Inquiry Laboratory Investigation: Using Bacterial Contamination to Reinforce the Importance of Hypotheses in the Scientific Method of Inquiry

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Introduction

The domain Bacteria has existed on Earth for approximately 4 billion years. Throughout these years, microbes have evolved more quickly and have become more creative, more capable as a population, more adapted to new environments, and more predictable (Bryson 2003, Knoll, 2003, Koneman 2002). For example, *Escherichia coli* is "one of the most widely studied of prokaryotes, most versatile in its extent of animal and human colonization, and most innovative in its varied expression of virulence mechanism" (Koneman, 2002, p. 11). Remarkably, they swap drug-resistance genes to survive, and use living organisms, including humans, as platforms for carrying them around. But it is these same gene transferable capabilities that make some bacteria less desirable in the human population. According to the U.S. Centers for Disease Control and Prevention, on the average, every year "about 76 million people in the United States become ill from pathogens (bacteria, parasites, or viruses), or disease-causing substances (natural and/or synthetic chemicals), in contaminated food and drinking beverages. Of these people, about 5,000 die" (NIH Publication No. 07–4730 - May 2007). One memorable example is the 2006 spinach contamination by a particular strain of *E. coli* (O157:H7), which not only causes cramps and bloody diarrhea typically associated with infections, but can also lead to serious complications such as hemolytic uremic syndrome (HUS).

Inquiry Laboratory Investigation

In this activity, students will engage in a hands-on laboratory experiment to learn more about the microscopic bacteria all around us. They will 'view' bacteria as they grow in colonies on petri plates containing nutrient agar. By carefully noting the numbers of bacterial colonies on each plate, the students can act as microbiologists and generate an idea of how many bacteria are present (Alonzo 2008, Spangler 2008, Johnson and Case 2007). In addition to this laboratory allowing students to note the high prevalence of bacteria all around us (including on our hands), they will be noting their ease of transferability. Via a touch of their thumb, the student will see just how easily transference occurs; and with this realization they will gain a greater understanding and respect for these microscopic life forms that have been in existence for eons and comprise the Domain Bacteria. Along with gaining this understanding, students will be able to see the effectiveness of preventative measures against bacterial contamination by using various disinfectants, antimicrobial agents, and other cleaners. This laboratory will allow students to see why cleanliness is such a great issue in the food industry, and the relevance for the government bodies that aim to keep the human population safe from such an unseen possible adversary. In the end, we hope that the students will be able to utilize this laboratory situation as a means of seeing the bigger picture of these mostly hidden life forms in our midst. With knowledge of their existence, we attempt to offer students some solid understanding of this Domain; via actual observation of ease of transference, and possible means of preventing such occurrences, we are bringing science 'alive' to our students.

Note: This laboratory is designed for high school and 1st year college students; with this comes a desire to generate a laboratory situation with minimal equipment needs. But, optional activities could follow the initial laboratory experiment. For instance, the various shapes of bacteria could be determined via the use of staining techniques and oil immersion microscopy. But again, this is dependent on the materials and equipment available. The following material has been written in such a way that it may be given directly to students. Blank data tables are also provided which may be duplicated for student use.]

I. **Objectives** By completing this laboratory activity, you will:

1. *Observe* microbial life, hands on, noting the different shapes of microorganisms and presence or absence of a nucleus
2. *Predict* how various disinfectants inhibit growth of microorganisms
3. *Utilize* proper laboratory culturing techniques
4. *Understand* the spreading of disease by microbes and ways to combat microbial proliferation.

II. **Background**

Microbial life was the first life form on the planet Earth. In fact, when life on other planets comes into speculation, most scientists believe that microbes (or microorganisms) of some sort should be searched for. But moving back to planet Earth, how does one actually view various microbes, and what is more, how does one battle the more fierce, and sometimes, deadly microbes?

