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Outbreak! Scenario-Based Instruction in Microbiology

Jan Blom[1], Angela M. Seliga[2], Xiaojuan Khoo[3], Matthew L. Walker[4], Nathan Rycroft[5]

[1,2,4,5] Boston University, Department of Biology, 5 Cummington Mall, Boston MA 02215 USA
[3] Boston University, Department of Biomedical Engineering, 44 Cummington Mall, Boston MA 02215 USA
(**blom@bu.edu; amseliga@bu.edu; xiao@bu.edu; mwalk79@bu.edu; nrycroft@bu.edu**)

With recent outbreaks of swine flu and the emergence of superbugs, it is important to introduce students to the organisms responsible for infectious diseases and how easily they are spread. In this scenario-based learning (SBL) module, students will take part in a simulated epidemic and model the outbreak of an unknown pathogen. Through a six-part series of guided investigations, students will identify the "pathogen" responsible for the epidemic using simple microbiological assays and determine the best treatment to eradicate the pathogen. At ABLE, two of these activities were presented, as well as the development of this module.

Keywords: Infectious disease, synthetic epidemic, antibiotic testing, bacterial morphology, bacterial physiology

Introduction

The Outbreak! module is a multi-part laboratory exercise designed to provide students the opportunity to participate in active, collaborative, and inquiry-based learning in infectious disease epidemiology and microbiology. This module was developed and offered as part of the Biology Inquiry & Outreach with Boston University Graduate Students (BIOBUGS) program, an educational outreach program sponsored by the BU Learning Resource Center (LERNet). The mission of the BIOBUGS program is to encourage Boston-area high school students to become excited about science by (i) exposing them to sophisticated scientific equipment, (ii) providing interaction with senior undergraduate and graduate students, and (iii) introducing students to a university campus. More information on the BIOBUGS program is available at: **http://www.bu.edu/lernet/biobugs/index.html.** Although Outbreak! was designed for a high school audience, the laboratory exercises as well as the scenario can be easily adapted for undergraduates.

The Outbreak! module adopts a scenario-based learning (SBL) approach in which students are placed in a hypothetical situation and exposed to relevant issues, challenges and dilemmas. They are then expected to apply their knowledge and skills relevant to the situation to navigate through the exercise. Students will play the role of prospective trainees at the fictional "Boston University Center for Disease Control" (BUCDC). During the course of the trainee orientation, unidentified "pathogenic" bacteria are accidently "released" into the lab, causing an outbreak of an infectious disease. Through a series of six hands-on lab activities (Table 1), students will simulate the release and transmission of the pathogenic bacteria, and attempt to characterize, identify, and treat the "unknown" pathogen. Each activity is designed around the overall scenario to maintain engagement and achieve the program objectives. Besides introducing major concepts in infectious disease and microbiology, this module also exposes students to basic, but essential, microbiology laboratory skills including microscopy, catalase testing and gram staining (Brown, 2012).

Table 1: Six-part format of the Outbreak! module.

#	*Activity*	*Description*
1	BUCDC Ice-breaker/ Synthetic Epidemic	Ice breaker activity for students. Simulate "unknown" pathogen transmission and highlight key concepts in infectious disease epidemiology.
2	Introduction to the Light Microscope	Teach students to mount samples onto slides and how to operate a basic microscope.
3	Gram Staining	Compare morphological and structural differences to distinguish between different types of bacteria. First step to identifying "unknown" pathogen
4	Catalase Test	Compare physiological differences to distinguish between different types of bacteria. Second step to identifying "unknown" pathogen
5	Identifying the Unknown Pathogen	Summarize data from Activities 3 & 4 to conclusively identify "unknown" pathogen. Illustrate pathogen diversity.
6	Antibiotic Testing	Compare bactericidal activity of three antibiotics. Identify the best drug to treat "unknown".

The BIOBUGS Outbreak! module is typically offered over one week of each semester, with a different group of students in each daily session. Since its inception in 2009, the module has been held for ten sessions, with approximately 20 students per session. Completion of the module objectives can readily be accomplished in 3 hours; 2 hours for activities and 1 hour for guided inquiry and discussion. However, the multi-part design of the module allows simple modification to expedite or extend any activity to suit any audience. For example, BIOBUGS attendees have ranged from English as second language students in 10th grade currently taking Biology who have never had the opportunity to use microscopes to Advanced Placement Biology students in 12th grade who have covered infectious diseases. Outbreak! has also been extended to a 6-hour module for high school students in the Upward Bound Math Science program at BU, which is a federally funded TRIO program to prepare low-income and first-generation college bound students for success in post-secondary degrees in these subjects (Fields, 2001).

The Student Outline is an abbreviated version of the worksheets included in a 27-page laboratory packet given to student participants. Detailed protocols are not found within the original worksheets, nor provided to the students in advance of the module. Due to the variation of the population of high school students who are invited to participate in the BIOBUGS program, the prior background and skills of the students are unknown to the instructors in advance. Therefore, background information, detailed protocols, and discussions of results and implications are presented in the form of a PowerPoint slideshow. This gives the module leader some room to adapt the depth and length of instruction and discussion to audience needs. Sample pages from the student packet are included in Appendix A.

The Materials section lists the necessary reagents and equipment required for each session. Generally, the lab activities require minimal preparation and utilize affordable consumable materials which can be ordered in bulk for several laboratory sections. Specialized materials and equipment (e.g., microscopes) can be borrowed from existing undergraduate labs. Several bacterial cultures need to be grown 18-48 hours before the day of the lab. Preparation details are provided in Appendix B.

The Notes for the Instructor is an abbreviated version of the detailed protocols (including classroom prep instructions) distributed to all instructors and volunteers at an hour-long pre-lab prep session. Modifications are included within the descriptions of the activities in this section. Generally, each session will have one graduate student assigned as the lead instructor to present the slides, manage the timing of activities and improvise according to the audience. Additionally, there will ideally be one senior undergraduate or graduate student volunteer at each bench of 6 high school students to guide the students through the procedures, facilitate mini-group discussions, and communicate with the instructor on student progress.

We have also prepared a Teacher's Edition packet as a supplement to the Outbreak! module. This packet includes, for each of the six lab activities, an outline, background information, pre- and post-lab questions and activities, as well as relevant references and links. If desired, this packet can be sent to the high school teacher before the lab to equip the teacher with helpful resources to better prepare students for the module. The post-lab questions can also serve as a means to assess student understanding or stimulate further discussion following completion of the Outbreak! module.

The complete student laboratory packet, PowerPoint presentation, materials list, instructor pre-lab protocols, Teacher's Edition packet, as well as teacher/student evaluations and volunteer surveys, are available at **http://www.bu.edu/lernet/biobugs/outbreak/index.html.**

Student Outline

Activity 1: BUCDC Ice Breaker

1.1 Activity Objective

We will create a synthetic epidemic to show the ease with which microorganisms can be spread across a population, an ever-present problem in the clinical area. One employee will unknowingly act as the epidemic initiator. His/her glove is contaminated with an infectious pathogen. The process of shaking hands will simulate the contact that is necessary for an epidemic to spread. By the end of this activity, you should be able to track down the original source of the "infection" using deductive reasoning, an approach similar to that used by public health officials and epidemiologists.

