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# Unseen Life: Engaging Non-Science Students through Microbiology*

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## Introduction

Microbiology was used as a tool to engage, educate and excite non-science students to the basic principles underlying scientific investigations (in general) and basic tenets of microbiology (specifically). Microbes are an excellent tool for this population. The interest of the students can immediately be stimulated when topics such as anthrax, HIV or smallpox are discussed. These topics serve as the introduction to the course; the goal is to get the students interested and "buy in" to the content to be delivered over the length of the course.

Most anyone that has taught will say that hands-on activities are enthusiastically embraced by students. We took advantage of this fact and involved students in science from the start. In addition to a basic introduction to microbiology, scientific methodology, both inductive and deductive reasoning, was explored. While some experiments had defined protocols, others were left open ended, relying on the students to design their own procedures.

To facilitate basic microbiological inquiry students assembled their own culture collection, procuring microbes from water samples (Westhampton Lake on the University of Richmond campus), a hand washing exercise and isolates from environmental sites. Culturing these diverse areas assured that there would be varied populations of microbes for further examination.

Basic microbiological techniques were performed on the student cultures. Students learned aseptic technique, streak isolation and the utility of the Gram stain. Further characterization, using these isolates, investigated salt & temperature range tolerance, oxygen requirements and motility. The role of disinfectants was also explored.

Antibiotic resistance is a significant health care concern, and the topic was incorporated into this course. Students were able to perform minimal inhibitory concentration analysis to selected antibiotics for microbes in their culture collection.

The majority of the topics presented included ancillary material from *Scientific American* articles. These articles provided a "real-life" situation where the concepts discussed could be applied. Other reading was from a support text (Other End of the Microscope: The Microbes Tell Their Own Story), on-line web sites and "The Hot Zone" by R. Preston.

Evaluation in this course was determined by two exams, 2 papers (one on the efficacy of hand washing and one on any area of interest concerning either "The Hot Zone" of "Outbreak). In addition, pairs of students did oral reports on one of the following: Microbes use in bioremediation**,** Genetically modified organisms (especially Bt)**,** Microbes found in the arctic and deep sea thermal vents: Can there be microbes on Mars?**,** Epidemiology: representative case studies**,** Development and Marketing of new pharmaceuticals**,** Pathogens to fight cancer**,** Microbes: fermentation and food**,** Pharmaceuticals and other materials made by bacteria**,** and Prions and Mad Cow disease. The intent was to have students research a microbiological topic from an area they were interested in. One final component of the course required that each student present a microbe we did not discuss in class. The presentation was to cover basic micro, habitats, and what it is known for. The students were encouraged to be creative in their presentations.

## Materials

- Various soaps – natural soaps, antibacterial soaps, and heavy strength
- Various disinfectants used in households
- Antibiotics (1 mg /ml): Ampicillin, penicillin, kanamycin, tetracycline, chloramphenicol, and cephalosporins (cephalotin, cefoxitin as examples) or other antibiotics
- Gram stain: crystal violet, Gram's iodine, ethanol, safranin
- Saline (Sterile) for dilutions
- Slides
- Sterile materials: swabs, test tubes, small paper discs
- Incubators
- Microtiter plates and a plate reader (if available for MIC's)

**Media:**

- Nutrient agar plates used for: hand washing, dilution plating of water samples, environmental isolates, streak isolation, disinfectant assay, growth conditions (temperature)
- Nutrient agar plates with 1, 2, 5 and 10% NaCl
- Nutrient broth
- Nutrient agar stabs
- Coliscan medium: Coliscan Easygel (25001) *E.coli* / coliform growth medium (Micrology Laboratories - http://www.micrologylabs.com/

## Overview of the experiment

The students collect the water at the beginning of the semester. The microbes they isolate are used for all subsequent tests. The experiments are divided into basically 3 different areas:

1. Establishment of culture collection: water sampling, fecal coliforms, Gram stain
2. Physical parameters: growth requirements and efficacy of selected disinfectants
3. Minimal inhibitory concentration determination

The students are encouraged to do research on the land use that surrounds the body of water that was sampled.