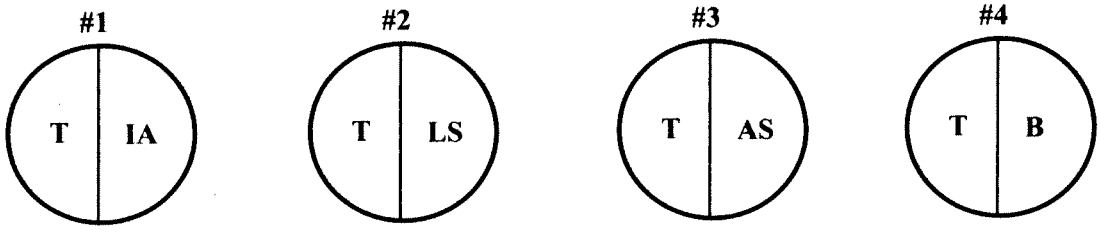
In this laboratory experiment, you will see standard colonies of bacteria on petri dishes, that you have "plated" (i.e., the placement of organisms on nutrient-rich agar via cell culturing techniques, such as swabbing) yourself. Going further, you will observe the effectiveness of some disinfectants to halt the spread of these microorganisms you have cultured. In addition, you may observe microbial life of various kinds via microscope, utilizing correct staining techniques if desired. Lastly, you may note that some of the microorganisms have resistance to many disinfectants.

III. Lab Activity

Materials Needed for: **Individual students**	Materials Needed for **Groups of 2 students**	Materials Needed for: **Whole class**
1. Protective gloves 2. Protective eye goggles 3. Apron 4. Student information sheet and data sheet for Activity One.	1. Isopropyl alcohol 2. Liquid soap 3. Antibacterial liquid soap 4. Bleach solution 5. Media in bottle 6. Petri dishes 7. Pipets 8. Microscope slides & cover slips 9. Methylene blue 10. Swabs 11. Waterproof markers	1. Standard light microscope. 2. Warm water bath (for example, a hot plate on low temperature with large glass containers of water placed on it)

Procedure: Before you start, make sure you are wearing your protective gloves and goggles. Also, remember that this laboratory involves the growth of microorganisms, and because of this, precautions must be taken to not spread these organisms via careless handling of the Petri dishes, slides, and/or swabs. Also, note that when this laboratory is completed, you should wash your work area as directed by your teacher.

1. (Do this only if your teacher requests this to be done. He or she may have already done this part of the laboratory for you.) Insert the sealed media bottles in the warm water bath for at least 20 minutes. This amount of time is necessary to liquefy the solid medium.
2. Put your, and your partner's initials and information on the bottom of four Petri dishes (not the lids) with the waterproof markers provided



Note: **T** = thumb, **IA** = thumb washed with isopropyl alcohol, **LS** = thumb washed with liquid soap, **AS** = thumb washed with antibacterial soap, **B** = thumb washed with bleach solution

