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CURE BIOLOGY: ANALYZING MICROBES USING A COLLABORATIVE STEM APPROACH

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CURE Biology: Analyzing Microbes Using a Collaborative STEM Approach

Synopsis:

We developed a Course-based Undergraduate Research Experience (CURE) to emphasize applied STEM and promote scientific communication. Students use the scientific process, microbiology, and analytical chemistry to characterize microbes encountered in their daily lives. They communicate their findings in an exercise that illustrates the collaborative nature of science and the supporting curriculum.

CURE Biology: Analyzing Microbes Using a Collaborative STEM Approach

ABSTRACT

Applied science, interdisciplinary modeling, and scientific communication are core competencies for biological education (AAAS, 2011). An evolving method to develop these competencies is through Course-based Undergraduate Research Experiences (CURE). CUREs, while variable in content and approach, promote the application and synthesis of increased knowledge along with improved analytical and collaborative skills (Corwin, Graham, & Dolan, 2015). Studies support departing from traditional quizzes to incorporate models and reports, which provide an intermediate level of difficulty for faculty assessment and a mid- to high-potential gains for students (Batzli & Long, 2010). Similarly, inquiry or model-based learning (Buckley, 2000; Ebert-May, Brewer, & Allred, 1997) or authentic research (Dymond, *et al*., 2009; Bednarski, Elgin, & Pakrasi, 2005) tends to improve overall biological comprehension. With this in mind, we endeavored to generate a CURE for an introductory biology course to augment the liberal arts curriculum and enhance microbiology skills through a practical application of the scientific method.

We developed a CURE entitled "Microbes Among Us" that leverages skills in microbiology, chemistry, and mathematics via hypothesis generation, experimentation, interpretation, and scientific communication. In this guided exercise, students select several sampling locations to gain experience in microbial isolation, culturing, and aseptic technique. Applying the scientific method, students make predictions and assemble findings in a structured format, complete with photographs, figures, and charts. This enables students to understand the

extent of microbial presence and diversity. An optional extension allows the identification of isolates supporting characterization of the microbiome. Further, this requires students to apply content from multiple disciplines, particularly biology, chemistry, and mathematics, to interpret and summarize their findings and communicate results through an authentic research experience.

Key Words: CURE, STEM collaboration, microbial isolation, scientific communication, biology education

INTRODUCTION

The core competencies in biology education emphasize applications of science, interdisciplinary modeling, and scientific communication (AAAS, 2011). An evolving method to develop these competencies is through a Course-based Undergraduate Research Experience (CURE). CUREs, while variable in content and approach, promote the application and synthesis of increased knowledge along with improved analytical and collaborative skills (Corwin, Graham, & Dolan, 2015). Students using CUREs have achieved gains in technical skills and the scientific process similar to students involved in research internships (Corwin, Graham, & Dolan, 2015; Jordan, *et al.,* 2014; Lopatto, 2008). These mechanisms resonate with students by increasing "situational awareness." That is, instructors provide contextual framework and a general question for students to expand upon by asking their own related questions. This contributes to students designing experiments and collecting information for analysis and interpretation. Studies support departing from traditional quizzes and incorporating models and reports, which provide an intermediate level of difficulty for faculty assessment and relative mid- to high-potential gains for students (Batzli & Long, 2010) thereby enhancing learning. Similarly, inquiry or model-based learning (Buckley, 2000; Ebert-May, Brewer, & Allred, 1997) or authentic research (Dymond, *et al.,* 2009; Bednarski, Elgin, & Pakrasi, 2005) tend to improve overall biological comprehension.

With this in mind, we endeavored to generate a CURE for biology to augment our liberal arts curriculum and enhance microbiology skills through a practical application of the scientific method.