Table 2. Employee ID numbers of possible infected individuals after handshaking.

ID #	Round 3	Round 2	Round 1	Round 0
1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	4	4	4	4
5	5	5	5	5
6	6	6	6	6
7	7	7	7	7
8	8	8	8	8
9	9	9	9	9
10	10	10	10	10
11	11	11	11	11
12	12	12	12	12
13	13	13	13	13
14	14	14	14	14
15	15	15	15	15
16	16	16	16	16
17	17	17	17	17
18	18	18	18	18
19	19	19	19	19
20	20	20	20	20
21	21	21	21	21
22	22	22	22	22
23	23	23	23	23
24	24	24	24	24
Number Infected				

1.2 Results

Circle the employees that were infected for each round in Table 2.

1.3 Discussion Questions

1. Who is Patient Zero?
2. What is the most likely route of transmission for this pathogen? Explain.
3. How would the transmission of the disease change if it were airborne? Waterborne?

Activity 2: Introduction to the Light Microscope

2.1 Discussion Questions

1. Calculate the power of magnification for each objective on your microscope in Table 3. Multiply the power of the objective lens by the power of the eyepiece lens.

Table 3. Magnification power of classroom microscopes.

Objective Power	Eyepiece Lens Power	Total Power of Magnification
4x	10x	
10x	10x	
40x	10x	
100x	10x	

2. What happens to the view of an image as the power of magnification is increased?

Activity 3: Gram Staining

3.1 Activity Objective

Gram staining is a method commonly used to determine the chemical structure of the cell wall of bacteria, and is a very helpful technique for bacterial identification. Bacteria have a cell wall that is composed of a sugar-protein compound called peptidoglycan. The bacterial cell wall can stain either positive or negative, depending on its structure. Gram-positive cell walls consist of several layers of peptidoglycan. Gram-negative cell walls have one layer of peptidoglycan surrounded by a lipid-based outer membrane. If the bacteria stain positive, they will retain a purple/blue color. If the bacteria stain negative, they will not retain the purple/blue color, but rather have a pinkish/red color. Each bench will perform Gram staining on four known bacteria AND the unknown pathogen, and will then use a light microscope to compare the morphology of the samples. Aside from staining the peptidoglycan layers, the Gram stain can also reveal one of the three characteristic shapes of bacteria in the samples: coccus (spherical or oval), bacillus (rod-like), or spiral.

3.2 Results

Draw and label your observations of the gram stains for all FIVE bacteria at 100x and 1000X magnification in Table 4. Use the oil immersion technique with the 100X objective to increase the resolution of the microscope. Place a drop of immersion oil on the center of the viewing area on the slide and rotate the nosepiece such that the 100X objective is rotated into the oil. Refer to the PowerPoint slide for examples of the different shapes of bacteria and Gram-positive and Gram-negative cells.

3.3 Discussion Questions

1. What bacterial characteristics can be determined using a Gram stain?
2. What can happen to make Gram-positive cells appear Gram-negative?

Table 4. Identification of bacterial morphology.

Label	Draw (100x)	Draw (1000x)
Bacteria Sample: Sample A *Gram Stain Color:* _____ *Gram Stain (+/-):* _____ *Shape:* _____		
Bacteria Sample: Sample B *Gram Stain Color:* _____ *Gram Stain (+/-):* _____ *Shape:* _____		
Bacteria Sample: Sample C *Gram Stain Color:* _____ *Gram Stain (+/-):* _____ *Shape:* _____		
Bacteria Sample: Sample D *Gram Stain Color:* _____ *Gram Stain (+/-):* _____ *Shape:* _____		
Bacteria Sample: Sample U *Gram Stain Color:* _____ *Gram Stain (+/-):* _____ *Shape:* _____		

Activity 4: Catalase Test

4.1 Activity Overview

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O (water) and O_2 (oxygen).

$$2\ H_2O_2 + \text{catalase} \rightarrow 2\ H_2O + O_2$$

Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is harmful to a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the hydrogen peroxide. One of those ways is to make catalase. We will now test for the presence of catalase in the bacteria as a means to distinguish them.

4.2 Results

Record your observations of the catalase tests in Table 5. Refer to the PowerPoint slide for examples of positive and negative catalase results.

4.3 Discussion Questions

1. Which bacteria tested positive for catalase? Which tested negative?
2. What conclusions can you make about the unknown pathogen?
3. What is the purpose of using new slides and sticks for each bacterial species?

Table 5. Identification of bacterial physiology.

Bacteria Sample	Observations	Result (+/-)
Sample A		
Sample B		
Sample C		
Sample D		
Sample U		

Activity 5: Identifying the Unknown Pathogen

5.1 Activity Overview

Now we can finally put together all the information that we have obtained from the earlier activities to positively identify the unknown pathogen that is infecting our group. Summarize your results in Table 6 and use your deductive and reasoning skills to identify the unknown sample.

5.2 Results

Fill in Table 6 with the information from the various tests that you have performed. Refer to the PowerPoint slide for the scientific names of our bacteria.

Table 6. Summary of final results from all bacteria tests

Bacteria Sample	Scientific Name	Shape	Gram Staining (+/-)	Catalase Test (+/-)
Sample A				
Sample B				
Sample C				
Sample D				
Sample U				

5.3 Conclusions

1. Summarize the characteristics of the unknown bacteria in 1 paragraph. Be descriptive.
2. By comparing its characteristics to known bacteria samples, we have identified the unknown infectious pathogen as:

_____.

Activity 6: Antibiotic Testing

6.1 Activity Overview

Antibiotics are a class of antimicrobials produced by living organisms, which, even in tiny amounts, can inhibit the growth of or kill bacteria. In order to find an effective cure for the pathogen infecting our facility, we have to determine which antibiotic compound is the most effective against the infectious unknown pathogen. This is done using the Kirby-Bauer test. In this test, bacteria are inoculated onto an agar medium, after which discs containing antibiotics are placed onto the surface of the agar so that the antibiotic will diffuse into the agar. Following incubation, inhibition of the organism can be seen as a clear region around the antibiotic disc, called a "zone of inhibition", in which no bacterial growth has occurred. The diameter of the zone of inhibition for an antibiotic indicates how effective it is for use in treating that particular infectious organism. We will now test the unknown pathogen against three different antibiotics and recommend the most effective one by measuring their zones of inhibition.

6.2 Results

Record your measurements of the zones of inhibition in Table 7. Refer to the PowerPoint slide for examples of zones of inhibition.

Table 7. Measurement of zones of inhibition to determine appropriate antibiotic treatment.

Antibiotic Sample	Zone of Inhibition (mm)		Average (mm)
	Trial 1	Trial 2	
Sample X			
Sample Y			
Sample Z			

6.3 Discussion Questions

1. Determine if the bacteria is resistant, intermediate or susceptible to each of the three antibiotics.
2. Which antibiotic has the most potential for treating this infectious disease?

