Introduction to Scientific Investigations and Hand Washing Lab

Resource Type: Curriculum: Laboratory

Publication Date: 2/17/2005

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Abstract

The students investigate the scientific method by a prompt in the form of a letter asking them to design a hand washing experiment. This experiment asks them to test antimicrobial chemicals for use in soaps. This is a workshop and experiment designed to instruct students on the scientific method. They learn to create a hypothesis, decide on variables, and determine the control. They then design the procedure for their labs, write their materials and methods, and finally conduct the experiment.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Objectives.
At the completion of this activity, students will be able to:

- understand the scientific method.
- write a hypothesis.
- design their own experiment with appropriate hypothesis, variables (independent and dependent), and controls.
- conduct their own laboratory.
- learn how to reach proper conclusions.
- interpret results.
- understand the need for hand washing.

Background.
Students need previous knowledge of normal microbiota of the skin. They must understand and be able to use aseptic techniques while designing and conducting the experiment. Students should also address safety issues for their experiment. Prior to conducting their experiments, students must submit materials and methods to the instructor for approval. In addition, it may help to have students with previous knowledge of procedures test antiseptics and do standard plate counts. The students may be directed to a biology lab manual or text book for the scientific method and ideas for testing effectiveness of antiseptics for hand washing. Refer to http://www.washup.org for American Society for Microbiology information on hand washing.

PROCEDURE

Materials.
Each student group must create and write a list of the materials and equipment they will need to conduct the lab they have created. Students must see the instructor for help with materials and other suggestions. As every lab is slightly different, it is challenging to guess ahead of time what the students will need. Over time, I have come up with a list of materials that works for my students. Every instructor will need to develop their own list according to the type of experiments they encourage. The list I have is:

- Several different types of soaps, including antibacterial soaps
- Several antiseptics: Cepacol, Zepiran, Hibiclines, hand sanitizer, Providone iodine, etc.
- 70% alcohol
- Sterile water
- Sterile basins or large beakers
- Sterile paper towels
- Nutrient agar plates
- Nutrient agar deeps and petri dishes
- Nutrient broth
- Sabouraud dextrose agar deeps
- L-shaped glass spreader
- Sterile swabs

- Sterile filter paper disks
- Sterile pipettes, 1.0 ml and 0.1 ml, or micropipettors and tips
- Plastic wrap
- Cultures: *Staphylococcus aureus* and *Escherichia coli*

Student Version.
See laboratory write-up hand out (PDF).

Instructor Version.
It is recommended that the students submit a write-up of their hypothesis, materials and methods, and procedures. This must be done with sufficient time prior to the lab for two purposes. First, the lab materials must be assembled for the students prior to conducting the lab. Second, the instructor must review the student submission to suggest safer and more appropriate alternatives to the student procedure. Any live organisms must be cultured in plates, no ultraviolet light should be used directly on hands, etc. Also, many students leave out the control.

Suggested answers to workshop portion of lab:
Hypothesis:

1. If the number of hours of study is related to the test score, then studying more hours will result in a higher percentage score on the test.
    - Independent variable: hours
    - Dependent variable: percentage score on test
2. If ultraviolet light is related to death of bacterial cells, then a longer exposure of bacteria to UV light will result in a decrease in bacterial cells.
    - Independent variable: time under UV light
    - Dependent variable: number of bacteria that die
3. If bacterial growth is affected by temperature, raising the temperature will result in an increase in bacterial growth.
    - Independent variable: temperature
    - Dependent variable: number of bacteria
4. If exposure of bacteria to antibiotics is related to antibiotic resistance, then increasing the time of exposure to antibiotics will result in an increase in the number of bacteria that are resistant to the antibiotic.
    - Independent variable: time of exposure of antibiotic to microbe
    - Dependent variable: number of resistant bacteria

Control in hypothesis:

1.

- Independent variable: concentration of salt
- Dependent variable: osmotic pressure in red blood cells
- Control: isotonic solution

2.

- Independent variable: amount of enzyme
- Dependent variable: speed of reaction
- Control: no enzyme

3.

- Independent variable: sugar type
- Dependent variable: amount of alcohol produced
- Control: no sugar

4.

- Independent variable: UV light
- Dependent variable: number of bacteria
- Control: no UV light

Safety Issues.

Staphylococcus aureus is a level 2 microbe. Also, students may be plating microbes from their hands, so you cannot be certain which microbes will be grown. If students bring in antiseptics from home or work, care must be taken that they are used according to directions.

ML Safety Statement regarding Environmental Isolates

The Curriculum Resources Committee recognizes that isolated organisms can be a powerful learning tool as well as a potential biological hazard. We strongly recommend that:

- Environmental enrichment laboratories should only be performed in classes in which students have been trained to work at a BSL2.
- Direct environmental samples (eg. soil, water) which are known to contain infectious organisms should be handled according to the biosafety level of that infectious agent.
- Cultures of enriched microorganisms, derived from environmental samples, should be handled using Biosafety Level 2 precautions.
- Mixed, enriched or pure cultures of microorganisms from environmental samples with a significant probability of containing infectious agents should be manipulated in a biosafety cabinet if available.