## Student Procedures

**Water collection**. Lake water was collected by placing a sterile 50 ml blue conical tube in a metal carriage and submerging in the water. The carriage was removed from the water, the cap closed and the sample was brought to the laboratory.

**Screening for fecal coliforms**. 3.0- and 5.0-ml aliquots of lake water were added to Coliscan medium and immediately poured into the provided Petri dishes, allowed to solidify and were incubated ($37^oC$) overnight. Water samples were plated in triplicate. Alternatively, the plates can be incubated at room temperature for approximately 5 days.

**Hand washing**. Students designed their own protocols. Materials (swabs, sterile saline, soap and agar plates) were made available to them.

**Establishing their culture collection**. The students isolated bacterial colonies as pure cultures using aseptic technique. Each group (of 4 students) needed to include two colonies of the following: *E. coli* (blue on Coliscan medium), Gram + cocci (based on Gram stain reaction), Gram + rods (based on Gram stain reaction) and Gram – rods (non-blue colonies picked from Coliscan plates).

**Growth requirements.** Each bacterial sample was then exposed to various growth conditions. Agar plates were divided in four parts and the bacterial samples were inoculated by making an "X." Temperatures tested included: $0^o$ C, $4^o$ C, $25^o$ C, $37^o$ C, and $55^o$ C. Ability to tolerate NaCl concentration was determined by inoculating 4 colonies onto nutrient agar containing 0% NaCl, 1% NaCl, 2% NaCl, 5% NaCl & 10% NaCl. Oxygen requirements were evaluated by stabbing nutrient agar a tube to the bottom.

**Evaluation of Common Disinfectants.** Sterile culture broth was inoculated with a single bacterial sample with the broth becoming slightly turbid. Using a sterile swab, the culture was applied to the surface of a sterile agar plate. To apply the disinfectants, they would add 50 µl of a selected disinfectant to a sterile disc, let it absorb and place it on the agar plate. Students could ask various questions when doing this exercise: [1] Does the concentration of disinfectant change efficacy demonstrated by smaller or no zones of inhibition, [2] Does the concentration of the bacterial sample change efficacy

demonstrated by smaller or no zones of inhibition? [3] Does the efficacy of a disinfectant vary with bacterial morphology?

**Minimal inhibitory concentrations to determine antibiotic resistance.** Overnight samples of bacteria are diluted to a concentration of approximately 1000 colonies/mL and added (125 µl) to twelve different wells in a microtiter plate or (1.0 ml) to 12 sterile test tubes. Each antibiotic (at 1mg/mL) will be prepared and the antibiotic will be added (125 µl) into the first well, mixed, 125 µl removed and added to the next well. This will continue until the antibiotic has been added to 11 wells. The last well will be used as the growth control. If using test tubes add 1.0 ml of antibiotic to the first tube, mix well, remove 1.0 ml of the sample and transfer to the next tube. Continue the serial dilution through tube 11. The last tube will serve as a growth control. This technique can determine the amount of antibiotic that is needed in order to inhibit the growth of the bacteria tested. These results will allow the students to compare different antibiotic resistance profiles of the varied microbes in their collection.

## Results

This laboratory experience was effective in allowing non-science students to conduct open ended experiments. All procedures were performed on bacterial isolates that they collected.

**Water collection and screening for fecal coliforms**. Students were able to easily identify colonies that were probably *E. coli.* They could easily differentiate between different microbes in the water sample and were able to calculate the percentage of fecal coliforms present (using concentration from dilution plating). This experiment easily leads into conversations concerning water safety and pollution.

**Hand washing.** Since this is student designed, the results are quite varied. To supplement this experiment, students read secondary literature dealing with adequate hand washing. These results lead to discussions about experimental design, use of controls, variables and factors that are difficult to define (water pressure, how vigorous you wash your hands, ETC). Students become aware of the necessity of adequate hand washing to try and prevent spread of colds, and when handling raw foods (chicken).