Materials

This section lists the equipment and supplies required for five lab sessions held over the week that BIOBUGS is offered. We can accommodate up to twenty-four students in each section, with six students assigned to each of the four lab benches in the classroom. This seating arrangement is conducive to the sharing of lab materials and equipment. Student stations will be set up ahead of each activity by program volunteers. Bacterial cultures will need to be grown 18 to 48 hours before the day of the lab. Only one culture per species is needed if all of the preparation is performed at one sitting. Cultures cost ~$10 each and can be stored for up to five days at 4ºC. Bacteria smears (Activity 3) and antibiotic sensitivity test plates (Activity 6) will also have to be prepared in advance. Detailed preparation notes and protocols are included in Appendix B.

Activity 1: BUCDC Ice Breaker

Supplies

- Paper towels ("Bounty Select-A-Sheet"; four sheets per student station)
- Paper plates (one plate per station; reusable across sessions)
- Baby powder (two 22 oz containers; refill paper plates as needed)
- Glo Germ™ powder (BBhealthy #B0006ZH45I; ~$16 per 4 oz)
- Latex or nitrile laboratory gloves (one box each of S, M, L)
- Aluminum foil (to cover paper plates)
- Lab tape (to label benches)
- Sharpie markers (two per bench)

Equipment

- Portable handheld black lights (one per bench; Penny Lane Gifts #BLA7; ~$8 per unit; requires 2 AA batteries)

Activity 2: Introduction to the Light Microscope

Equipment

- Light microscopes (three to five per bench; with 10x and 100x objectives and 10x eyepiece)

Activity 3: Gram Staining

Supplies

- Glass microscope slides (5 per bench per session)
- Nutrient Broth powder (100g; Fisher Scientific, #0003-15-0; ~$50 per unit)
- 18-hour cultures grown in nutrient broth and smeared on slides at least an hour before the activity: three cultures of *Staphylococcus epidermidis* (labeled: samples B, D, U; Ward's Natural Science, #85 V 1035), one culture of *Escherichia coli* (labeled: sample C; #85 V 0400), and 1 culture of *Bacillus megaterium* (labeled: sample A; # 85 V 1154)
- Sterile swabs
- Student Gram staining kit: crystal violet, iodine, and safranin in dropper bottles, ethanol and sterile dH_2O in squeeze bottles, pipeclay triangles, and blotting paper (one kit per bench)
- Disposable sterilized inoculating loops (Fisher #22-363-602)

Equipment

- Bunsen burner
- Flint lighters
- Light microscopes (see Activity 2)

Activity 4: Catalase Test

Supplies

- Glass microscope slides (five per bench per session)
- Nutrient Agar powder (100g; Thermo Fisher Scientific, #0001-15-2; ~$56 per unit)
- 18-hour cultures grown in nutrient agar, one set per bench: three plates of *S. epidermidis* (labeled: samples C, D, U; Ward's Natural Science, #85 V 1035) and two tubes of *Myobacterium smegmatis* (labeled: samples A and B; Ward's Natural Science, #85 V 11671)
- Glass microscope slides (five slides per bench)
- Wooden transfer sticks (five slides per bench)
- 3% hydrogen peroxide (two dropper bottles per bench)

Activity 5: Identifying the Unknown Pathogen

- No materials are needed

Activity 6: Antibiotic Testing

Supplies

- Muller-Hinton II agar (500 g, Thermo Fisher Scientific, #211438; ~$121 per unit)
- 100 cm plastic Petri dishes (two dishes per bench)
- *S. epidermidis* culture (Ward's Natural Science, #85 V 1035)
- Disposable sterilized inoculating loops (Fisher #22-363-602)
- Antibiotic sensitivity discs for ampicillin (labeled X; Thermo Fisher Scientific, #230705, ~$21 per 50 discs), streptomycin (labeled Y; #230942, ~$22 per 50 discs), and penicillin (labeled Z; #230918, ~$22 per 500 discs)
- Rulers (two per bench)

Notes for the instructor

The Outbreak! module is developed as a multi-part SBL exercise. The student packet is presented in the form of a "BUCDC New Employee Information Packet". Aside from lab notes and worksheets, the packet also contains components such as an employee welcome letter and a lab safety guide to place students within a hypothetical laboratory setting in which the outbreak occurs. Each activity is also designed around the overall scenario to maintain student engagement. Instructors and volunteers adopt various roles in the context of the scenario to help navigate students through the exercise. This section provides general instructions for the Outbreak! module for Activity 1 through Activity 6 and is broken down into activity overview, pre-lab setup, in-lab instruction, as well as possible discussions and extensions. We also review potential safety issues at the end of this section. Although this three-hour module was designed for high school students, it can also be expanded to fit into a semester-long curriculum for undergraduates. The inspiration for Outbreak! came from two undergraduate microbiology courses for both biology majors and non-majors at Boston University. All of the techniques in this module are basic microbiology techniques found in Benson's Laboratory Manual (Brown, 2009). There are many other activities in this manual that could be used to modify Outbreak! for undergraduates. The heart of Outbreak! is the morphological and physiological identification of an unknown bacteria, activities done in the beginning of both microbiology courses. Specific modifications or expansions of these activities for an undergraduate course can be found below.

Activity 1: BUCDC Ice Breaker (45 min)

1.1 Activity overview

This synthetic epidemic, disguised in the form of a "BUCDC ice breaker," is ranked as the favorite activity of the students and teaching staff based on the feedback obtained. It is designed for 24 participants, but can be modified to accommodate a class of a different size, with 3 rounds of hand shaking of pre-determined partners for each round (Table 8). It is important to know the number of students beforehand to best design the activity such that everyone shakes hands with a different person in each round. It is possible to allow truly random hand shaking, but the likelihood of powder contamination increases.

1.2 Pre-lab setup

Each student station (with 6 stations per bench) is labeled with an "Employee ID" and set up with four sheets of paper towels labeled Round 0, Round 1, Round 2, and Round 3 on lab tape, as well as a paper plate containing a thick layer of powder (Figure 1). Cover the plates with aluminum foil to prevent powder contamination. Stations are set up identically with the exception of one that has a paper plate of Glo Germ powder instead of baby powder. The Glo Germ station could be randomized across sessions, but the Instructor must know which is the "contaminated" station beforehand. Glo Germ has a similar consistency to baby powder but is visible under UV light. The student who sits at the Glo Germ station is patient zero and will spread the infection during the rounds of hand shaking. Student lab packets are also distributed to each station.

Table 8. Pre-determined partners for 3 rounds of hand shaking.