Designing the CURE

An overarching goal of our biology curriculum is to cultivate leaders skilled in critical analysis who can solve ill-defined problems. In support of this goal, we strive to incorporate applications and models of course material that migrate beyond cursory depth of explanation in didactic learning. CUREs are activities that integrate learning into these types of models to meet course objectives. Educational literature provides several examples of projects designed to meet these objectives and discusses the benefits of group work when solving complex or open-ended problems (Wilson, Brickman, & Brame, 2018; Scager, Boonstra, Peeters, Vulperhorst, & Wiegant, 2016) with increased student motivation and gains (Kirschner, Paas, & Kirschner, 2011) when the problem is relevant to their lives outside the classroom (Wilson, Brickman, & Brame, 2018; Schmidt, Rotgans, & Yew, 2011). For our student population, predominately non- science majors, we considered the CURE literature of probable outcomes (Corwin, Graham, & Dolan, 2015) and wanted to increase awareness of the importance of teamwork and analytical skills (Shaffer, *et al.,* 2014; Bascom-Slack, Arnold, & Strobel, 2012; Shaffer, *et al.,* 2010). We also wanted to promote faculty interaction (Hanauer & Dolan, 2014; Hanauer, Frederick, Fotinakes, & Strobel, 2012). We further developed an extension exercise, designed for our science majors, that was more career and technically specific, a recommendation of previous CURE-related authors (Shaffer, *et al.,* 2014; Drew & Triplett, 2008), which has the potential for student authorship by characterizing the microbial diversity of our campus.

We developed a CURE entitled "Microbes Among Us" that leverages skills in microbiology and analytical chemistry via hypothesis generation, experimentation, interpretation, and scientific communication. In these guided exercises students working in teams of two are encouraged to identify several locations that they encounter on a daily basis and prepare samples for analysis (e.g. dorm rooms, classrooms, sporting equipment, etc.) to gain experience in microbial isolation, culturing, and aseptic technique. The full description of this exercise is located in Enclosure 1. In brief, students selected locations or objects particularly meaningful to their daily lives and made predictions regarding microbial content and diversity between their selected locations. After sample collection, they plated samples on agar media prepared with and without antibiotics. Following incubation, students compared growth under both conditions making observations regarding quantity, size, shape, and structure. Further, logical conclusions on the effectiveness of select antibiotics are included for discussion to reinforce coursework on antibiotic resistance. Students communicated their findings in a structured format, complete with photographs, figures, and charts. Journeying into the "invisible" microbial realm, students gained an understanding of the volume and diversity of microbes that are encountered on a daily basis. Further, this required students to apply content from multiple disciplines, particularly biology, chemistry, and mathematics, to interpret and summarize their findings and communicate results.

The assessment of these outcomes was through direct measurement (grades shown in Figure 1) and surveys (Tables 1 & 2). In particular, we wanted to assess their abilities to design an experiment, make predictions, how they utilize technical skills to generate data and, most importantly, analyze results. The secondary outcome was the ability of students to work as a team. This included scheduling sampling, analyzing, and writing outside of the classroom to package results and interpretations.

Pre-requisite Knowledge & Facilitator Notes

Minimal prerequisite knowledge is required to implement this CURE. Our students were enrolled in a one-semester introduction to biology course for non-majors. Prior to this exercise, they had a basic understanding of prokaryotic and eukaryotic organisms along with laboratory familiarization with microscopy and species diversity. They had a basic understanding of organisms at the microscopic level but many did not apply it to their daily lives in a meaningful context. All of our students have completed one semester of general chemistry as a prerequisite, such that they are familiar with basic laboratory safety. Standard guidelines (ASM, Guidelines, 2012) were followed to include the wear of safety glasses, lab coats, and gloves at all times when working with the samples in the laboratory. All electronic devices and books were kept on the lab periphery to preserve the benchtop for specimen observations and any manipulation. This is the first time students encountered concentrated, potentially pathogenic, organisms. It is important to minimize unnecessary exposures and exercise caution when inoculating or transferring isolates. Groups designated a recorder, who did not handle the specimens, to make any notes or photographs of the materials or results. Additionally, as this is the first time students may have worked with bacterial cultures and media, aseptic technique is introduced in classroom discussions.

ASM guidelines indicate that BSL1 is suitable for plating environmental samples so long as they are sealed and observed unopened. However, any manipulation or sub-culturing must be accomplished within a BSL2 laboratory (ASM, Appendix, 2012). The non-science majors were provided a sterile sample pack to take to their sampling locations containing Eppendorf tubes with pre-aliquoted buffered solution and sterile swabs (described in Enclosure 1).

