“Investigating the Effectiveness of Antibiotic Soap”

Experimental Design

The word “science” is derived from a Latin verb scientia, meaning “to know.” Science is a way of knowing about the world. There are many other ways of knowing – in other words, of finding truth – including faith, philosophy, and cultural tradition. All of these ways of knowing help us understand different aspects of our world. An idea or topic under investigation in science is called the experimental question.

The essence of science as a way of knowing is the formulation and testing of statements called hypotheses. A hypothesis is a tentative explanation of how something works or of the cause of an event. Scientific hypotheses must be testable. In other words, hypotheses must be made in such a way that observations of the natural world can help us support or reject them. An observation is any information gathered using our senses. Hypotheses are tested via objective observations; that is, observations that when made by many different, independent people would produce the same results.

Many cleansing products on the market today advertise that they “kill germs” or, more specifically, that they are “antibacterial.” This label typically indicates that these products contain triclosan or another related antiseptic chemical. Consumers concerned about food poisoning or other bacteria-caused illnesses preferentially purchase these products based on their assumption that the following hypothesis is true:

*Antibacterial soap kills more bacteria on hands than standard hand soap.*

Good scientists are usually skeptical of untested hypotheses. For instance, there are reasons to doubt the hypothesis that antibacterial soap is a more effective cleanser. The action of hand washing physically removes bacteria from hands. It is not clear that the chemical makeup of the soap (outside of the fats, oils, and alkali that all soaps contain) is an important factor in disinfecting hands. Without testing the hypothesis, there is no way to determine whether it is correct.

The first step in testing a hypothesis is making a prediction about the observations one would expect to make if the hypothesis was correct. You can think of a prediction as the “then” part of an “If . . . then . . .” statement. In other words, “If this hypothesis is true, then I expect to observe . . .” A prediction forms the basis for evaluating the truth of any statement. The prediction of the hypothesis that antibacterial soap kills more bacteria on hands than standard hand soap is that *hands washed with antibacterial soap will have fewer bacteria on them than hands washed with standard soap.* Many (but not all) scientific hypotheses can be tested through experimentation. An experiment is a situation set up by a researcher solely for the purpose of testing a hypothesis. The hypothesis about antibacterial soap is testable by experiment.

When a hypothesis can be tested through experimentation, the most effective way to remove ambiguity (the chance that results could be interpreted in more than one way) from the results is to design a controlled experiment. Control indicates that the researcher works to ensure that all subjects in the experiment are treated identically (except for the experimental treatment). In other words, a control helps to verify that the effect of an experimental treatment is due to the treatment itself and not some other factor. The treatment the experimenter changes intentionally, is called the independent or manipulated variable. The resulting change in the experiment is called the dependent or responding variable. Measurements collected from tests of hypotheses are called data. Data can come as quantitative, being expressed as numbers (ex. 35 pounds, 10 degrees Celsius, 10 meters long), or qualitative, which is merely descript (red, pungent, presence/absence of smoke, bubbles).

After collecting your results of an experiment using data from observation, you can make inferences or assumptions based on the interpretation of data. The assumptions and inferences made are called conclusions, which may or may not support your initial hypothesis. Oftentimes, scientists repeat the experiment many times to increase the reliability and credibility of their data.
Suppose you observed that a cricket outside your window seems to be chirping every night, but some nights it chirps faster than others. To investigate this **experimental question** you decide to generate and test hypotheses. A friend of yours told you once that you can use the sound of a cricket chirp to tell the temperature. Curious, you decide to design an experiment. First you must create a **hypothesis**; here are some examples of possible hypotheses:

- The frequency of cricket chirps will change as the temperature changes.
- As the temperature decreases, a cricket will chirp fewer times.

Either hypothesis will work, the important thing is that you can test the hypothesis by doing an experiment which will confirm or deny the statement.

To set up the experiment, you go out to your yard and capture a few crickets. You bring them inside and place them in a container. But wait, if you have a bunch of crickets together, what if they chirp based on how many crickets there are nearby. The goal in designing an experiment is to eliminate all the variables except the one you are testing. This means all your cricket subject must be housed in the same environment (same lighting, same food, same water, etc). Okay, so you get that set up and take the temperature of your room. Now you must wait for the crickets to start chirping. You observe how many times the cricket chirps for a 5 minute period and record the information into a **data table**.

Now you have to compare that number with the chirps that occur at different temperatures. You may use a heating pad, or ice or any other way to lower or raise their temperature. You would then take data for 5 minutes at the new temperature.

In your experiment, the **manipulated variable (independent variable)** is the thing you changed — the temperature. The **responding variable (dependent)** is what you are measuring that happens as a result of that change - the number of chirps.

After you have taken data, you can then draw a **conclusion** about whether your hypothesis is accepted (correct) or denied (incorrect). To give my study more credibility, I could repeat my cricket experiment over and over to examine if my results were the same or different.

**PREPARING TO DESIGN AN EXPERIMENT**

In the introduction to the lab, we put forth the following scientific hypothesis:

*Antibacterial soap kills more bacteria on hands than standard hand soap.*

Discuss the following questions with your lab partners and be prepared to share your answers with the lab instructor and other students.

