Supplemental Materials

for

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

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Savvy Consumer lab pre/post-quiz

NAME: _________________________

Circle ALL the correct answers. **Questions might have more than one right answer.**

1. You have received a moisturizer sample contaminated with *E. coli* bacteria. How can the presence of these organisms be confirmed?
   a. Looking at the sample under the microscope
   b. Extracting and sequencing DNA from the sample
   c. Using a specific stain to visualize *E. coli*
   d. Plating part of the sample in selective and differential microbial media

2. Microorganisms are normally identified based on their
   a. Shape
   b. Color
   c. Biochemical properties
   d. Genetic composition (DNA sequence)

3. A good scientific hypothesis
   a. Definitively proves a scientific question right or wrong
   b. Is based on prior observations
   c. Will always be confirmed
   d. Must be testable but also falsifiable (can be proved wrong)

4. Are “natural” products safer to use than comparable types made from synthetic materials?
   a. Yes, because synthetic chemicals are harmful to humans
   b. Yes, because natural products have had more extensive testing prior to their release
   c. No, because natural products may still contain ingredients that cause allergic reaction, and may be less pure than their synthetic counterparts
   d. No, because they often lack preservatives, and thus may spoil or become more easily contaminated with bacteria

5. Some microbial media contain dyes that change color upon acid production. Why is this type of test important to microbiologists?
   a. Because acid production kills harmful bacteria
   b. Because acid production allows isolation of fungi
   c. Because acid production is a property commonly used to distinguish between different types of bacteria
   d. Because acid production stimulates microbial growth and facilitates bacterial isolation

6. Circle the set of steps that best describe the treatment of a cosmetic sample for microbial analysis
   a. Extraction, dilution, plating, isolation
   b. Isolation, extraction, dilution, plating
   c. Plating, isolation, dilution, extraction
   d. Dilution, extraction, plating, isolation

7. Which of the following is an example of a non-selective (general isolation) medium?
   a. Mannitol salt agar (MSA)
   b. Eosin methylene blue agar (EMB)
   c. Nutrient agar (NA)
   d. Sabouraud dextrose agar (SDA)
8. Cosmetic products provide a favorable environment for microbes to grow because
   a. Their chemical composition provide nutrients for microbial growth
   b. Bacteria and fungi get immobilized and are unable to leave cosmetic products’ surfaces
   c. The surface of the cosmetic product provides protection for bacteria
   d. Cosmetic products contain substances considered microbial attractants that facilitate colonization by fungi and bacteria

9. Which of the following microbial media would be more effective at detecting fungal (mold and yeast) contamination of a given cosmetic product?
   a. Mannitol salt agar (MSA)
   b. Sabouraud dextrose agar (SDA)
   c. Eosin methylene blue agar (EMB)
   d. Nutrient agar (NA)

10. When using cosmetics, best practice means
    a. Using expired products as long as they don’t smell or look bad
    b. Not sharing the cosmetic with anyone else
    c. Adding water to hydrate it if the product has dried out
    d. Not using in-store “testers” to apply products
    e. Following the storage directions
    f. Washing your hands before use

11. The salt content of mannitol salt agar (MSA) allows microbiologists to detect contamination with the following organisms:
    a. *Escherichia coli* and *Salmonella*
    b. Fungi and bacteria
    c. *Escherichia coli*
    d. *Staphylococci* species

12. When grown on eosin methylene blue (EMB) medium, some bacteria appear to have green coloration. What does the color change indicate?
    a. Death bacterial cells
    b. Sugar (lactose) fermentation and generation of acid products
    c. Presence of bacteria that are reproducing rapidly
    d. Decomposition of the purple dye in the medium

13. What is the purpose of expiration dates on cosmetics?
    a. They are a gimmick used by companies so consumers will buy their products more often
    b. They guarantee a time frame in which the cosmetic is safe to use
    c. They indicate the time frame where the cosmetic ingredients are relatively stable and should not have broken down/spoiled
    d. They indicate the date after which a product will become dangerous or ineffective to use?
Biology of Women: Savvy Consumers Lab (15 points)

Awareness of problems or deceptions associated with the products we buy is necessary for us to be advocates for safer cosmetics. When looking for cosmetics, we often find that right next to a well-known brand, such as Olay®, there sits a generic product. Are they the same thing in less fancy packaging? Or is there a significant difference between the products that justifies their price difference? Who regulates the ingredients that go into these products? Who regulates their safety? Additionally, manufacturers frequently make claims about things their products can do to alter our bodies. How realistic are these claims, biologically speaking? Are we being duped?

Most cosmetics are expensive, and as such, we may be reluctant to buy multiple brands to compare with one another. Some of us have cosmetics lying around that we thought would be a good purchase, but then turned out to be a waste of money when we discovered we didn’t like the product. Or we may have old products that are past their expiration date that we are unwilling to throw out, because it doesn’t seem to justify the cost to throw away a half-used product. But are these products still safe if they have expired?

The objective of this lab is two-fold. First, you will become more aware of the presence of microorganisms in your environment (and possible your cosmetics!), and recognize that their presence is often overlooked or conveniently ignored. Secondly, by learning to read product packaging and understanding the biology of beauty products, you will become more aware of the common “tricks” manufacturers use to influence your purchase. In this manner, you will be better equipped to make your own, informed conclusions about the safety of the products you use.

COSMETICS

Cosmetics are used to cleanse the body or to alter the body’s appearance or odor. They include lotions and creams, make-up, perfumes, sunscreens, hair dyes, toothpastes, and deodorants. Many of these products claim to improve our appearance. While there is science (chemistry and biology) that goes into the design of beauty products, many of the claims made by companies regarding their products are misleading. Additionally, cosmetics can be a source of irritation to the skin, and sometimes cause allergic reactions or infections. Fragrances and preservatives in the cosmetics can cause skin problems such as rashes or acne, and may harbor bacteria, molds, fungi, or viruses that could infect the body.

Chemicals:

The FDA does not require cosmetics companies to test their products prior to releasing them on the market. It is illegal to sell cosmetics known to contain poisons or other harmful materials that would injure consumers when used normally, or to sell products that are rancid or unclean. However, cosmetics manufacturers may use any ingredient in their products other than those materials that are prohibited, and they do not have to test the safety of these ingredients. More than half of cosmetics available for purchase have no toxicity information, which leaves us in the dark regarding the safety of their ingredients for human use. Additionally, companies are currently not required to report if a product has harmed a consumer; reports are strictly voluntary. There are some clues to help us, though. If the manufacturer has not tested the product, they must include a label on the packaging that states: "WARNING—The safety of this product has not been determined."