Following sample collections, specimens were transported back to the laboratory in sealed bags, unopened until inoculation during the scheduled laboratory period. Once cultured, students minimized unnecessary agitation or contact with isolates by sealing the plates with parafilm.

To streamline the process, instructors demonstrated the concepts of sampling techniques, aseptic transfer and plating. This can be accomplished through videos or in-class demonstrations. Introducing these concepts early decreased the confusion during sampling and while plating. Another recommendation is for students to clearly label everything (tubes, plates, etc.) with their names and contents prior to introducing or transferring any content. Additional recommendations regarding safety and best practices are included in the lab protocol (Enclosure 1).

For advanced students, an extension of this activity allowed the identification of isolates, described in Enclosure 2, which is more appropriate for students trained in the safe use and handling of potentially pathogenic microorganisms in a BSL2 designated space. The students used the source material, colonies on agar plates prepared by the introduction to biology students. Isolates were analyzed using a Matrix-Assisted Laser Desorption/Ionization Time-of- Flight Mass Spectrometer (MALDI-TOF MS) and identified using the Shimadzu iD^{Plus} Advanced database platform. This allowed students to not only characterize the microbes found on campus but apply analytical skills from upper-level coursework. Together this CURE, entitled "Microbes Among Us" is a package that can be adopted for introductory or advanced biology courses where students gain practical experience with microbial isolations, aseptic technique, and scientific communication to enhance connections across the curriculum.

RESULTS

When assessing the early implementation of our CURE, we examined our lesson objectives and how they related to course outcomes. We focused on student's ability to effectively make predictions and generate hypotheses with subsequent analysis and reflection based on their experimental data. The laboratory report and formal report designed for our non- science major course, specified within Enclosure 1, demonstrates this guided progression from consumer to producer of knowledge. Overall, students averaged 90.9% (range 60-100%) on the event (Figure 1). We also considered whether faculty experience influenced student performance by categorizing the relative faculty teaching experience within the course as: less than 5 years; 5- 10 years; or more than 10 years of experience. As shown in Figure 1A, the average performance was slightly lower in students within sections of teachers with the fewest years of experience compared to faculty with 5-10 years of experience. While the range of scores within sections of the more experience faculty (10+ years) was larger, the scores were not significantly different from the new to mid-level experienced instructors. Overall, students performed slightly better on the CURE (0.91 average; range 0.60-1.0) than the course average (0.84; range 0.66-1.0), Figure 1B.

As a secondary assessment, we embedded survey questions within the laboratory report to obtain qualitative student feedback on this exercise. Students stated that they had a sense of "ownership", increased "appreciation for the scientific method", and better "application of what we hear in class". Representative comments are shown in Table 1, which suggest an appreciation for the application of the course content and the scientific process. We also surveyed students at the end of the course for feedback, summarized Table 2. Keeping in mind our course outcome to cultivate leaders skilled in critical analysis who can solve ill-defined problems, seventy-six percent agree that they can solve problems from a multi-disciplinary perspectives and that fellow students contributed to their learning process. Instructors encouraged students to be responsible for their

own learning (95%) and used effective techniques (91%). Notable, 78% of these non- science majors have increased motivation to learn, attributable to their biology instruction.

Another valuable interaction for this exercise is the dialogue between faculty and cadets as students examined their plates and recognized the volume and diversity of microbial life surrounding them every day. Initially, for some students, this CURE was challenging as it required time management and was not a standard "fill in the blanks" type of laboratory exercise (Table 1). Some were interested in further identification or analysis of their isolates. These observations and further questions illustrated invaluable examples of how applied STEM encourages learning through faculty to student mentorship and was the genesis of an analytical extension exercise where students can work with science majors to further characterize isolates.

We partnered with an analytical research group at the Academy to facilitate the development of protocols to address this interest to further characterize samples. This exercise, available at Enclosure 2, was designed for students majoring in life science to foster collaboration with non-science majors to support interdisciplinary dialogue. Samples collected across campus by the non-science majors were provided to advanced students who employed analytical skills using a Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) and the Shimadzu iD^{Plus} Advanced database platform to identify isolates. Briefly, a small amount of cells from a culture prepared on an agar plate are transferred to a MALDI sample plate. On the sample plate, the cells are combined with a matrix solution. The sample plate is then loaded into the instrument, analyzed, and the resulting spectrum is matched against a database for identification (Freiwald & Sauer, 2009). During sample collection, it is important for the non-majors to carefully document the specimen sample location and date to cross-reference with samples selected for further characterization.