1. What objective measures could we use to test the hypothesis about the cleansing power of antibacterial soap?

2. If the hypothesis is correct, what would you predict the outcome of the test to be?

**DESIGN AND PERFORM A CONTROLLED EXPERIMENT**

The last discussion exercise should have led you to consider some of the factors you will need to control when testing the hypothesis that antibacterial soap kills more bacteria on hands than standard hand soap. Now you should be prepared to design a well-controlled experiment to test this hypothesis. First, you will need a short lesson on how bacteria levels can be counted.
Bacteria or are single-celled organisms without a nucleus (Prokaryotes) that are much too small to be seen with the naked eye, and many can only be seen under the highest magnification of a typical light microscope. Bacteria reproduce rapidly when in contact with a nutrient source. If an individual bacterial cell is transferred to a gel-like nutrient source, the cell will multiply into millions of descendants, producing a colony of cells that is visible to the naked eye. A bacterial colony that arose from a single cell typically appears as a distinct circular dot on the surface of the gel-like nutrient source (called agar). Thus, the number of bacteria on a given surface can be estimated by transferring those bacteria to a petri dish filled with nutrient agar gel, giving those cells 24-48 hours to multiply, and then counting the number of visible colonies on the plate.

A. Work with your lab partners to design a controlled experimental test of the hypothesis.

Materials available:
- Liquid hand soap: one containing triclosan and a similar formula soap minus triclosan (Note: most of these soaps instruct users to rub the lather on their hands for 30 seconds to get the maximum effect).
- Dishes filled with nutrient agar (two per lab team)
- Sterile cotton swabs for transferring bacteria from hands to petri dishes
- Sterile water to swab dirty hands with
- Markers

B. Write an outline of your experiment in the format below. Be prepared to share this design with the lab instructor and/or your classmates.

“Title”

Experimental Question:

Hypothesis:

Procedure:

Data:

<table>
<thead>
<tr>
<th>Type of Soap</th>
<th>Number of Colonies</th>
<th>Class Average (Mean)</th>
<th>Class Mode</th>
<th>Class Median</th>
<th>Standard Deviation</th>
<th>Other Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (Just Water)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibacterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Antibacterial</td>
<td></td>
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</tbody>
</table>

*In addition include a graph of your data.

Conclusions:
1. Include an interpretation of your data that supports or refutes your hypothesis.
2. Describe any experimental errors that could have affected the results.
3. Refer to quantitative and/or qualitative data to support your conclusions.

Extension:
1. Describe the independent and dependent variables in your experiment.
2. What was your treatment group?
3. Why do you think we included a treatment using just water if we were testing the difference between two types of soap?
4. How did we increase the validity or reliability of our data?
5. For each statement, determine whether it is a scientific hypothesis as written, and if not, why not.

- If the statement is not a scientific hypothesis, try to modify the statement so that it is testable.
  a. It is wrong to perform medically unnecessary cosmetic surgery.
  b. Biology lab is more fun than a barrel of monkeys.
  c. Plants that are spoken to regularly grow more rapidly than plants that are not spoken to.

**Standard Deviation**

**How to Calculate Standard Deviation**

Standard deviation ($\sigma$) is a statistical measure of how precise your data is. It is calculated using the following equation, where $\bar{x}$ is the data average, $x_i$ is the individual data point, and $N$ is the number of data points:

$$
\sigma = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}}
$$

To calculate the standard deviation, you would begin with calculating the quantity $(x_i - \bar{x})$, which is the deviation of each data point from the average. You would square each one, then add them up and divide by one less than the number of data points. Finally, you would find the square root of this value.

**EXAMPLE:**

The volume of a liquid was measured five times, the results being 4.5 mL, 4.4 mL, 4.5 mL, 4.2 mL and 4.3 mL. The deviations $(x_i - \bar{x})$ are shown below:

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Deviations $(x_i - \bar{x})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 mL</td>
<td>$(4.5 - 4.4)$ mL = 0.1 mL</td>
</tr>
<tr>
<td>4.4 mL</td>
<td>$(4.4 - 4.4)$ mL = 0.0 mL</td>
</tr>
<tr>
<td>4.5 mL</td>
<td>$(4.5 - 4.4)$ mL = 0.1 mL</td>
</tr>
<tr>
<td>4.2 mL</td>
<td>$(4.2 - 4.4)$ mL = -0.2 mL</td>
</tr>
<tr>
<td>4.3 mL</td>
<td>$(4.3 - 4.4)$ mL = -0.1 mL</td>
</tr>
</tbody>
</table>

Average ($\bar{x}$) = 4.4 mL

$$
\sigma = \sqrt{\frac{(0.1)^2 + (0.0)^2 + (0.1)^2 + (-0.02)^2 + (-0.1)^2}{5-1}} \text{mL} = \sqrt{\frac{0.07 \text{mL}^2}{4}} = 0.13 \text{ mL} \approx 0.1 \text{ mL}
$$

We generally would rather go on and calculate the relative standard deviation, so that we can see whether 0.1 mL is a small or large quantity compared to the average value (4.4 mL). This done by finding the percent standard deviation:

$$
\text{relative standard deviation} = \frac{\text{standard deviation}}{\text{average value}} \times 100
= \frac{0.1 \text{ mL}}{4.4 \text{ mL}} \times 100 = 2 \%
$$