The FDA does require testing and adherence to rules regarding the use of colorants derived from coal tar. Coal tar derivatives have been shown to cause cancer in animals, but are allowed in cosmetics because they are only applied to the skin and aren’t being directly injected into humans (as they are in laboratory animals). On packages, coal tar derived colorants are listed as:

- FD&C – color that can be used only in foods, drugs, and cosmetics
- D&C – color that can be used only in drugs and cosmetics
- External D&C – color only to be used in drugs and cosmetics applied to the skin surface
Some cosmetics contain ingredients that are drugs. These also require FDA testing. These “cosmeceuticals” include fluoride-containing toothpastes, antiperspirants, dandruff shampoos, and moisturizers and make-up with sun protection. These are identified as “active ingredients.” It is important to be aware of active ingredients, as their use may be associated with dangers such as increased sun-sensitivity or even poisoning if too much of the active ingredient is consumed.

It is a good idea to follow the directions for proper storage of a cosmetic; increased heat may cause ingredients in the cosmetic to break down. If the ingredients degrade, they may irritate the skin or cause the product to separate or change consistency. Alterations in texture or odor of a cosmetic are a sign that it has gone bad and should be disposed of.

Cosmetic terms to be aware of:

- “Cruelty-free” or “not tested on animals”—just because the company itself did not test their final product on an animal does not guarantee that in the evolution of the product’s ingredients no animal was tested or harmed.
- Hypoallergenic—claim that the product causes less allergic reactions than similar products. These products typically have the most common irritants removed, but doesn’t mean that you won’t get acne or other skin irritations. The FDA does not ask companies to prove the product is allergen-free—therefore, the claim means whatever the manufacturer wants it to mean.
- For “sensitive skin”—people have different skin conditions (acne, rosacea, allergy-prone skin), but these products are marketed to all people with skin that is easily irritated.
- Natural—A product that does not contain synthetic ingredients. Be cautious, as many natural compounds can still cause severe allergic reactions or irritation. (i.e. Poison ivy is “natural” but it’s not good for you!) Some natural oils can cause acne outbreaks.
- Non-comedogenic—this means the product will not close the pores of the skin, so it should be less likely to cause acne. Pore-blockage alone is not the sole cause of acne, however, and these claims are not necessarily scientifically tested.
- Dermatologist tested—this simply means some dermatologist, somewhere, tested the product. It does not mean that dermatologists recommend it, or that it won’t cause acne.

Bacterial and fungal contaminants:

Some cosmetics have expiration dates, and the rest have “recommended” expiration dates that can be found through a simple Internet search. A random look through someone’s cosmetics collection would likely show that most people don’t heed these expiration dates. Considering that the product may not have been used up by the expiration date, and the fact that cosmetics are expensive, many of us continue to use expired cosmetics. However, there is good reason for adhering to expiration dates: bacteria and fungi from our bodies and the environment can grow in cosmetics.

Bacteria are small, single-celled organisms that reproduce rapidly. A basic feature of bacteria is their prevalence and persistence in all environments with which man comes in contact. Some bacteria belong where they are found, comprising a normal flora in balance with that particular environment. Others may be transitory, competing more or less effectively—or barely surviving—and are found in these places merely as a consequence of their ready dispersal. Individual bacterial strains may be restricted to highly specific environments, growing only when selective conditions are encountered. Nonetheless, even these bacteria may show a surprising ability to survive in unfavorable situations. It is essential to recognize that microorganisms are everywhere because even highly specialized and finicky pathogenic (disease-causing) bacteria may become widely dispersed and survive long enough to infect.

Bacteria can cause cosmetics to spoil, break down the ingredients, and cause infection in their users. While the FDA may not require testing of cosmetic ingredients, it is the best interest of the manufacturers to be sure that their products will not readily harbor bacteria. Cosmetics are recognized to be ideal
environments for the survival and development of a large variety of microorganisms, since they possess some of the nutrients that facilitate their growth (lipids, sugars, proteins, amino acids, alcohol, and vitamins.) Also, the features we want from our cosmetics (inability to separate, silky or creamy consistencies, pleasant textures or odors, etc) require the addition of oils, oxygen, and other additives that favor microbial growth.

Because of this, microbiological analyses are routinely conducted in industrial labs to determine the quality of a cosmetic product and test for fungi and potentially pathogenic bacteria. Preservatives or antimicrobial agents may be added to prevent or slow the growth of bacteria in the product. In fact, products that are considered “natural” because they have no added preservatives may be more likely to grow bacteria, as they often lack ingredients that would normally kill microbes.

While the manufacturers may take steps to prevent bacteria or fungi from growing in their products before they reach the consumer, it is also important to recognize that as consumers, we need to be responsible in our use and storage of these products to minimize their contamination.

In this lab, we will be testing cosmetics to see if they indeed harbor bacteria or fungus. How do we determine if our cosmetics are contaminated? Microbiologists use a variety of media to grow and isolate microbes. Culture media consists of liquids or gels that favor the growth of cells. There are many different types of culture media available, depending on which organism you wish to grow. Some media allow a wide range of organisms to thrive. Others are specialized to restrict growth only to particular species with specific nutritional or environmental needs.

The following specialized agar will be used in this lab:

- **Nutrient agar (NA):** This is a general purpose culture medium because it is of rich composition, and provides a variety of nutrients appropriate for most bacteria and fungus to grow.

- **Mannitol salt agar (MSA):** This red-colored medium contains a high salt concentration which inhibits the growth of most bacteria other than the staphylococci. Staphylococci are mostly benign, and are found on our skin and mucous membranes. A few species have been identified to cause human infection, particularly *Staphylococcus aureus*, which can cause minor infections such as acne to major, life-threatening abscesses, toxic shock, and blood poisoning (septicemia). Mannitol is a sugar alcohol, and bacteria capable of fermenting it produce acid that cause a color change in the media. Mannose-fermenting staphylococci exhibit a yellow zone surrounding their growth; staphylococci that do not ferment mannitol will not produce a color change.

- **Eosin methylene blue agar (EMB):** This medium contains a purple dye that inhibits growth of Gram-positive organisms, allowing a variety of Gram-negative bacteria to grow. Many Gram-negative bacteria are pathogenic to humans, causing gastrointestinal problems, STDs, and meningitis. In EBM medium, lactose-fermenting bacteria metabolize the lactose (a milk sugar) in the media and produce acid, causing a color change. Strong acid production by organisms such as *E. coli* results in a metallic green sheen. Weaker fermentation of lactose results in colonies with a pinkish-purple color. Colonies of non-lactose fermenters remain colorless.