Round 1		Round 2		Round 3	
Employee ID#	Shakee's ID#	Employee ID#	Shakee's ID#	Employee ID#	Shakee's ID#
1	16	**1**	8	**1**	4
2	15	**2**	7	**2**	3
3	14	**3**	6	**3**	2
4	13	**4**	5	**4**	1
5	12	**5**	4	**5**	16
6	11	**6**	3	**6**	15
7	10	**7**	2	**7**	14
8	9	**8**	1	**8**	13
9	8	**9**	16	**9**	12
10	7	**10**	15	**10**	11
11	6	**11**	14	**11**	10
12	5	**12**	13	**12**	9
13	4	**13**	12	**13**	8
14	3	**14**	11	**14**	7
15	2	**15**	10	**15**	6
16	1	**16**	9	**16**	5

1.3 In-lab instruction

Instruct students to sit down without touching anything at their stations. Begin the slideshow and introduce students to the scenario of a BUCDC new employee orientation. After welcoming students to the BUCDC (this can be played up as much as the instructor desires), hand out gloves, and demonstrate the following procedures for Round 0: place the right palm on the powder for 5 sec and immediately, but carefully, place the right palm on the paper towel labeled Round 0 for 5 sec. The procedures for Rounds 1 - 3 are a form of "Simon Says", whereby students are guided through each round together as a class. In each round students will hold his/her right hand palm-side up, find their assigned "shakee", shake right hands for 5 sec, then return to their stations and place the palm on the appropriately labeled paper towel for 5 sec. When all rounds are complete, students should remove and dispose of gloves in the trash bins provided.

Once all hand shaking rounds are completed, the instructor will advance the PowerPoint presentation to trigger a warning alarm and slide to inform students that an air contaminant has been detected and the classroom is in quarantine. To enhance credibility, a program volunteer can claim

responsibility for this lab accident. However, he/she is unsure which pathogen the classroom has been exposed to. Students are informed that this "unknown" pathogen is genetically modified to be visualized with UV light. Volunteers scan the paper towels to identify who has been infected in each round, starting with Round 3, while the Instructor tallies the data on the slide. If the handprint is visible, the student should stand up and remain standing. With 24 participants, 8 people should be standing in Round 3. During subsequent rounds, volunteers only need to scan the infected students, who should sit down if the handprint is not visible. By the end of three rounds, one student should remain standing and is designated "Patient Zero". In the context of the Outbreak! scenario, students are then told that they will be four identified samples (labeled A-D) of potentially pathogenic bacteria, and a sample of the unknown pathogen (labeled U) that they will have to identify.

1.4 Discussions and extensions

Basic discussion includes the concepts of patient zero, exponential growth, quarantine, routes of transmission, and epidemiology. Tested extensions included video clips from popular movies, historical examples of epidemics, personal accounts of Swine flu, and a brief activity where students made lists of every object they touched that day.

1.5 Modification for undergraduate level

The synthetic epidemic in Outbreak! could be used to engage the students for the morphological and physiological identification of an unknown. This opening activity can be done with two strains of bacteria as described in exercise 78 in Benson's laboratory manual (Brown, 2009). To stimulate interest and motivation, the instructor could generate a scenario similar to Outbreak! for students to identify an unknown that could cause a pandemic or epidemic.

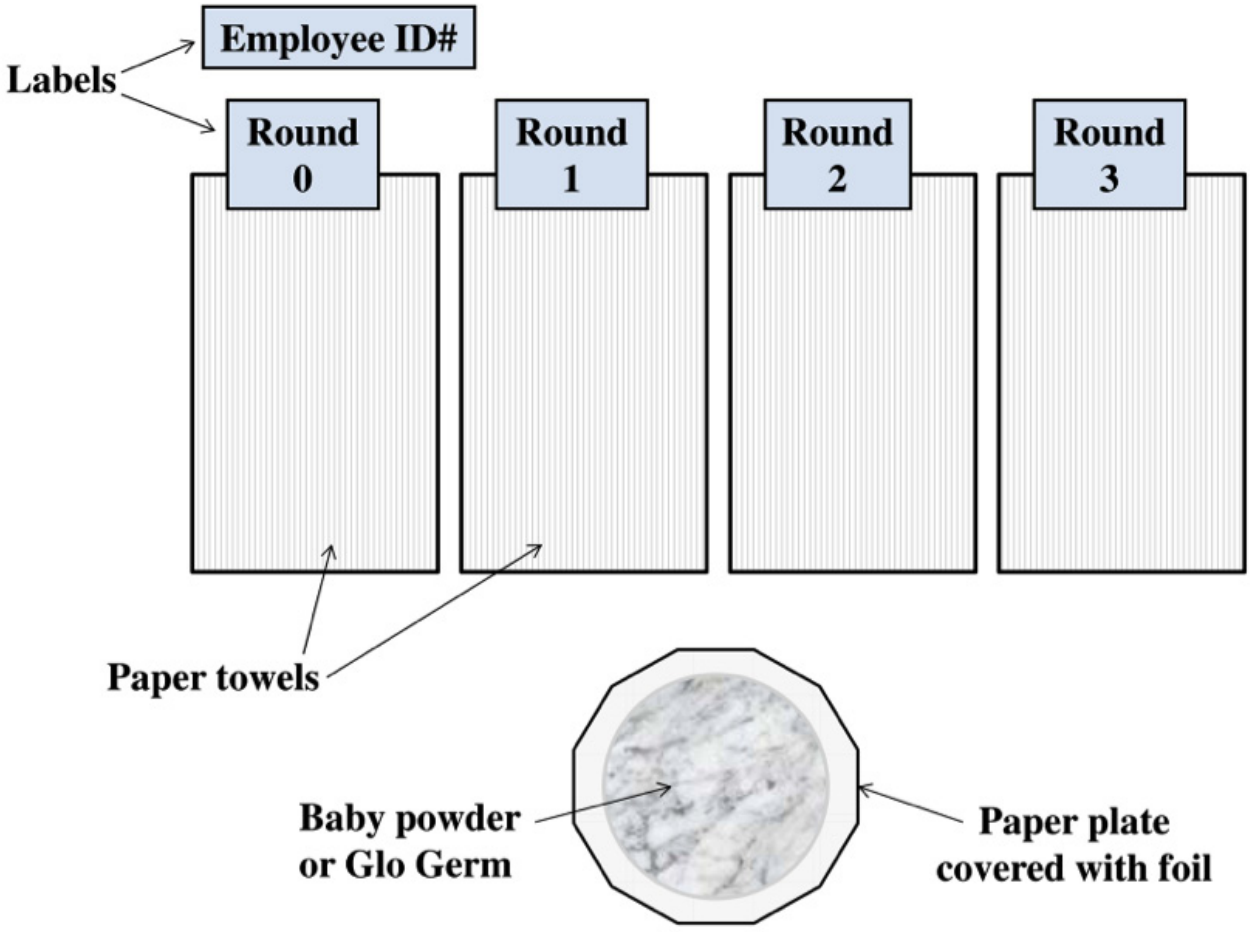


Figure 1. A sample station for Activity 1. Benches were labeled with Round 0, Round 1, Round 2, and Round 3 on lab tape. Paper plates were covered with a thick layer of baby powder or Glo Germ, then covered with aluminum foil. Paper towels need to be replaced every session and powder as needed.

Activity 2: Introduction to the Light Microscope (10 min)

2.1 Activity overview

This activity is used to instruct students on the use of a basic light microscope. Through discussion or blank diagrams, students learn or recall the parts of the microscope, specifically the components they will need to adjust during Activity 3. Students also practice calculating the magnification power and observe the changes in an image with increased magnification.