3. (Do this only if your teacher requests this to be done. He or she may have already done this part of the laboratory for you.) Pour approximately 1/3" of medium into the bottom of each labeled Petri dish. The medium can also be transferred via pipette. All dishes should be on a flat surface, and must not be touched during the solidification process of the medium. It will take 10 minutes for the medium to cool and solidify.
4. Both you and your partner need to take off your gloves. Then, press with your thumbs on the section of medium that is labeled **T**. One of you (student A) should use their right thumb in Petri dish #1, and their left thumb in Petri dish #2; the other (student B) should use their right thumb in Petri dish #3, and their left thumb in Petri dish #4. To reemphasize, each thumb print should be in the area labeled **T**.
5. One of you (student B) needs to put their gloves back on, to assist student A wash their right hand **only** with isopropyl alcohol. Then student A needs to press their right thumb on the portion of dish #1 labeled **IA**.
6. Now, student A needs to wash their left hand with liquid soap. Then he or she needs to press their left thumb on the portion of dish #2 labeled **LS**.
7. Student A now needs to assist student B wash their right hand **only** with antibacterial soap. Then student B must press their right thumb on the portion of dish #3 labeled **AS**.
8. Finally, Student B needs to wash their left hand with bleach solution. Then he or she needs to press their left thumb on the portion of dish #4 labeled **B**.
9. Put covers on the Petri dishes, and place them somewhere away from sunlight. Clean up your area with a solution that your teacher will give you.
10. Now you and your partner need to predict which of the cleaning methods will be most effective in preventing the growth of microorganisms, which will be least effective, and what the basis for your judgment is in each case. Write down your predictions and justifications for later reference. Use Table 2 to record your data.
11. (One day later), look at the growth of microorganisms. This can be done via (**Lab Activity A**) direct observation of the Petri dishes themselves or via (**Lab Activity B**) use of microscopes--- or both. Your teacher will tell you which Lab Activity to do next.
    - **Lab Activity A**: Look for and count little splotches, or colonies, of microorganisms, on the Petri dishes. They will look somewhat white or grey. Record observations on Lab Activity A Table. Note that each splotch is considered one colony of microorganisms, which is approximately a million organisms
    - **Lab Activity B**: Gently swab the colony and then transfer your sample to a microscope slide. (Your teacher may ask you to put a tiny drop of methylene blue on some samples before the cover slip goes on. This will allow you to observe some cellular components of the microorganisms.) Finally, place a cover slip over the top, making the sample ready to observe microscopically. Record observations on Lab Activity B Table Make note of shapes, possible cilia or flagella, and location of the absorption of methylene blue.
12. Compare your results to what you predicted by looking at your ideas in Table 2. How accurate were your starting hypotheses? Discuss why your actual outcomes might have been different from what you predicted; in doing this, generate revised hypotheses on why certain cleaners (isopropyl alcohol, liquid soap, antibacterial soap, bleach) were more or less effective in reducing bacterial contamination. Record your ideas in Table 3.
13. Perform an Internet search to deduce possible reasons for the effectiveness of the cleaners used. You should also research out other means of reducing transference of microbes.
14. Clean up your working areas completely, with Petri dishes disposed of properly and all equipment placed back where your teacher suggests.

Table 2: Student Hypotheses

Condition: Thumb washed with:	**Less Effective**	**More Effective**	**Reason**
IA: isopropyl alcohol			
LS: liquid soap			
AS: antibacterial soap			
BS: bleach solution			

Table 3: Revised Hypotheses

Condition: Thumb washed with:	**Reason for Effective**	**Internet Research Results**
IA: isopropyl alcohol		
LS: liquid soap		
AS: antibacterial soap		
BS: bleach solution		

Lab Activity A Table

Petri Dish		**Number of Colonies**	**Number of Microorganisms** (1 million organisms per colony)
#1	**T**: right thumb of student A		
	IA: thumb with isopropyl alcohol		
#2	**T**: left thumb of student A		
	LS: thumb with liquid soap		
#3	**T**: right thumb of student B		
	AS: thumb with antibacterial soap		
#4	**T**: left thumb of student B		
	BS: thumb with bleach solution		

Lab Activity B Table

Petri Dish		Shape of Microorganisms (e.g. circular, square, columnar, spiral)	Interesting Observations (e.g. cilia, flagella, nucleus)
#1	T: right thumb of student A		
	IA: thumb with isopropyl alcohol		
#2	T: left thumb of student A		
	LS: thumb with liquid soap		
#3	T: right thumb of student B		
	AS: thumb with antibacterial soap		
#4	T: left thumb of student B		
	BS: thumb with bleach solution		

Conclusion:

The learning activity included in this article is designed to help students understand the critical role of hypotheses in the scientific method of inquiry, the orderly means of investigation to answer questions and solve problems. The lab experiment we included gives students the opportunity to form meaningful hypotheses, based on direct experimentation and possible background research; via this inquiry based activity, students learn to predict, observe, experiment, and discover. The laboratory activity is a direct and easily interpreted means to understand phenomena--- even that which is microscopic, as in the case with microbes and microbial transference.