Fungi, unlike bacteria, are eukaryotes, and can exist as single cells or as multicellular organisms. While we use fungi for many important processes, such as cheese, bread, beer and wine making, enzymes for industrial chemicals, and medicines, they can also cause fungal infections ranging from cosmetic (athlete’s foot) to quite serious (respiratory infections, pneumonias, cancers, even death). To test for the presence of fungi in your products we will be using Sabouraud dextrose agar:

- **Sabouraud dextrose agar (SDA):** SDA is a medium used for growing fungi because the nutrients and slightly acidic environment encourages fungal growth and survival. Fungi such as yeast grow well on SDA, and it can also be used to grow molds.
Week 1 Cosmetics Experiment:

First, observe your cosmetic products. Look at the labels and the physical qualities of the product, noting texture, odor, and other observations. Following your analysis, answer the questions below:

1. When making decisions about a beauty product, what factors do you take into consideration when deciding to purchase the product?

2. Does your product make any claims about what it will do for your appearance? Do you think there is a biological basis for the claim, or is it just a marketing gimmick? Why do you think so?

3. Do any of the products contain coal tar derivatives? Which ones, and how do you know this?

4. Do your products contain anything that suggests they are “cosmeceuticals” (a cosmetic that contains a drug?) How do you know this? If they do, what is the drug?

5. Cosmetics may become contaminated with microbes. When considering different cosmetics:
   a. Which products would you predict to have bacteria? Why?
   b. Which ones might you expect to contain fungi or molds? Why?
   c. Where do these contaminants come from?

Following discussion of your observations and predictions, use the protocol below to extract microbes from your product.

Protocol to Isolate Bacteria from Cosmetics

1. Using an inoculating loop, take a sample of the cosmetic product that you need to analyze and spread it onto one plate of each of the following microbial media:
   a. NA (Nutrient agar),
   b. SDA (Sabouraud Dextrose Agar)
   c. EMB (EosinMethyleneBlue Agar)
   d. MSA (Mannitol Salt Agar).

2. Weigh 0.5 grams of your sample and mix it with 5 ml of the sterile diluent. Shake vigorously to mix and extract the microbes from the sample. Label this tube as #1

3. Take 0.5 ml of Tube #1 and place it in 4.5 ml of sterile diluent. Mix as described. This sample has been diluted 100 times. Label this tube as #2

4. Take 0.5 ml of tube #2 and place it in 4.5 ml of sterile diluent. Mix as described. This sample has been diluted 1000 times. Label this tube as #3

5. Plate 50 μl of each diluted sample (tubes#1, #2 and #3) onto individual NA, SDA, EMB and MSA plates. Gently spread the samples across the surface of the agar using a glass “hockey stick.” You will have 16 plates total by the time you are done with steps 1-5.

6. Allow the solutions to absorb into the agar, seal them with Parafilm, and then place the plates upside down in a 37°C incubator. (To avoid spreading spores, leave the SDA plates right-side up.) You will observe the plates next week in lab.
**Week 2 Cosmetics Experiment:**

Observe, *but do not open*, the plates you set up last week. Count any bacterial colonies or fungi that have grown on plates inoculated with cosmetic samples. You will need to describe the bacterial or fungal growth observed. It will be useful to learn the terminology for describing common colony types. The following outline will help you to communicate the appearance of observed colony growth.

**Form** – The form refers to the shape of the colony. These forms represent the most common colony shapes you are likely to encounter:

- **a. Size** – The size of the colony can be a useful characteristic for identification. The diameter of a representative colony may be measured. Tiny colonies are referred to as *punctiform*.
- **b. Surface** – Bacterial colonies are frequently shiny and smooth in appearance. Other surface descriptions might be: *veined, rough, dull, wrinkled/shriveled, or glistening*.
- **c. Texture** – Several terms that may be appropriate for describing the texture or consistency of bacterial growth are *dry, moist, mucoid, brittle, viscous, or butyrous (buttery)*.
- **d. Color** – It is important to describe the color or pigment of the colony. Also include descriptive terms for any other relevant optical characteristics such as *opaque, cloudy, or translucent*.

You will be provided with a table to record information about your plates.

1. Based on the plates you set up, did your product(s) harbor bacteria or fungus?
   - a. Were there bacteria in your product(s)? If so, what kinds?
   - b. Was there fungus present in your product(s)?
   - c. Did your results surprise you? Why or why not?
   - d. If you predicted a product to contain bacteria or fungi, and you didn’t see growth on your plate, what explanation might there be? Provide at least two possibilities.

2. What steps can you take to minimize infections from cosmetics? Propose at least 3.

3. Based on your results, would you continue to use this product in the future? Why or why not?

**References:**

1. Arvidson, Cindy. 2009. Cultivation Media for Bacteria. MicrobeLibrary

*Special thanks to Dr. Betsy Martinez-Vas (Hamline University) for her assistance in writing the microbiology information and protocol.*
Mr. Ag Lafley, CEO
Procter and Gamble

Dear Mr. Lafley:

This letter is in reference to inspections of your OTC human drug products and cosmetics manufacturing facility Olay LLC., Puerto Rico, conducted between August 25 to October 1, 2008 and November 5 to November 12, 2008, by an investigator from the Food and Drug Administration (FDA). The inspection revealed that your firm's manufacture, processing, packing, or holding of human OTC drug products deviate from the Current Good Manufacturing Practice Regulations (CGMPs), rendering the drugs adulterated within the meaning of the Federal Food, Drug, and Cosmetic Act (the Act). The manufacture and processing of your OTC drug products do not conform with CGMPs to assure that the drug products meet the requirements of the Act as to safety, and have the identity and strength and meet the quality and purity characteristics that it purports or is represented to possess. The inspection also revealed that your OTC drug products have been prepared, packed and held under insanitary conditions whereby they may have been contaminated with filth or rendered injurious to health, and are, therefore, adulterated within the meaning of the Act.

Additionally, several of your cosmetic products have been prepared, packed, or held under insanitary conditions whereby they may have become contaminated with filth, or whereby they may have been rendered injurious to health, and are, therefore, adulterated within the meaning of the Act.

At the conclusion of the inspections a List of Inspectional Observations was issued. We addressed these responses below, in relation to each of the noted violations where appropriate.