2.2 Pre-lab setup

Before students arrive, ensure that all microscopes are working and contain 10X and 100X objectives and a 10X eyepiece. During the discussion of Activity 1, volunteers transition the student benches to set up for Activity 2, which requires three to five microscopes at each bench, and Activity 3, which requires the Gram stain kits (see Activity 3).

2.3 In-lab instruction

Beginning with this activity and continuing through the remaining activities, students will work in groups of four to six students at their assigned benches. Due to the varied audience in the program, this activity is designed to be brief if students are comfortable with microscopes or can be extended if students cannot answer basic questions of the parts of a microscope and magnification. Unfortunately, our instruction is minimal for this activity and the main reason we require one volunteer per six students.

2.4 Discussions and extensions

The only extension activity tested had students first label a blank diagram of the microscope and make wet mounts of words cut out from the newspaper and draw what is viewed with each possible magnification of the microscopes.

2.5 Modification for undergraduate level

Students will need to be introduced to basic laboratory practices including microscopy, aseptic technique, and culturing bacteria. Instead of the traditional teaching these techniques and then doing the activity, instructors can introduce the techniques as students are culturing their unknown organism.

Activity 3: Gram Staining (50 min)

3.1 Activity overview

Students will use microscopy and Gram staining to identify the morphological and structural characteristics of the "unknown" pathogen. This activity requires the most amount of time and is the most challenging for the students.

3.2 Pre-lab setup

Before students arrive, the bacteria smears will need to be prepared from the 18-hour cultures. Using a sterile swab, spread a generous amount of bacteria from the nutrient broth onto a glass slide. Grasp the end of the slide with a clothespin, and flame the slide from underneath the smear gently, which means three or four quick passes over the flame, to dry out the smear and fix the bacteria to the slide. Create enough slides such that each group of four to six students can Gram stain all five samples (labeled A, B, C, D, and U). The smears require at least an hour before initiating the Gram stain, which is perfect for the design of the Outbreak! module. The smears are stored on a tray away from the student benches until needed.

Gram stain kits were created to include all of the supplies needed in transportable boxes, one per student bench, and contain enough consumable reagents for all of the sessions. The kits can be placed at the student benches during Activity 2.

3.3 In-lab instruction

While wearing gloves, place slides on a pipeclay triangle over a waste container. The procedure is a series of staining and washes, but advise students not to wash the bacteria off the slide. First, apply one drop of crystal violet to stain all cells purple, wait for 1 minute and rinse with water. Second, apply one drop of Gram's iodine to stain peptidoglycan layers (Gram+), wait for 1 minute and rinse with 95% ethanol to remove the dye from Gram- cells. Finally, apply one drop of safranin to stain all colorless cells pink, wait for 30 seconds and rinse with water. Students should blot the slides and examine under the microscope. Students require a lot of guidance to locate and focus on the sample and observations are difficult due to incomplete staining or over-rinsing. However, Activity 5 allows sharing of class data for proper identification of bacterial morphology. Often, benches are treated as diagnostic teams in competition with one another for the most correct identifications, fueling debate and repetition of procedures among team members. The competition continues throughout the remaining activities. When the activity is completed, glass slides should be thrown in the glass box, gloves in the biohazardous box, and the waste containers emptied in the sink.

3.4 Discussions and extensions

Discussions during the background can include a more in depth look into the structural characteristics of bacterial cell walls. The only extension activity tested had students prepare their own bacterial smears prior to Activity 2. Since this involves Bunsen burners, lab safety should be discussed and carefully monitored.

3.5 Modification for undergraduate level

In addition to the gram stain, students can do a series of stains (i.e. acid fast, spore stain) to help identify the cellular structure. The techniques are outlined in exercises 16 and 17 in Benson's laboratory manual (Brown, 2009).

Activity 4: Catalase Test (20 min)

4.1 Activity overview

Students will use a simple catalase test to explore the physiological characteristics of the "unknown" pathogen. This activity is quick to explain and complete.

4.2 Pre-lab setup

During the discussion of Activity 3, volunteers transition the student benches to set up for Activity 4, which requires a set of the 18-hour cultures of the five samples (labeled A, B, C, D, and U), clean glass slides, wooden transfer sticks and dropper bottles of H_2O_2.

4.3 In-lab instruction

As written, while wearing gloves, students handle the bacteria directly to test bacterial physiology through a simple catalase test. Like Activity 3, students can test one sample, or time-permitting, they can each test all five samples. Using a wooden stick, students can smear a small amount of bacteria from the culture onto a clean and labeled microscope slide. Then, add three drops of H_2O_2 and record whether the sample bubbles (catalase +) or not (catalase -). Waste disposal is similar to Activity 3, but wooden sticks should also be thrown in the biohazardous box.

4.4 Discussions and extensions

Discussions during the background can include the differences in physiological characteristics of bacteria, the tools that make them different, and H_2O_2 as an anti-microbial agent. No extensions activities have been tested.

4.5 Modification for undergraduate level

Similar to the modifications for the activity above, students can do a series of tests that will allow them to explore the metabolism of their unknown by incorporating techniques outlined in exercises 41 and 42 in Benson's laboratory manual (Brown, 2009).

Activity 5: Identifying the Unknown Pathogen (5 min)

5.1 Activity overview

Typically, timing is limited and students want to find the 'cure' to the infection (Activity 6), so this activity is often condensed in order to finish Activity 6.

5.2 Pre-lab setup

As this is a discussion activity, there is no pre-lab setup required.

5.3 In-lab instruction

Each diagnostic team is asked to share data, either by test or by sample. Students discover they have been infected with the "deadliest" of our pathogens. The results students would record in Table 6 can be found in Appendix D.

5.4 Discussions and extensions

No extensions activities have been tested with high school students.

5.5 Modification for undergraduate level

As the students are collecting data from the morphological and physiologically characteristics, they can use the Bergey's Manual found in exercise 44 of Benson's laboratory manual (Brown, 2009) to start to narrow down the genus and species of their unknown organism.

Activity 6: Antibiotic Testing (30 min)

6.1 Activity overview

As stated previously, timing is usually limited by this activity, and there is only time for qualitative observations of the antibiotic treatments.

6.2 Pre-lab setup

During the discussion of Activity 5, volunteers transition the student benches to set up for Activity 6, which requires at least two Muller-Hinton plates with three antimicrobic discs each (labeled X, Y, and Z) per bench.

6.3 In-lab instruction

However, with advanced students, measurements of zones of inhibition for three different antibiotics are possible. Students measure the diameter of the emptiness surrounding the medicinal discs of two plates to quantitatively determine which antibiotic is most likely to cure the infection. Regardless of the time remaining, students should indicate whether the organism is resistant, intermediate or susceptible to three antibiotics.

6.4 Discussions and extensions

Discussions can include the need for several types of antibiotics, antibiotics that are commonly available and mechanisms of action and antibiotic resistance. The only extension activity tested had students measure zones of inhibition for multiple bacterial samples and create a graph of class data.