Our overall purpose is to generate meaningful activities to link the concept of microbial transference to that of food contamination, never losing sight of its relevancy to public health. Food contamination, foodborne illness, and health and safety have become an urgent matter in the United States. By engaging today's students in realistic learning activities, we create environments that promote active learning, critical thinking, collaborative learning, and knowledge creation--- habits that are urgently needed in the next generation of physicians, researchers, communicators and public policy makers. They will need this knowledge base, as they attempt to deal effectively with issues such as nutrition, health, safety, and wellness for their, and successive, generation(s).

Instructional guide for Teachers: A teacher's guide for this laboratory (Appendix #1) can be obtained electronically by contacting the authors via e-mail: Abour H. Cherif, Ph.D. *acherif@devry.edu*, Linda O. Michel, Ph.D. *lmichel@devry.edu*, Farahnaz Movahed Zadeh, Ph.D. *fmovahedzadeh@ccc.edu*, JoElla Eaglin Siuda, M.S. *jsiuda@aii.edu*, Gerald Adams, Ph.D. *gadams@colum.edu*, Robert Aron, Ph.D. *baron@devry.edu*

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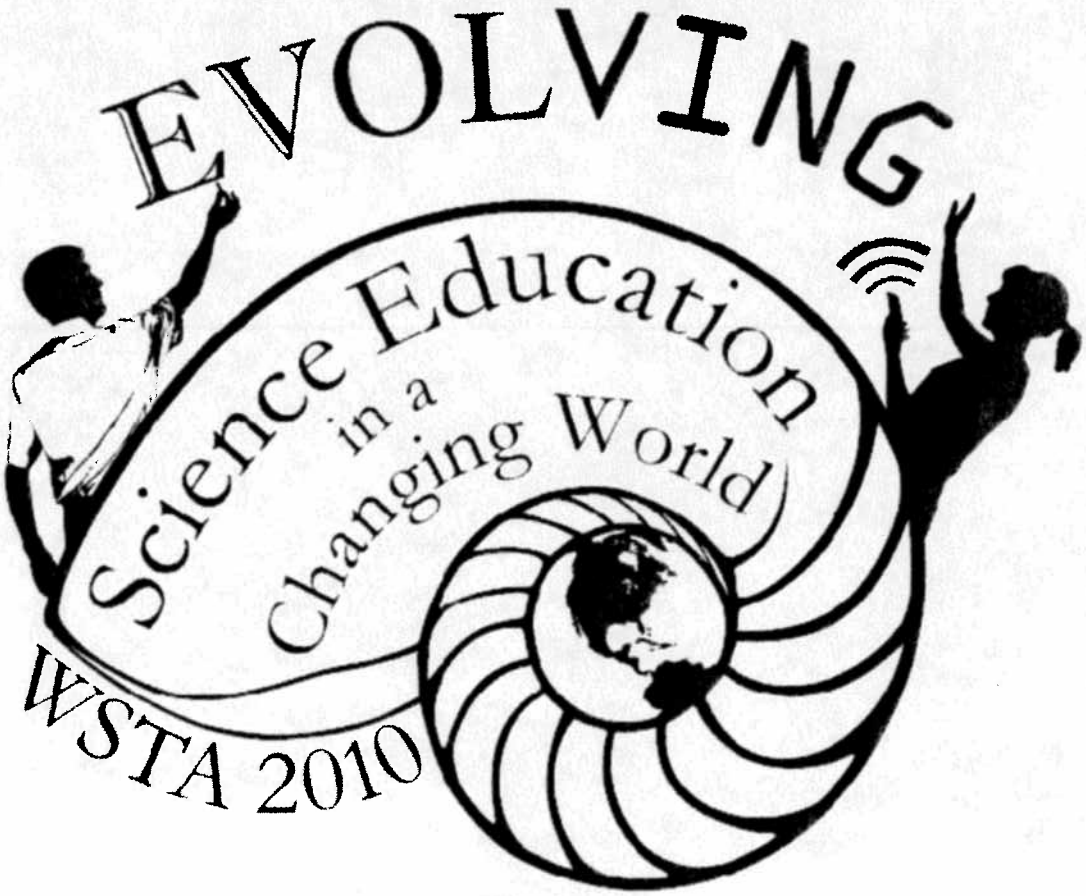

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