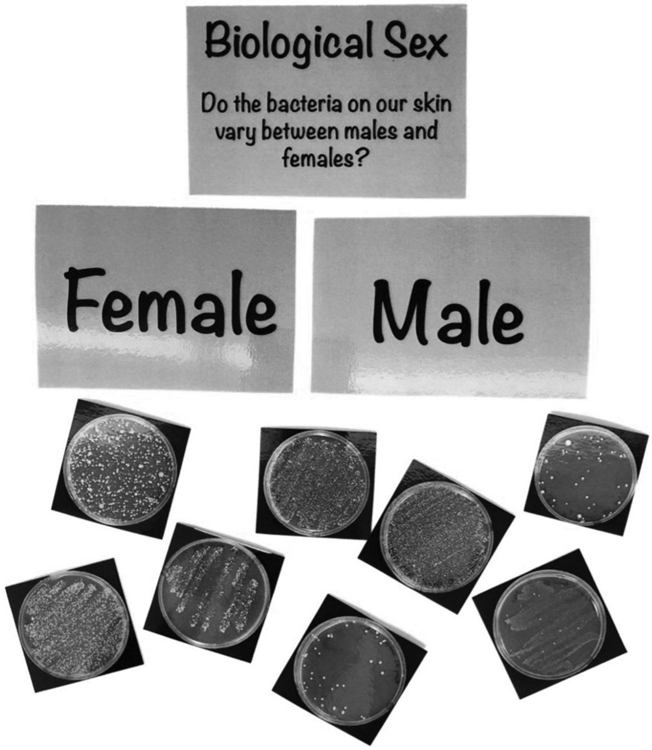

Figure 2. Sample and question labels.

subject #5 we see differences in the morphology, number, and color of the bacteria even though they were taken from the same subject and location. This helps to emphasize how samples taken from the same population can still differ and have a large variation of samples within the same group.

○ Modifications for Limited Class Time

The original exercise did not take more than 10 minutes to do because it was in the context of a farmer's market. In a classroom, the additional time available could be used to set up the experiment and explain the variables and the study. Students should choose one variable—age, body part, or gender—and generate a prediction and a reason for it, the two elements of hypothesis testing. Then they receive the cards relevant to their experiment, sort them according to their prediction, and then turn them over to see if their predictions were supported. For example, if they choose to compare mouth and hair and predict there will be more bacteria in the mouth because food goes into it, then they would receive cards for mouth and hair from one gender and one age group. If there is enough time, they could receive more cards from other genders or age groups. They would not receive cards for body parts not in their experiment since they would not be relevant to their question. Afterward they should summarize what they found. This should take no more than 30 minutes.

○ Detailed Instructions for a Class

1. Introduce the topic of skin bacteria. We all have many bacteria on our skin. Most are beneficial, but sometimes they get out of balance. There are different bacteria on different parts of the skin. Different people have different bacteria on their skin. In this exercise, students will think about whether there might be differences according to age, body part, or gender. We will not look at all of these at once, but students will think about possible differences and come up with a hypothesis in their group, using one of the categories, age, body part, or gender. A hypothesis has a directional prediction and a reason. *A* increases with *B* because of biological reason *C*. This exercise is about hypothesis testing with this sample and is not about who actually has the most bacteria or where, since we would need a much bigger sample for that.
2. Once the students have been introduced to the topic, have them discuss the question and write down their hypotheses. They should tell the teacher which cards they will need to test their hypothesis.
3. Give the students the necessary cards to test their hypothesis. For example, if they predict there is more bacteria in the mouth than the armpit, then they only need cards for mouth and armpit and it can be from all ages and genders for those two body parts, and if they predict older people have more bacteria than younger people, then give them only cards of females since they do not have older subjects of another gender.
4. Have the students sort the cards according to their hypotheses. Tell them not to turn the cards over until they are sorted, so they do not know if a hypothesis was supported or not until they have sorted the cards. They should sort them according to their predictions. If they predicted more bacteria in the mouth than the armpit, then they should put the images with more bacteria in the mouth pile.
5. Have the students turn over their cards and see if their hypotheses were supported. We do not say *right*, just *supported*. Let them discuss why this might be. Have them write a few sentences on the results.

○ Assessment

To assess the success of our activity, we trialed it at the Ferguson Farmers Market in Missouri so that we could engage children and adults who were not scientists. Over the two weekends we presented our activity, over 300 people came to our booth and completed our activity. Participants ranged from grade-school children to teenagers to adults.

We presented each participant with two options to engage in the activity. They could fill out worksheets that stepped them through the activity with guiding questions, or they could work with one of the activity facilitators to verbalize their thought process. We found that most opted for the second option, saying that the worksheet seemed too formal and that they preferred the more personal verbal communication.

Most participants found it daunting to create their own scientific hypothesis. We saw that by breaking down the process of creating a hypothesis and asking the guiding questions in Table 2, participants were more willing to slow down and reflect on what factors they recognized from their own interactions with their microbiome that could affect the bacterial populations. We also found that because each participant was testing their own hypothesis, they had a higher level of ownership over their results, which led to increased curiosity about why their hypothesis was or was not supported.

Overall, we saw that participants were able to not only engage in scientific activities but also grasp the individual steps necessary to formulate a hypothesis and use experimentation to determine whether it is supported.

○ Extensions

After students finish the activity, teachers can ask them to create a short presentation about their findings and present it to the class. This can be an important introduction into the world of modern scientific research in which peer review is a fundamental feature. Important facets that each student should emphasize include the question they are asking, the hypothesis and how they form it, how they test their hypothesis, and ways they could improve future experiments. After each presentation, teachers should leave opportunity for class discussion. Teachers should try to focus the conversation on not only what the student did well but also what they could do to improve the experiment. Overall, this presentation should help refine students' communication skills, challenge them to draw conclusions from their results, and teach them how to incorporate feedback from their peers in future experiments.

Although this activity was designed to introduce students to engaging in hypothesis-driven research, extensions of the activity could provide high school and undergraduate students the opportunity to develop wet-lab and statistical skill sets they need for scientific critical thinking. For example, rather than providing students with a picture of premade bacterial plates, students could culture samples from their own bodies using a sterile technique and agar plates. This could introduce them to wet-lab procedures necessary for future biological experimentation. Procedures for creating agar plates and taking bacterial samples can be found in Supplement 2 available with the online version of this article.

Instructors could also use the activity to show students basic statistical analyses necessary for hypothesis-driven research. For example, the instructor could separate the class into two groups based on a single categorical variable that could affect their skin microbiome populations. One easy example is half the class washing their hands with antibacterial soap and then each student culturing their own bacterial samples and taking quantitative measurements (i.e., number of bacterial colonies, morphology, color). The instructor could then show how to utilize statistical tests, such as the Mann-Whitney U test, to determine whether there are statistical differences between groups.

○ Supplemental Material

The following supplements are available with the online version of this article:

- Supplement 1: Illustrations of sampled body regions from one of the participants. Copy and cut out, perhaps pasting on card stock so each group of students has a set.
- Supplement 2: Images of the petri plates in a form so they can be cut out and folded over with the identifying information on the back. Each group of students should be given the cards that fit their hypothesis. For example, if it is about age, they should be given only women cards since there are no old men in their sample. If their hypothesis is about hair compared to nose bacteria, they should be given only images of petri plates that came from hair or nose samples.

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