6.5 Modification for undergraduate level

Along with antimicrobic sensitivity testing, students can also explore other methods used to control bacteria, such as alcohols and detergents as described in exercises 37 and 38 of *The Benson's Laboratory Manual* (Brown, 2009).

Laboratory Safety and Awareness

Since the Outbreak! module includes a "wet" lab where students perform actual microbiological testing on bacterial cultures, we emphasize general lab safety, chemical safety and biological safety to ensure smooth running of the module. The instructor-led PowerPoint presentation contains several slides as an oral quiz to discuss lab safety guidelines, personal protective equipment, and proper behavior presented in the context of a "New Employee Orientation". The student packets, or "New Employee Information Packets", also contain a one page Lab Safety Guide which explicitly lists student responsibilities and chemical safety guidelines. Students are required to read through this document, then read and sign a Lab Safety Contract (see Appendix A) on the next page to acknowledge their understanding of these rules. To further ensure student compliance and to address any student concerns, at least one program volunteer will be stationed at each bench of six students. During an hour-long pre-lab prep session, program volunteers will be briefed on lab safety as well as alerted to any potential issues that may arise with each activity.

It is also important to note that the bacteria species used in this module are chosen for their low pathogenicity and prevalent usage in college microbiology labs. As such, we do not expect students to face any issues with the handling of these organisms. As mentioned earlier, students will be instructed to work with five bacteria samples; one unknown sample (labeled U) and four known bacteria cultures (labeled A-D). However, it should be noted that in the actual preparation of the lab, we have chosen not to use four different bacterial species to span the range of four morphological and physiological combinations discussed in the lab (Gram +/-; catalase +/-). Only three organisms were used for Gram staining (*Staphylococcus epidermidis, Bacillus megaterium*, and *Escherichia coli*) and two for catalase testing (Staphylococcus epidermidis and *Myobacterium smegmatis*). Reasons for this include the limited availability of samples from our undergraduate microbiology courses and the Biosafety Level 1 of our biology laboratories. Furthermore, these bacteria are non-pathogenic and easy to culture and stain, which simplifies the prep and set up of the lab for subsequent BIOBUGS instructors (who may not have the relevant expertise). While the actual bacterial identifications are not entirely accurate, the use of these organisms remains appropriate for the scope of an engagement lab, but perhaps not an evaluative lab without extensions to highlight the scientific errors in the identification themselves as a part of a more technical class.

Acknowledgments

This module was developed as part of Biology Inquiry & Outreach with Boston University Graduate Students (BIOBUGS) program. This project was supported by Boston University's Learning Resource Network and the National Science Foundation. The authors are grateful to numerous graduate and undergraduate students in the BIOBUGS program who volunteer their time and provide feedback, and to the Lab Supervisors, Katie Clifford for preparing the cell cultures and Lauren Tereshko for preparing the classroom for our week-long semi-annual program.

Literature cited

Brown, Alfred E. 2012. *Benson's Microbial Applications*. Twelfth Edition. McGraw Hill, New York, New York, 415 pages.

Fields, Cheryl D. 2001. Can TRIO and GEAR UP continue to coexist? *Black Issues in Higher Education*, 18: 26-30.

Appendix A

Sample Pages from the Student Laboratory Packet

Figure A1. Lab safety contract.

Page 4 of 27

Lab Safety Contract

While I am an employee in the BUCDC facility, I agree to:

- Follow oral and written instructions.
- Protect my eyes, face, hands and body with appropriate safety gear when involved in science experiments
- Keep my work area clean and neat to avoid accidents and contamination.
- Contact the instructor immediately when help is needed fast.
- Know the locations of first aid equipment, eyewash, fire blanket and fire extinguisher.
- Act in a responsible way at all times so as to ensure the safety of others, as well as well as my own.

I, [*print name*)_____, have been instructed in the lab safety and emergency techniques needed for the science facility. I understand and agree to follow the lab safety regulations set forth above and in the Lab Safety Guide as stated by the instructors, lab manuals, and specific experiment instructions. I am aware that my safety and the safety of my colleagues depend on my behavior in the laboratory. With this in mind, I will closely follow the oral and written instructions provided by the instructors and/or the administration.

_____
Student Signature/Date

_____
Student Name (please print)

_____
Student Email Address

BUCDC
The Center for Disease Control
@ Boston University

Figure A2. Notes and instructions for Activity 4 - Catalase Test.

ACTIVITY 4: CATALASE TEST

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O (water) and O_2 (oxygen).

$$2\ H_2O_2 + \text{catalase} \rightarrow 2\ H_2O + O_2$$

Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is harmful to a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. One of those ways is to make catalase. We will now test for the presence of catalase in the bacteria as a means to distinguish them.

MATERIALS SUPPLIED (work individually)

- 5 labeled petri dishes containing bacteria samples (A-D, Unknown)
- Glass slides
- 5 Wooden sticks
- 3% hydrogen peroxide solution
- Glass dropper
- Sharpie marker

PROCEDURE

1. Place a small amount of bacteria growth from your culture broth onto a clean microscope slide using a wooden stick.
2. Add a few drops of H_2O_2 solution onto the smear. If needed, mix with a wooden stick.
3. A **positive** result is the rapid evolution of O_2 gas as evidenced by bubbling.
4. A **negative** result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container. Dispose of the wooden sticks in the biohazard sharps container.
6. Repeat experiment for all bacteria samples, using new slides and sticks.

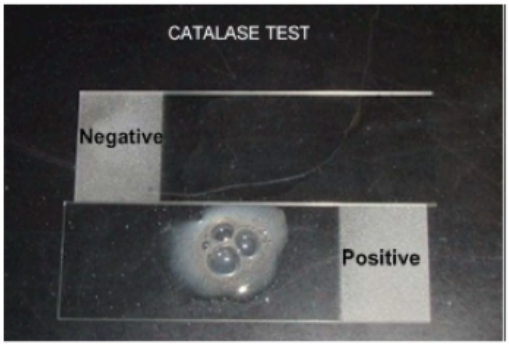
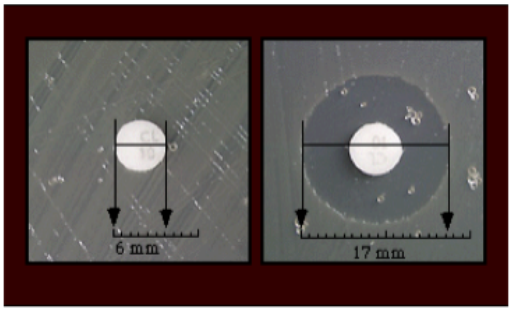


Figure A3. Worksheet for Activity 6 - Antibiotic Testing.

Example zone of inhibition measurement:



RESULTS

Record your data in the following table:

Type of Antibiotic	Zone of inhibition (mm)		Average Zone of Inhibition (mm)
	Trial 1	*Trial 2*	
Drug **X** -			
Drug **Y** -			
Drug **Z** -			

DISCUSSION QUESTIONS

1. Determine if the bacteria is **resistant**, **intermediate** or **susceptible** to each of the three antibiotics.

2. Which antibiotic has the most potential for treating this infectious disease?

Appendix B

Detailed Preparation Notes and Protocols

All techniques described here can be found in detail in The Benson's Laboratory Manual (Brown, 2012).