- Where possible, media used for the enrichment of environmental isolates should contain an appropriate anti-fungal agent.
- Instructors should be aware if they are teaching in regions with endemic fungi capable of causing systemic infections, and should avoid environmental isolations.

ASSESSMENT AND OUTCOMES

Suggestions for Assessment.
A complete laboratory report for the experiment can be written and turned in for grading. Use a rubric to check the report and give the students the rubric in advance. Suggestions for rubric: title, statement of hypothesis, identify variables and controls, purpose of experiment, clear procedure, materials and methods present, data, conclusion. Also, students may give a brief oral presentation of the experiment, with procedures and results, to the class.

At the end of the lab is a good time to discuss aseptic techniques, normal flora, transient flora, nosocomial infections, and use of gloves for allied health professionals.

Field Testing.
The activity has been conducted in Allied Health Microbiology labs with 24 students. Nonmajor science students have also done a similar lab. The lab is done midsemester or at the end of the semester. The students are organized into groups of four for creating and conducting the experiment. The students enjoy creating their own lab, after having followed written labs. It may help to give the students the material prior to the laboratory session when they are required to create their own lab.

Pitfalls encountered include the following:

- Students who do not turn in their materials list.
- Students who let one person in the group take over while they just watch or talk.
- Students who do not turn in their lab in time for the instructor to suggest changes.
- Students who do not follow the scientific method, especially forgetting to use a control.
- Students who choose the easiest possible experiment (press fingers onto nutrient agar plates) so they can complete the lab in 15 minutes and leave.
- Students who do not label the plates properly.

Student Data.
Some students choose to do standard plate counts after washing their hands in antiseptic for varying times. Others choose to put disks saturated with the antiseptic on lawns of chosen bacteria. The less creative students wash their hands and then press their fingertips into a nutrient agar plate.

Students usually conclude the following: hand washing with soaps is ineffective in removing normal flora, gloves are necessary to prevent nosocomial infections, not all antiseptics are effective in reducing microbes on the hands, and dry fingers show fewer bacteria than wet fingers.

One student recognized a similar cultural characteristic of his unknown bacteria to the bacteria growing on the nutrient agar plates when the liquid soap from the classroom dispenser was used. This student requested materials to do unknown determination of the bacteria from the hand washing lab. He determined our liquid class room soap was growing *Pseudomonas aeruginosa*. The student received an "A" on the project, and I switched to dry soap in the classroom.

SUPPLEMENTARY MATERIALS

Possible Modifications.

Creative students can come up with many ways to test the effectiveness of hand washing antiseptics.

If students desire to test level 2 microbes, you may suggest they use gloves during the experiment.

Sources of Information.

http://www.washup.org
Microbiology lab manual to give students ideas for procedures
Biology text book or lab manual for the scientific method

ACKNOWLEDGEMENTS

This project was supported in part by the Division of Undergraduate Education at National Science Foundation, grant number DUE - 0127800 to the San Francisco Bay Area Collaborative for Excellence in Teacher Preparation, known locally as MASTEP (Mathematics And Science Teacher Education Program).

INTRODUCTION TO SCIENTIFIC INVESTIGATIONS

Scientists use the process of scientific investigation to establish the principles and rules of science. Scientists want to know how or why something works in a particular way. A scientist must define a question. What this involves is a hypothesis. A hypothesis is an educated guess about how or why something works in a particular way. The experiments the scientist conducts involve a test of the hypothesis. Scientists must present the work at scientific meetings or in publications, showing the evidence from observations and experiments that support their hypothesis or explanation of the biological principle. These scientific investigations are called the scientific method. (While the word used here, "scientist," is singular, most scientific investigations use teams of researchers working closely together and coordinated by a team leader.)

The scientific method begins with observations of a biological process, followed by questions. Then the scientist proposes possible explanations to answer these questions. A tentative explanation or educated guess for the biological process is called a **hypothesis**. It is a **testable** statement that may include a prediction. A hypothesis is only accepted when it cannot be disproved after repeated testing through observation, experimentation, models, and accumulation of data from a variety of sources.

Why are hypotheses used?

Hypotheses are used because they are testable. A test is performed to show how two variables might be related. This is what makes a real experiment. You are testing variables. A hypothesis is usually based on some previous observation such as noticing that in November many trees undergo color changes in their leaves and the average daily temperatures are dropping. Are these two events connected? How?

Correct form for the Hypothesis

A hypothesis must contain the words, **if** and **then**. They are necessary in a formalized hypothesis. Consider the statement, "Temperature may cause leaves to change color." To create a hypothesis, the words, if and then, must be used. “**If** temperature is related to leaf color change, **then** exposing plants to low temperatures will result in changes in leaf color.”