**Drug Products:**

Our investigator documented significant violations of CGMP regulations including, but not limited to, the following:

1. You did not evaluate if at least 4 lots of OTC drug products (Olay C&C daily scrub, Olay TE Antiblemish Cleanser) out of approximately 50 lots of products were contaminated with objectionable microorganisms. A gram negative bacilli was detected in the beginning and fourth hour sample for microbial growth (filling/packaging process) of finished product Olay Total Effects Revitalizing Daily Foam. A retest confirmed the initial results. Those potentially affected lots were released from July 14, 2008 to August 12, 2008.
We reviewed your October 31, 2008 response letter which addressed this observation. You indicated that the corresponding finished product microbial content testing confirmed no recovery of the microorganisms initially detected on the filling equipment monitoring samples. You concluded that "this is indicative of a low level transient contamination that was killed off by the product preservatives system." We find this conclusion highly objectionable because the purpose of adding preservatives to drug and cosmetic products is not to kill microorganisms present in your finished products due to poor manufacturing practices, but rather to prevent the growth of microorganisms in products manufactured in compliance with good manufacturing practices.

2. Inspectional evidence shows that from June 30, 2007 to August 31, 2008, your firm received 198 health effect-related complaints for the Vick Sinex product that could indicate a failure of the product to meet its specifications. None of these were referred for a manufacturing investigation.

3. You indicated that a formulation change is being pursued for your Sinex Long Acting Nasal Spray, but you do not mention whether you are planning to validate the new manufacturing process, and you have made other changes to the formulation of this product without validating their effectiveness. We find it highly objectionable that you continued manufacturing and distributing this product. We would like to remind you that it is unacceptable for a manufacturer of drug products to distribute products prior to completing its validation.

The justification provided in your response for failing to conduct an investigation of about complaints reporting is inadequate. You indicated these complaints were not investigated because they did not meet your criteria in terms of complaints reported per month required for initiating a manufacturing investigation. The number of complaints received should not be the only factor considered to initiate a manufacturing investigation, especially when manufacturing issues could be related to the complaints.

In addition, our investigators found that your OTC drug products have been prepared, packed and held under insanitary conditions whereby they may have been contaminated with filth or rendered injurious to health, and are, therefore, adulterated. As noted above, at least two lots each of two of your firm's OTC drug products were manufactured which included product contact surfaces that were later found to be contaminated with multiple species of potentially injurious gram-negative bacteria. This indicates that the sanitization practices employed by your firm were insufficient to prevent contamination with these organisms. Although you had evidence of possible contamination in your manufacturing equipment prior to the manufacture of these products, you proceeded to use this filling line to manufacture drug products and did not quarantine the products or provide a rationale for allowing the products to remain in distribution when this contamination was later confirmed.

Cosmetics:

Our inspection also revealed that several of the hair care and skin care cosmetics manufactured in your facility, including your Olay Total Effects Revitalizing Daily Foam, Olay Regenerist Micro Sculpting Cream, Olay Moisture Foaming Face Wash, and Olay Moisture Rich Cream Cleanser, are adulterated within the meaning of Section 601(c) of the Act because they have been prepared, packed, or held under insanitary conditions whereby they may have become contaminated with filth, or whereby they may have been rendered injurious to health. Specifically, the inspection revealed your current procedures are not adequate to prevent your cosmetic products from being contaminated with gram-negative organisms.

Your firm issued several Quality Notices in 2008 because you found growth of the gram-negative organisms in several portable tanks even after sanitizing each tank. These tanks are used to hold cosmetic products prior to filling. Even though you had evidence of microbial contamination in your manufacturing
equipment, you released the products based on acceptable bulk and finished product testing. Our investigator determined that, despite evidence that your portable tanks had been contaminated with these organisms, you continued your practice of monitoring these tanks on a monthly basis and did not modify your cleaning and sanitization procedures to prevent such contamination from recurring.

We reviewed your October 31, 2008 response. Your response indicates that the contamination was at a low level. Although the level of contamination may have been low, the presence of any gram-negative organisms may render products held in your tanks injurious to health because, if tanks are contaminated with these organisms, these organisms can be incorporated into finished products, and any amount of gram-negative organisms in a finished product has the potential to cause illness.

You also indicate in your response that your finished-product microbial content testing did not detect the organisms initially detected in the monitoring samples because they were "killed off" by preservatives in the product. However, this statement does not address your failure to adopt procedures sufficient to prevent contamination of your cosmetic products by gram-negative organisms. These test results do not guarantee that these products are not contaminated. Preservatives in these products may fail to eliminate gram-negative organisms and, therefore, your finished cosmetic products may be rendered injurious to health by such organisms even if finished-product testing does not indicate their presence.

The violations cited in this letter are not intended to be an all-inclusive list of the deficiencies that may exist at your facility. You are responsible for investigating and determining the causes of the violations identified above and for preventing their recurrence or the occurrence of other violations. It is your responsibility to assure that your operations at this facility and all other facilities under your control comply with all requirements of federal law and FDA regulations.

You should take prompt action to correct the violations cited in this letter. Failure to promptly correct these violations may result in legal action without further notice, including, without limitation, seizure, and injunction. Other federal agencies may take this warning letter into account when considering the award of contracts. Additionally, FDA may withhold approval of requests for export certificates, or approval of pending new drug applications listing your facility as a manufacturer until the above violations are corrected. A reinspection may be necessary.

Within 15 working days of receipt of this letter, please notify this office in writing of the specific steps that you have taken to correct violations. Include an explanation of each step being taken to prevent the recurrence of violations as well as copies of related documentation. If you no longer manufacturer or market the above mentioned products, your response should so indicate, including the reasons that, and the date on which, you ceased production.

Your reply should be sent to the Food & Drug Administration, San Juan District Office, San Juan, PR, to the attention of Margarita Santiago, Compliance Officer.

Sincerely, Maridalia Torres
District Director
San Juan District

cc: Mr. Ezio Garciamendez
Plant Manager
Olay LLC
Writing a Scientific Hypothesis

A scientific hypothesis is a statement that proposes an explanation for observable phenomenon. In a sense, it’s an educated guess based on prior observations that can be tested by the scientific method. In the scientific method, a hypothesis is made regarding prior observations or data collected, and then an experiment is designed to test it. With data in hand, the hypothesis can then be supported or refuted and revised. Scientific theories are backed up by a great number of such hypotheses that have been tested and supported by many different scientists. Theories are always evolving, and as long as the hypotheses supporting the theory are not falsified, the theory is generally regarded as the truth. When designing a sound hypothesis, it is important to remember a few key points:

- A hypothesis must be falsifiable. That is, it must be able to be proven wrong through experimentation. For example, if you made the statement, “The government replaced my brother with an identical clone,” the statement cannot be falsified. As ridiculous as it sounds, how are we to test or refute this statement? On the other hand, a statement such as, “Cloned animals behave identically to their parent” is falsifiable, because it can be tested. All you have to do is observe the behavior of the clones in comparison to their parent. Falsifiable does not mean “false,” it just means that you can test the hypothesis and collect data that allow you to make a conclusion to support or not support your claim. Falsifiability is what separates science from non-science.