Preparation of nutrient broth and plated nutrient agar

Eight grams of nutrient broth powder (Thermo Fisher Scientific, Pittsburg, PA; #0003-15-0) were added to 1 L of distilled water until completely dissolved. 5 mL of broth was dispensed into each test tube and capped. Test tubes were autoclaved for 15 min at 121 °C, and placed in 4 °C for storage. Nutrient agar plates were prepared by adding 11.5 g of nutrient agar powder (Thermo Fisher Scientific, Pittsburg, PA; #213000) to 500 mL of distilled water and heated until boiling and then autoclaved for 15 min at 121 °C. Plates (approx. 25) were poured and left to stand at room temperature and stored at 4 °C. Both media types can be made in advance and stored at 4 °C until needed every day.

Preparation of bacterial stock cultures

Bacterial stock cultures were prepared by inoculating nutrient broths with *Staphylococcus epidermid*is (Ward's Natural Science, Rochester, NY; #85 V 1035), *Bacillus megaterium* (Ward's Natural Science, Rochester, NY #85 V 1154), and *Escherichia coli*, (Ward's Natural Science, Rochester, NY #85 V 0400) or nutrient agar plates with *Staphylococcus epidermidis* and *Mycobacterium smegmatis* (Ward's Natural Science, Rochester, NY #85 V 1167) according to Tables 9-11. Fresh cultures were incubated at 37 °C the night before every session. Note that the cultures used for the gram stain activity should ideally be grown for no more than 18 hours.

Antibiotic resistance testing

Muller-Hinton II (MHII) agar plates were prepared by adding 19 g of MHII powder (Thermo Fisher Scientific, Pittsburg, PA; #211438) to 500 mL of distilled water and heated until boiling. Agar solutions were autoclaved for 15 min at 121 °C, then poured into plates (approx. 25) and left to gel at room temperature. Plates were inoculated with *S. epidermidis* using a sterile swab, then one antimicrobial disc each of ampicillin (labeled X; Thermo Fisher Scientific, Waltham MA #230705), streptomycin (labeled Y; Thermo Fisher Scientific, Waltham MA #230942), and penicillin (labeled Z; Thermo Fisher Scientific, Waltham MA #230918) carefully placed into the center of each plate. Plates should be incubated at 37°C overnight to allow for bacterial growth, then stored at 4 °C until needed. Plates can be reused for multiple sessions if stored at 4 °C in between each use.

Table B1. Preparation of Gram stain samples for Activity 3.

Sample Label	Actual Organism	Gram +/- Results	Morphology
A	*B. megatarium*	Gram +	Rod
B	*S. epidermidis*	Gram +	Cocci
C	*E. coli*	Gram -	Rod
D	*S. epidermidis*	Gram +	Cocci
U	*S. epidermidis*	Gram +	Cocci

Table B2. Preparation of Catalase samples for Activity 4.

Sample Label	Actual Organism	Catalase +/- Results
A	*M. smegmatis*	-
B	*M. smegmatis*	-
C	*S. epidermidis*	+
D	*S. epidermidis*	+
U	*S. epidermidis*	+

Table B3. Preparation of MHII plates for Activity 6.

Antimicrobic Label	Actual Antimicrobic	Results
X	Ampicillin	Sensitive (S) Small/no zone of inhibition
Y	Streptomycin	Intermediate (I) Small to medium one of inhibition
Z	Penicillin	Resistant (R) Large zone of inhibition

Appendix C

Sample Survey Forms

Figure C1. Student evaluation form.

Student form

Your School:_____ Grade level: _____

BIO BUGS

Biology Inquiry & Outreach with BU Graduate Students

Your feedback and ideas are important to improving this program for future students. We appreciate you taking the time to help us!

1. What was your favorite lab activity in lab today? Why?

2. What activity in lab today did you dislike? How can we make it better?

3. Please rate the following based on how they facilitated your learning. (5=Great! Really helped me learn, 3=OK. I could take it or leave it, 1=Bad. Needs improvement.)

Teacher's knowledge of material	1	2	3	4	5
Teacher's speaking voice	1	2	3	4	5
Teacher's presentation	1	2	3	4	5
Lab worksheets	1	2	3	4	5
I'd recommend this lab to a friend	1	2	3	4	5

4. Anything else you'd like us to know?

Figure C2. Volunteer evaluation form

BIOBUGS Evaluation Spring 2011

Volunteer Evaluation

Your feedback and ideas are important to maintaining and improving this program for future students. We appreciate you taking the time to help us!

Name: _____ Major/Program:_____

Check One **Undergrad**: ☐ Junior ☐ Senior **Grad Years**: ☐ 1st-2nd ☐ 3rd-4th ☐ 5th+

volunteer days this semester: _____ # semesters volunteered: _____

all titles of labs you've volunteered for: _____

Would you be interested in teaching a lab (use the powerpoints)? ☐ Yes ☐ No (Spring and/or Fall?)

Would you be interested in designing a new lab for May 2012? ☐ Yes ☐ No

Please rate each of the following components of this laboratory using a scale of 1-5.