Also the hypothesis must state a tentative relationship. There must be two variables. One is an **independent** variable and the other is a dependent variable. The independent variable is the one the scientist controls or varies during the experiment. The dependent variable is the one that the scientist observes and/or measures. The independent variable in the above hypothesis is temperature. The scientist varies the temperature during the experiment. The dependent variable is the leaf color change. The leaf color is dependent on the temperature. The scientist does not control the leaf color. The scientist measures or observes the leaf color. A good hypothesis will force the scientist to think about the results the experiment will measure.

Exercise: write hypotheses
Read the following statements. Rewrite each of them as a hypothesis.
Circle the independent variable.
Put a square around the dependent variable.

1. If I study, then I will get a good grade.
2. Ultraviolet light may kill bacterial cells.
3. Bacterial growth may be affected by temperature.
4. Constant exposure to antibiotics causes antibiotic resistance.
5. Write your own original hypotheses here:

1. ____
____

2. ____
____

3. ____
____

4. ____
____

5. ____
____

The Controlled Variable

A microbiologist wants to investigate the relationship between antibiotic resistance and antibiotics in animal feed. A hypothesis could be "**If** antibiotic resistance in microbes is related to use of antibiotics in animal feed, **then** exposing animals to increasing levels of antibiotics in their feed will result in an increase in antibiotic resistant microbes." The dependent variable, antibiotic resistance, can be measured in many ways. The microbiologist can measure the different species of antibiotic resistant microbes, the number of antibiotic resistant microbes, etc. There can be several dependent variables. But there must be only one independent variable, antibiotics in the animal feed. All other independent variables, such as other food, type of enclosure, water, etc., must be held constant. These variables kept constant are called **controlled variables**. This eliminates the possibility that other factors will affect the outcome of the experiment.

Designing the Procedure

The hypothesis has been written. The independent and dependent variables selected. Now the scientist must establish a procedure or method to measure the dependent variable. For example, the amounts of antibiotic in the feed to be used must be determined. The type of antibiotic must be established. In addition, the number of repetitions of the experiment must be determined. Repetitions are needed because variations in monitoring biological systems occur, so averages must be taken. Lastly, an important aspect of the experiment, the control, must be established. The controlled variables must be kept the same in all experiments so that the only effect will be on the dependent variable, the variable being measured. Once the controlled variables have been established, a **control treatment** is set up to demonstrate that the dependent variable is the only variable being tested. A control is a treatment in which the independent variable is eliminated or set to a standard value. The control in the animal feed experiment would be to add no antibiotics to the feed. If the bacteria became more resistant in the animals with no antibiotic feed, then the experimental procedure is not valid.

Exercise: determine a control in the following hypotheses

The following are hypotheses. Give the independent and dependent variables in an experiment designed to test each hypothesis. Then give the control treatment.

1. If the concentration of salts in solution surrounding the red blood cell is related to the osmotic pressure in the red blood cell, then decreasing salt concentrations of the solution will cause the cell to swell and burst.

2. If the amount of enzyme is related to the rate of the chemical reaction, then increasing the amount of enzyme will increase the speed of the reaction.

3. If the type of sugar provided to growing yeast is related to the amount of alcohol produced by the yeast, then growing yeast in different sugars will result in different amounts of alcohol produced.

4. If the time under UV light is related to bacterial death, then increasing the time under UV light will increase the number of bacteria killed.

Designing the Experiment

You will now design your own experiment as a microbiologist working for Soaps Dot Com. The public is becoming more aware of bacteria in the environment. People are aware that bacteria can be transferred from the hands to food, eyes, mouth, etc. The company wants to expand into the area of germicidal soaps for hand washing. You must design an experiment to find a way to test the effectiveness of antiseptics used to wash hands with the company soaps.

Complete the following:

1. Establish your hypothesis with the dependent and independent variables.
2. Determine your controlled variables.
3. List in numerical order the exact steps of your procedure.
4. Be sure to include your controlled treatment.

5. Make a list of supplies needed. Submit the supply list to the workshop leader.
6. Collect the supplies.
7. Set up the experimental apparatus.
8. Perform the experiment in class.
9. Record the data.
10. Use the results to establish tables or graphs.
11. Interpret the results.

Soaps Dot Com
Antibacterial Avenue
Staphylo, Co 11111

Dear Dr. Vaccinia,

Profits this year are terrible. Our soaps come in wonderful colors, exotic fragrances, and easy to use dispensers. However, the public no longer buys ordinary soaps. We must add antibacterial chemicals to our soaps to keep up with the competitors. Below are the questions I need to answer. We would appreciate if you would conduct experiments to answer question #2. If you can help us, we will pay you $1,000,000.

1. What antibacterial chemicals can we add to our soaps without altering the color, texture, and consistency of the product?

2. Which of these antibacterial chemicals are most effective in killing bacteria on the hands?

3. Which of these chemicals are least likely to cause damage to the skin, such as dryness, redness, or allergic reactions?

4. What amount of antibacterial chemical is most effective in killing bacteria but least likely to increase the cost of our products?

We appreciate your help. You come to us highly recommended. We know we can expect a full written report with excellent measurements and details of how you did your experiments.

Respectfully yours,
Ms. Pamela Prion