- A good hypothesis will make a prediction following a proposed relationship between two variables. A poorly written hypothesis fails to predict what you expect as an outcome. For example, if you hypothesize, “Increasing the temperature may affect the cells,” any result you get could be interpreted as supporting your hypothesis. But at the same time, you have provided no indication of how you will test the hypothesis. What will you measure? On the other hand, if you hypothesize, “If cells perceive heat as stress, then increasing the temperature will cause the cells to produce stress-response proteins,” you then have a good hypothesis from which you can perform experiments, collect data, and determine if the hypothesis was supported or not.

- It’s OK for your hypothesis to be incorrect. A hypothesis is simply a prediction, and sometimes our predictions are incorrect. We typically don’t say the hypothesis was “proven,” because it may be that future data refutes the hypothesis. Hypotheses are typically “supported” or “rejected.”

- A hypothesis may not always be confirmed. Suppose we hypothesize, “If girls enter puberty sooner than boys, then 10-year-old girls are taller than 10-year-old boys.” If we measure a population of children, we might find that the average height of girls compared to boys is statistically similar enough that we cannot support or refute our hypothesis. We would then need to revise our hypothesis.
**Hypothesis Workshop:**

1) Based on the letter to Olay LTD as well as the lab handout, write a hypothesis regarding the safety of unopened cosmetic products.

2) Read the following question and hypothesis below. Then, design a simple experiment you could perform to test the hypothesis.

   **Question:** Does sharing lipstick increase its microbial contamination?
   
   **Hypothesis:** Communal usage of lipstick does not affect the microbial content of the product and presents no risk of bacterial contamination.

3) For the following hypotheses, determine whether the hypothesis is strong. If it is not a strong hypothesis, explain why, and re-write the hypothesis so that it is improved.

   Hypothesis #1: Hand soap is bad for bacteria.

   Hypothesis #2: Use of antibacterial hand sanitizer is better at reducing the spread of germs.

   Hypothesis #3: *E. coli* are present in raw hamburger, and will be evidenced by a green sheen when cultured on EMB agar.
4) For the purposes of today’s lab, formulate a scientific question and hypothesis regarding microbial contamination in a cosmetic product that you own. You will be using the tools available in this lab to design an experiment to answer your question and test your hypothesis.
<table>
<thead>
<tr>
<th>Plate/Sample</th>
<th>Product</th>
<th># of colonies</th>
<th>Colony size (diameter in mm)</th>
<th>Colony color (opaque, cloudy, translucent)</th>
<th>Colony texture (smooth, slimy, rough, mucoid)</th>
<th>Morphology (circular, irregular, filamentous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry—NA</td>
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<tr>
<td>NA—dilution 3</td>
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**For group experiment**
<table>
<thead>
<tr>
<th>Plate/Sample</th>
<th>Product</th>
<th># of colonies</th>
<th>Colony size (diameter in mm)</th>
<th>Colony color (opaque, cloudy, translucent)</th>
<th>Colony texture (smooth, slimy, rough, mucoid)</th>
<th>Morphology (circular, irregular, filamentous)</th>
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<tbody>
<tr>
<td>Dry—NA</td>
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<td>Dry—NA</td>
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**For individual student’s plates**
<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Incomplete (0)</th>
<th>Fair (0.5)</th>
<th>Good (0.75)</th>
<th>Excellent (1)</th>
<th>Score</th>
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<tbody>
<tr>
<td>Concept (2)</td>
<td>Hypothesis is illogical; does not answer scientific question (or none proposed)</td>
<td>Hypothesis proposes reasonable variables to be compared but lacks detail</td>
<td>Proposes a logical hypothesis based on general knowledge on the topic.</td>
<td>Proposes a sound hypothesis based on evidence from reading and/or lecture</td>
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<tr>
<td>Does not list variables to be compared and not falsifiable</td>
<td>Unable to be tested in lab as stated (lacks variables or not falsifiable)</td>
<td>Hypothesis can be tested in the lab.</td>
<td></td>
<td>Clearly designed to answer the scientific question proposed using lab materials; testable/falsifiable.</td>
<td></td>
</tr>
<tr>
<td>Prediction of measurable outcome (2)</td>
<td>Fails to predict an outcome</td>
<td>Hypothesis states prediction but does not include information to support it</td>
<td>Includes an explanation of why the proposed results are expected based on general knowledge</td>
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<tr>
<td>Fails to propose a way to measure the outcome</td>
<td>Does not suggest how results will be measured</td>
<td>Measurable outcome not clearly defined</td>
<td></td>
<td>Indicates a clear, measurable outcome</td>
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</tr>
<tr>
<td>Format (1)</td>
<td>Is written as a question</td>
<td>Written as a statement, but uses inappropriate language (proven, true, false, etc.) or vague descriptors</td>
<td>Written in proper format</td>
<td>Uses an “if…then” or “because…we predict” style of phrasing</td>
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**Total out of 5**
Grading Key for Assessment Test

Students should circle ALL the correct answers. For questions with more than one right answer, partial credit is given. Points are indicated in parentheses.

1. You have received a moisturizer sample contaminated with *E. coli* bacteria. How can the presence of these organisms be confirmed?
   a. Looking at the sample under the microscope
   b. Extracting and sequencing DNA from the sample
   c. Using a specific stain to visualize *E. coli*
   d. Plating part of the sample in selective and differential microbial media
   *(0.5) each for C, D*

2. Microorganisms are normally identified based on their
   a. Shape
   b. Color
   c. Biochemical properties
   d. Genetic composition (DNA sequence)
   *(0.33) each for A, C, D*

3. A good scientific hypothesis
   a. Definitively proves a scientific question right or wrong
   b. Is based on prior observations
   c. Will always be confirmed
   d. Must be testable but also falsifiable (can be proved wrong)
   *(0.5) each for B, D*

4. Are “natural” products safer to use than comparable types made from synthetic materials?
   a. Yes, because synthetic chemicals are harmful to humans
   b. Yes, because natural products have had more extensive testing prior to their release
   c. No, because natural products may still contain ingredients that cause allergic reaction, and may be less pure than their synthetic counterparts
   d. No, because they often lack preservatives, and thus may spoil or become more easily contaminated with bacteria
   *(0.5) each for C, D*