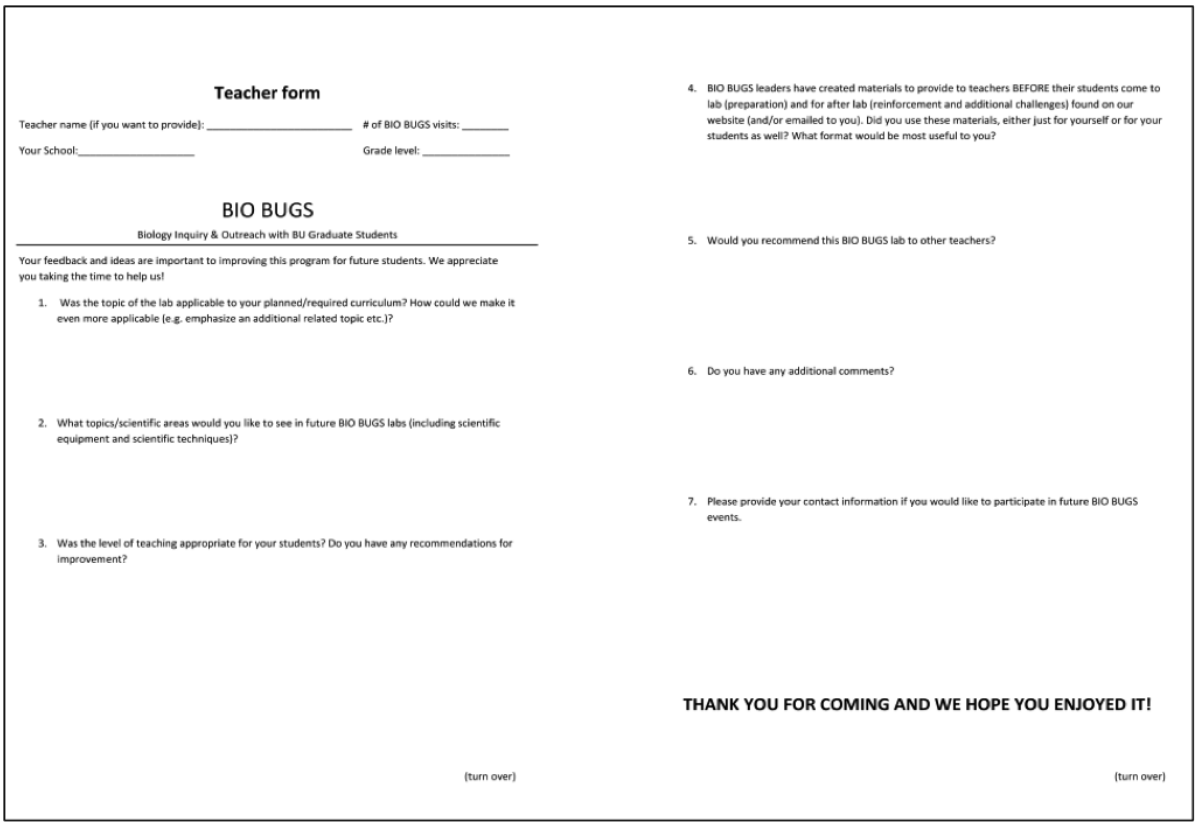
1. Clarity of the laboratory procedures.
   (1 = confusing; 5 = clear) _____
2. Quantity of background information presented in the lab text.
   (1 = too little; 5 = too much) _____
3. Relevance of background information presented in the lab text.
   (1 = mostly irrelevant; 5 = mostly appropriate) _____
4. Lab equipment and materials.
   (1 = major shortages: 5 = adequate supplies) _____
5. Quantity of material you were expected to cover in this lab.
   (1 = spare time available at end; 5 = too much, impossible to cover) _____
6. Adequacy of prep session for preparing you to volunteer.
   (1 = inadequate, major gaps; 5 = adequate, made me well-prepared) _____
7. Your enjoyment level in volunteering this lab.
   (1 = no enjoyment; 5 = extreme enjoyment) _____
8. Estimate of your student's enjoyment level in doing this lab.
   (1 = no enjoyment; 5 = extreme enjoyment) _____
9. Your estimate of the educational value of this lab.
   (1 = worthless; 5 = extremely worthwhile) _____

Use the reverse side of this sheet to tell me specifically about any of the following, keeping in mind what would be useful to know for future semesters (GOOD AND BAD):

- Ratings above: reasons for ratings
- Lab content: waste of the students' time, suggest improvements
- Staff: indicate areas where the prep sessions and setup can be improved
- Students/Teacher: comment on the abilities, behavior, etc

Thank you for volunteering and we hope you enjoyed it!

Figure C3. Teacher evaluation form.



Teacher form

Teacher name (if you want to provide): _____ # of BIO BUGS visits: _____

Your School:_____ Grade level: _____

BIO BUGS

Biology Inquiry & Outreach with BU Graduate Students

Your feedback and ideas are important to improving this program for future students. We appreciate you taking the time to help us!

1. Was the topic of the lab applicable to your planned/required curriculum? How could we make it even more applicable (e.g. emphasize an additional related topic etc.)?

2. What topics/scientific areas would you like to see in future BIO BUGS labs (including scientific equipment and scientific techniques)?

3. Was the level of teaching appropriate for your students? Do you have any recommendations for improvement?

(turn over)

4. BIO BUGS leaders have created materials to provide to teachers BEFORE their students come to lab (preparation) and for after lab (reinforcement and additional challenges) found on our website (and/or emailed to you). Did you use these materials, either just for yourself or for your students as well? What format would be most useful to you?

5. Would you recommend this BIO BUGS lab to other teachers?

6. Do you have any additional comments?

7. Please provide your contact information if you would like to participate in future BIO BUGS events.

THANK YOU FOR COMING AND WE HOPE YOU ENJOYED IT!

(turn over)

Appendix D

Results from Activity 5

In Activity 1 after the warning alarm announces an airborne pathogen has been detected, students are told BUCDC works with the four bacteria listed in Table 12 and that the unknown pathogen must be one of these four. As previously described, these four bacteria are not the samples students are working with, nor is Sample A always one of the five species that are prepared for each Activity. For preparation details, see Appendix B.

Table D1. Student results to identify unknown pathogen in Activity 5.

Sample Label	Organism	Morphology	Gram +/- Results	Catalase +/- Results
A	*Myobacterium tuberculosis*	Rod	+	-
B	*Streptococcus pneumoniae*	Coccus	+	-
C	*Escherichia coli*	Rod	-	+
D	*Staphylococcus aureus*	Coccus	+	+
U	**Staphylococcus aureus*	Coccus	+	+

About the Authors

Jan Blom earned his B.S. in biotechnology from Marywood University and his Ph.D. in molecular cell biology and biochemistry from Boston University. As a graduate student at BU he was awarded the teaching fellow of the year award and participated in the NSF funded GK-12 program. As a GK-12 fellow he taught AP Biology to juniors and seniors at Brighton High School, and designed the Outbreak! module as part of BIOBUGS. Jan currently works in medical communications at Millennium Pharmaceuticals.

Angela Seliga earned her Ph.D. in biology from Boston University and is currently the Physiology Laboratory Coordinator at BU, where she teaches, trains staff to teach, and develops curriculum for multiple physiology laboratory courses. As a graduate student at BU, she participated in the NSF funded GK-12 program where she taught biology and chemistry to high school sophomores through seniors with special needs. She designed or oversaw the design of laboratory modules for several outreach programs.

Xiaojuan Khoo earned her B.S. in bioengineering from the University of California, Berkeley and her Ph.D. in biomedical engineering from Boston University. As a graduate student at BU she participated in the NSF funded GK-12 program. As a Boston Urban Fellow, Xiaojuan taught biology to sophomores and juniors at Fenway High School, and developed a series of laboratory activities and science fair modules to support their existing curriculum. She co-designed the BIOBUGS Outbreak! module, and assembled a post-module panel to introduce participants to the general scientific community at BU. Xiaojuan is currently a postdoctoral researcher in the Laboratory of Biomaterials and Drug Delivery at Children's Hospital Boston, where she continues to participate in educational outreach.

Matthew Walker earned his B.A. in biology at Brandeis University and his currently working on his Ph.D. in molecular cell biology and biochemistry from Boston University. As a graduate student at BU he participated in the NSF funded GK-12 program and taught AP Biology and AP Chemistry to juniors and seniors at Noonan Business Academy. He also designed the Outbreak! module as part of BIOBUGS.

Nathan Rycroft is pursuing his Ph.D. in Marine Biology at Boston University. He has been involved in multiple avenues of outreach including the NSF GK-12 program, BIOBUGS, and hosting high school students in the laboratory of which he is a member. He has taught Introductory Biology, Animal Behavior, and Systems Physiology laboratories.

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