**Establishing their culture collection & growth requirements.** The students were able to see that microbes isolated from similar environments may have different morphology (Gram stain), or be more tolerant or less tolerant to varied growth conditions (temperature, oxygen availability, NaCl concentration). This experiment was used to segue into discussions of extremophiles.

**Evaluation of Common Disinfectants.** The students were engaged in this process as they assessed disinfectants routinely used.

**Minimal inhibitory concentrations to determine antibiotic resistance.** Bacterial antibiotic resistance is a major threat to health care today. Students were able to evaluate the resistance of their microbes to a selected panel of antibiotics. To supplement this experiment, basic modes of antibiotic resistance (by bacteria) were explored as well as new drug design. Bacteria's altruistic behavior, in sharing DNA, was also addressed. This was accomplished by using secondary literature.

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## About the Authors

**Paula B. Lessem** earned her doctorate degree in Biochemistry from Rutgers University, New Brunswick, New Jersey. She is a Director of Biological Laboratories at the University of Richmond where she is responsible with the development and implementation of laboratories for genetics and cell biology, the first year courses in the Biology sequence. She also teaches a non-major course in microbiology.

**Debra Wohl** earned both her masters degree in Entomology and her doctorate degree in Ecology from the University of Georgia, Athens, Georgia. She currently holds the position of Assistant Professor of Biology at Elizabethtown College, where she teaches both general education and biology courses. Courses include Biological Concepts, Introductory Biology, Microbiology, and Microbial Pathogenesis.

## Original poster:

# *Unseen Life: Engaging Non-Science Students through Microbiology*

by Paula B. Lessem & Debra Wohl

University of Richmond
Founded 1830

Microbiology is used as a tool to introduce non-science students to basic principles underlying scientific investigations (in general) and tenets of microbiology (specifically). Students monitor the bacterial level of Westhampton Lake over a 4 week period. Bacterial populations targeted include coliforms ( fecal and non-fecal) and other microbes (typically Gram positive rods and cocci). These isolates are the basis of their culture collection. During the course, they will categorize these microbes based on their Gram reaction, and growth requirements (oxygen, temperature, salt) to illustrate the diversity of habitats where microbes reside. Selected microbes will also be evaluated as to their susceptibility to common disinfectants (many of the isolates are common) and UV radiation. The final investigation will be the determination of the minimal inhibitory concentration to selected common antibiotics. As a result of the experience it is hoped that students will appreciate the roles played by microbes and humans.

Overall goals: Students will gain an appreciation and awareness of the role microbes play in their world.

Topics Discussed and Addressed Included:

Microbes use in bioremediation
Genetically modified organisms (especially Bt)
Microbes found in the arctic and deep sea thermal v
Can there be microbes on Mars?
Epidemiology: representative case studies
Development and Marketing of new pharmaceutical
Pathogens to fight cancer
Microbes: fermentation and food
Pharmaceuticals and other materials made by bacte
HIV, Smallpox & Ebola
Biowar

Resources used for this course include:

- "The Other End of the Microscope – the Bacteria Tell Their Own Story" by Elmer W. Koneman
- On-line microbiology resources and interactive sites
- Appropriate articles primarily from *Scientific American*
- Use of "The Hot Zone" by Robert Preston

Science knows no country, because knowledge belongs to humanity, and it is a torch that illuminates the world.
…Louis Pasteur

Laboratory experiments conducted included:

- Establishmen of a culture collection using microbes isolated from Westhampton Lake (on campus), a hand washing experiment and from environmental sampling
- Use of microbes in the culture collection to investigate diversity in bacterial physiology (Gram reaction), growth requirements of microbes, effect of various disinfectants, and determination of the minimum inhibitory concentration (for these microbes) to selected antibiotics
- Demonstration of the pivotal role played by microbes in molecular biology - PCR & restriction digestion