5. Some microbial media contain dyes that change color upon acid production. Why is this type of test important to microbiologists?
   a. Because acid production kills harmful bacteria
   b. Because acid production allows isolation of fungi
   c. Because acid production is a property used to distinguish between different types of bacteria
   d. Because acid production stimulates microbial growth and facilitates bacterial isolation
   *(1) for C*

6. Circle the set of steps that best describe the treatment of a cosmetic sample for microbial analysis
   a. Extraction, dilution, plating, isolation
   b. Isolation, extraction, dilution, plating
   c. Plating, isolation, dilution, extraction
   d. Dilution, extraction, plating, isolation
   *(1) for A*

7. Which of the following is an example of a non-selective (general isolation) medium?
   a. Mannitol salt agar (MSA)
   b. Eosin methylene blue agar (EMB)
   c. Nutrient agar (NA)
   d. Sabouraud dextrose agar (SDA)
8. Cosmetic products provide a favorable environment for microbes to grow because
   a. Their chemical composition provide nutrients for microbial growth
   b. Bacteria and fungi get immobilized and are unable to leave cosmetic products’ surfaces
   c. The surface of the cosmetic product provides protection for bacteria
   d. Cosmetic products contain substances considered microbial attractants that facilitate colonization by fungi and bacteria

(0.5) each for A, B

9. Which of the following microbial media would be more effective at detecting fungal (mold and yeast) contamination of a given cosmetic product?
   a. Mannitol salt agar (MSA)
   b. Sabouraud dextrose agar (SDA)
   c. Eosin methylene blue agar (EMB)
   d. Nutrient agar (NA)

(1) for B

10. When using cosmetics, best practice means
   a. Using expired products as long as they don’t smell or look bad
   b. Not sharing the cosmetic with anyone else
   c. Adding water to hydrate it if the product has dried out
   d. Not using in-store “testers” to apply products
   e. Following the storage directions
   f. Washing your hands before use

(0.25) each for B, D, E, and F

11. The salt content of mannitol salt agar (MSA) allows microbiologists to detect contamination with the following organisms:
    a. Escherichia coli and Salmonella
    b. Fungi and bacteria
    c. Escherichia coli
    d. Staphylococci species

(1) for D

12. When grown on eosin methylene blue (EMB) medium, some bacteria appear to have green coloration. What does the color change indicate?
    a. Death bacterial cells
    b. Sugar (lactose) fermentation and generation of acid products
    c. Presence of bacteria that are reproducing rapidly
    d. Decomposition of the purple dye in the medium

(1) for B

13. What is the purpose of expiration dates on cosmetics?
    a. They are a gimmick used by companies so consumers will buy their products more often
    b. They guarantee a time frame in which the cosmetic is safe to use
    c. They indicate the time frame where the cosmetic ingredients are relatively stable and should not have broken down/spoiled
    d. They indicate the date after which a product will become dangerous or ineffective to use?

(1) for C
Grading Key For Hypothesis Workshop:

1) Based on the letter to Olay LTD as well as the lab handout, write a hypothesis regarding the safety of unopened cosmetic products.

*Answers will vary. See hypothesis grading rubric in appendix 6.*

2) Read the following question and hypothesis below. Then, design a simple experiment you could perform to test the hypothesis.

*Question:* Does sharing lipstick increase its microbial contamination?

*Hypothesis:* Communal usage of lipstick does not affect the microbial content of the product and presents no risk of bacterial contamination.

*Answers will vary.*

3) For the following hypotheses, determine whether the hypothesis is strong. If it is not a strong hypothesis, explain why, and re-write the hypothesis so that it is improved.

Hypothesis #1: Hand soap is bad for bacteria.

*Unsound. Not falsifiable or vague (what does “bad” mean and how will it be measured?) Students’ re-written hypotheses will vary.*

Hypothesis #2: Use of antibacterial hand sanitizer is better at reducing the spread of germs.

*Unsound. Lacks a variable for comparison (better than what?) Students’ re-written hypotheses will vary.*

Hypothesis #3: *E. coli* are present in raw hamburger, and will be evidenced by a green sheen when cultured on EMB agar.

*This hypothesis is sound.*

4) For the purposes of today’s lab, formulate a scientific question and hypothesis regarding microbial contamination in a cosmetic product that you own. You will be using the tools available in this lab to design an experiment to answer your question and test your hypothesis.

*Again, answers will vary. See hypothesis grading rubric in appendix 6.*
Sample answers to student worksheet questions

Week 1 Cosmetics Experiment:

First, observe your cosmetic products. Look at the labels and the physical qualities of the product, noting texture, odor, and other observations. Following your analysis, answer the questions below:

1. When making decisions about a beauty product, what factors do you take into consideration when deciding to purchase the product?

   “Packaging—how attractive it looks. Commercials, price, and brand name.”

   “I usually choose products that are the cheapest or what my friends suggested.”

   “Some factors [our group takes] into consideration include whether or not it’s been tested on animals, dermatologist tested, hypoallergenic, won’t clog pores, etc. Also, the price and ingredients.”

2. Does your product make any claims about what it will do for your appearance? Do you think there is a biological basis for the claim, or is it just a marketing gimmick? Why do you think so?

   “My product says ‘soothing, cooling, refreshing.’ After using it, I know that it does leave a person with a cooling feeling, so it’s not just a gimmick.”

   “The product claims to make you more attractive. There really is no biological basis, it is simply marketing. Society tells them that women with features that stand out are more attractive.”

   “My lotion states on the bottle ‘leaves skin soft, silky, and scented.’ While the scent of the lotion is not a gimmick, the lotion doesn’t leave my skin soft and silky (maybe shiny and a little oily.)”

3. Do any of the products contain coal tar derivatives? Which ones, and how do you know this?

   “Mine doesn’t have the ingredients listed, so I’m not sure.”

   “The shaving gel contains the coal tar derivative FD&C Blue #1 and D&C #10.”

4. Do your products contain anything that suggests they are “cosmeceuticals” (a cosmetic that contains a drug?) How do you know this? If they do, what is the drug?

   “No. Nothing on the product indicated this.”

   “No, because there are no active ingredients listed.”

5. Cosmetics may become contaminated with microbes. When considering different cosmetics:
   a. Which products would you predict to have bacteria? Why?
   b. Which ones might you expect to contain fungi or molds? Why?
   c. Where do these contaminants come from?

   “Products that come into contact repeatedly with your fingers. Items that are moist. Bacteria are naturally occurring in our environments and we often contaminate these products ourselves.”

   “Any lip products. Each of these products is pressed to the user’s lips, and the mouth contains a lot of bacteria. The contaminants come from the surfaces that the product touches.”
Week 2 Cosmetics Experiment:
Observe, but do not open, the plates you set up last week. Count any bacterial colonies or fungi that have grown. You will be provided with a chart to record information about your plates. Please fill it out completely.

1. Based on the plates you set up, did your product(s) harbor bacteria or fungus?
   a. Were there bacteria in your product(s)? If so, what kinds?
   b. Was there fungus present in your product(s)?
   c. Did your results surprise you? Why or why not?
   d. If you predicted a product to contain bacteria or fungi, and you didn’t see growth on your plate, what explanation might there be? Provide at least two possibilities.

   “Yes, there was one colony of bacteria and one fungus. I was not surprised that not many were contaminated as that is what I predicted. The fungus probably accidentally got onto the plate. There might not be growth because it was not an optimal environment or it did not have enough time to develop.”

   “There was bacteria and fungus in my lip gloss. The fungus was probably accidental contamination. I figured there would be bacteria, but actually seeing it on the plate surprised me.”

   “No growth in the lotion. The lotion is within a closed tube and you have to squeeze it out. So it’s probably harder for things to get in.”

2. What steps can you take to minimize infections from cosmetics? Propose at least 3.

   “Wash your hands before you use them, do not share, and do not use beyond the expiration date.”

   “Wash brushes and applicators, don’t share with anyone, replace expired make-up.”

   “Follow the storage instructions. Try not to leave products open and exposed.”

3. Based on your results, would you continue to use this product in the future? Why or why not?

   “I would still use the product. You just have to try to be careful about how you use them.”

   “I probably would continue to use this product. The results were kind of gross, but people use chapstick everyday and don’t get sick.”

   “It’s probably time for a replacement.”

   “Probably not, since I’ve been pretty grossed out by the whole thing.”
Hypothesis Workshop:

1) Based on the letter to Olay LTD as well as the lab handout, write a hypothesis regarding the safety of unopened cosmetic products.

“If cosmetic products are created and packaged without being contaminated by bacteria then an unopened cosmetic product will contain no harmful bacteria.”

“If cosmetic products are developed and packaged in an unsanitary area, then they will produce more contaminants than products from a sanitary area.”

“Unsanitary conditions during the production process of cosmetics render them harmful to the health of consumers.”

“Unopened cosmetic products have just as much bacterial contamination as products that have already been opened and used.”

2) Read the following question and hypothesis below. Then, design a simple experiment you could perform to test the hypothesis.

**Question:** Does sharing lipstick increase its microbial contamination?

**Hypothesis:** Communal usage of lipstick does not affect the microbial content of the product and presents no risk of bacterial contamination.

“Have forty women use the same lipstick. First, swab the lipstick before anyone has used it and test for bacteria. Then swab the lipstick after all forty women used it to check for bacteria that could have collected while being passed from person to person.”

“I would create an experiment using dry swabs and diluted swabs on NA, MSA, and EMB. I would use a lipstick that was used communally and as a control a lipstick that was used by only one person.”

“Take 10 samples of lipstick that have never been opened as a control group. Then obtain 10 samples of lipstick used by women who do not share their cosmetics, and 10 samples from cosmetic sampling counters where products are shared. Label and test all products separately, using both NA and EMB agar to test for bacteria. Observe results and write up conclusions.”

3) For the following hypotheses, determine whether the hypothesis is strong. If it is not a strong hypothesis, explain why, and re-write the hypothesis so that it is improved.

**Hypothesis #1:** Hand soap is bad for bacteria.

“Unclear what bad means. Hand soap reduces the amount of bacteria on a person’s hands.”

“Not a sound hypothesis—‘bad’ is ambiguous and cannot be falsified. Softsoap antibacterial hand soap kills 99% of bacteria.”

**Hypothesis #2:** Use of antibacterial hand sanitizer is better at reducing the spread of germs.

“Better than what? Use of antibacterial hand sanitizer is better than hand soap at reducing the spread of germs.”

“Does not specify better than what—not falsifiable. Use of antibacterial hand sanitizer is effective at reducing 80% of germs.”
Hypothesis #3: *E. coli* are present in raw hamburger, and will be evidenced by a green sheen when cultured on EMB agar.

“This is a sound hypothesis.”

“No, which hamburger? *E. coli* are present in store-shelf ground beef.”

“This is a sound hypothesis. It can be measured as either supported by the presence of a green sheen or rejected by lack of a green sheen.”

4) For the purposes of today’s lab, formulate a scientific question and hypothesis regarding microbial contamination in a cosmetic product that you own. You will be using the tools available in this lab to design an experiment to answer your question and test your hypothesis.

“My lip balm will have a higher level of microbial contamination than the concealer I use, because I use the lip balm more and have to put my finger in it to use it.”

“Due to a lack of antimicrobial properties, the used women’s Degree Invisible Solid will contain bacterial contaminants.”

“A year of use, there will be a higher presence of bacteria compared to fungi or mold.”

“Older cosmetics are more likely to contain bacteria than new products.”

“Based on frequent physical contact with the cosmetic surface, Trublend Whipped Foundation will have bacteria present.”

“Based on our belief that the product is expired and has been shared, if tested for bacteria and brought into contact with a nutrient rich environment then it will yield bacteria.”
**Student Survey and Feedback:**

1. **Do you remember this lab? If so, what made it memorable?**

   (A) I definitely remember this lab. It was memorable because we used products we actually own instead of theoretical ones.

   (B) I remember doing this lab because the results were eye-opening. I never knew that my make-up was actually a breeding ground for bacteria and fungus. It kind of disgusted me that I was putting that stuff on my face every day.

   (C) I remember this lab. I was actually just thinking about it a couple days ago! I think the fact that I saw that the makeup that I use wasn't as unhealthy as I thought it would be. I was super surprised.

   (D) This lab was the most useful/informative lab I've ever done. [It was memorable for] the large amount of bacteria/fungi on people's cosmetics.

   (E) Yes, I remember the lab because it was very realistic science; almost all of us females put on makeup every day but have very little understanding about the bacteria that can be found in it. It was also informative because I learned 'all natural' and 'organic' usually don't contain preservatives, so they have a shorter shelf life.

   (F) Yes, the lab taught me that the expiration dates on cosmetics are there for a reason. I thought the expiration dates were partially a marketing technique, to possibly get you to continue buying more of a particular product.

   (G) I barely remember this lab. I only remember using different products and putting samples of them in to dishes to leave over night.

   (H) I do remember the Savvy Consumers lab, and talked about it with the women in my life. In biology, I feel it’s easy to forget the larger picture, and for those of us who are not bio majors, to simply understand what we’re doing in lab. Therefore, using materials (i.e makeup) that we use every day, even those that we possibly used personally that morning, was really neat addition to the course. That is what made it memorable; it "personalized" the lab and my understanding of the material.

   (I) I remember the lab because it made me consider something I hadn't before: that cosmetics might contain agents bad for our health or be safe but become contaminated by improper use.

   (J) Yes. This was actually my favorite lab of all the ones that we did. I think it was because we were testing the bacteria of our own products that WE use daily so it just opened my eyes to how important it is to replace cosmetics frequently and take good care to make sure that products are sealed correctly.

   (K) I do remember the lab. I thought it was a very interesting approach to test the fungi and bacteria content of such products.

   (L) I do remember the lab. 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 the assignments I get thrown I can't see how they relate to me at all, but this lab obviously did.

   (M) I remember this lab! One of the things that stands out to me is the information we got regarding the manufacturing of cosmetics. The fact that they can be filled with bacteria and the FDA does not test them before they come to our shelves unless there is a problem.
2. Do you think differently about cosmetic use following the lab? Did it change your practices? Why or why not?

(A) I did think differently about my products after the lab but I did not change my practices. Every time I double dip my mascara I remember how much germs are spreading. I think the reason I haven't changed my practices are just due to habit and convenience. (I still don't share my mascara)

(B) I think differently about cosmetic products now. Before, I didn't think twice about sharing make-up or leaving containers open (I didn't even know most products had expiration dates!) but now I make certain that nobody else uses my products and I'm careful to store cosmetics correctly.

(C) I definitely think differently. I don't used ANYONE else's make up, or allow anyone to use my makeup. The results of sharing [makeup] for me was a big eye opener.

(D) 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!

(E) Yes, I think differently about cosmetic use; after the lab I went through my mascaras and threw away the ones I barely use anymore, and replaced a few I like that have been drying out. I have, a few times, added water to my drying mascaras but ceased doing that after the lab. I will also be less willing to share makeup after learning about the bacteria and fungi.

(F) Yes, the lab changed my practices with cosmetics. Besides throwing away expired products I also avoid using products that involve your hands rather than an applicator as we saw increased bacteria growth in these products.

(G) I do not really think differently about cosmetics after this lab. I use the same products.

(H) There are a few things I am more cognizant about regarding makeup usage after doing the lab. The first is the practice of keeping/storing makeup. During the morning rush, I often left my eye shadow open on the counter and my brush lying on the counter (sometimes even in splattered water.) Knowing that this could be bacteria's "dream come true", I make sure things stay closed and clean. There was one brand of makeup that had lots of growth in the lab (I believe it was either Mary Kay or Mac), and I don't use that anymore. Lastly, during the lab, we discussed that the longer you have/keep your makeup, the more it can be affected by bacteria, so I make sure that I replace it with fresher/newer makeup. I don't keep my makeup any longer than necessary, whereas before the lab, I still had some from my teenage years! I actually went through everything and threw it out.

(I) I definitely think differently about cosmetics since the lab. For example, I look for liquid foundations with a pump to avoid bacteria entering the makeup. I'm also more careful about choosing cosmetics that contain less harmful chemicals, especially since I am a nursing mother now.

(J) Definitely. I am now more careful to not share products I use on my face since we saw how much bacteria form in products we use only by ourselves. I now replace my mascaras and eye makeup frequently. I actually threw away a ton of stuff right after our lab.

(K) I haven't thought differently about using any products since the lab. After remembering it now, I do think twice about using my lip gloss!

(L) After the lab I'm more aware and more conscious of using makeup and the ingredients in makeup. I don't use sample makeup in stores anymore. My own makeup use in my house hasn't, but that might be because I hardly ever use makeup.

(M) Yes I do think differently about cosmetics, they are just so unnatural! I never wore alot of makeup before but I am much more aware now.
3. Did the lab help you learn more about bacteria or fungi? If so, what do you feel that you learned?
(A) I definitely learned how much bacteria and fungi are spread through the skin and how make up can go bad just like food.

(B) The lab helped me to learn that bacteria and fungus can grow in virtually any place and to be aware of those environmental factors.

(C) I learned how bacteria and fungi can be transferred and the dangers of it; I don't remember specifics.

(D) Yes! When I walk through Macy's makeup department, I cringe! I'll never try their testers again. There are bacteria and fungi on all cosmetics even if you aren't sharing them with anyone. I remember that not all bacteria/fungi that grow on cosmetics will make you sick, but either way it's gross.

(F) The lab did teach me about bacteria and fungi. I think the most important thing I learned involved how bacteria grow and flourish.

(G) I always figured there were bacteria or fungi on the products I use so the results did not surprise me.

(H) 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).

(I) The lab made me more aware of how bacteria can be transported and what sort of environments encourage further bacterial growth. For example, bacteria in moist environments have higher and faster rates of spread.

(J) Yes! I can't quite remember the names of the bacteria, but I learned that it grows in dark, moist places quite fast such as cosmetics we use daily.

(K) I learned that bacteria can grow on things that I would have never even considered. It makes sense once you think about it.

(L) At the time I had better understanding of how bacteria/fungi could grow and the environment of where it could grow. Two years down the road I'm not remembering a lot about it though. I think makeup was a good environment because it was wet and damp and repeatedly touched.

(M) I learned about bacteria and fungi and how they can survive in different environments that we regularly expose ourselves to.

4. Any other comments you might have about the lab.
(A) This lab and every lab we did have stuck with me. I brought up one of our labs with my coworkers last week. I loved every minute of that class.

(B) I always think about how you told us that just because a product says "not tested on animals" it doesn't mean that the ingredients weren't tested on animals.

(C) I thought the lab was really interesting. I like that it was self engaging---the fact that I could use my own make up to test out what I was putting on my skin was really interesting and I definitely learned a lot about the products that are marketed towards women aren't necessarily the best products for us to use.

(D) This was a great lab! I think high-schoolers would really benefit from this lab - so common to share and harbor makeup during those years of your life!

(E) It was nice to be able to see the results of other students' labs.

(H) I really enjoyed the class, and honestly, it’s one of the ones I learned the most from at Hamline.

(J) I think all girls should take this lab. Seriously!

(M) It was a really fun and relevant lab especially for us non-science majors!