Given that collection and preparation of the cultures require the greatest time investment, upper-level students will greatly benefit from the extensive variety of samples collected and prepared by students participating in the introductory biology course. Not only does this investigation answer what is growing on their object or location of interest but it allows students to take the exercise further and uniquely identify microbial diversity on a particular surface by leveraging a multi-disciplinary team.

CONCLUSION

In conclusion, the CURE: Microbes Among Us is a tool to apply biological theory in a manner relevant to non-scientists. Focusing on the process of science, this experience has the potential to stimulate interest in developing the analytical skills necessary in a non-majors biology course or within the confines of an advanced section. It also affords the opportunity for students to pursue independent avenues of inquiry addressing emerging issues of microbial diversity. We have provided this framework such that introductory courses can facilitate integration of this project with minimal cost while promoting core scientific competencies and applications across disciplines.

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FIGURE AND ENCLOSURE LISTING

Figure 1. CURE Scores and Course Average

Table 1. Student Comments Specific to this CURE

Table 2. End of Course Feedback

Enclosure 1. CURE Protocol & Specific Instructions

Enclosure 2. Analytical Extension Protocol

A) **B)**

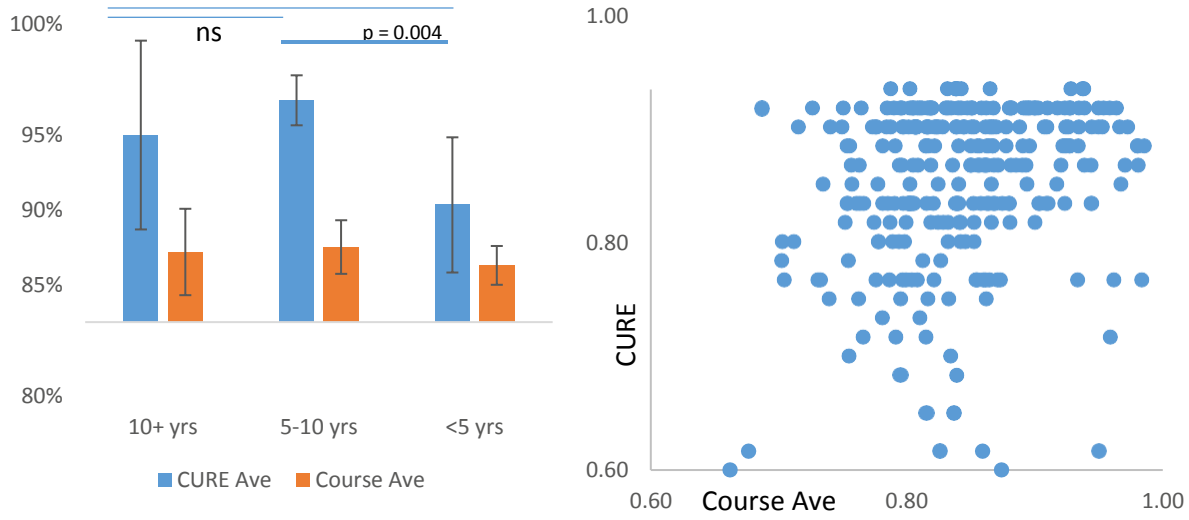

Figure 1. CURE Scores and Biology Course Average. A) The overall percentage for this event, based on years of instructor teaching experience, was compared to final course averages. The fifteen sections were categorized by: 10+ years of teaching (two instructors, three sections); 5-10 years teaching (two instructors, five sections); and <5 years teaching (three instructors; seven sections). Columns indicate average scores across these categories with bars reflecting standard deviation of the average. A one tailed Students' T-test was performed with non-significant differences between course average and the 5-10 year and 10+ year faculty categories (n=284 cadets). **B)** The individual student performance on the CURE (y-axis) is plotted against the final course average (x-axis) in non-majors biology.

Enjoyed or positive comments regarding module

"We liked being able to choose our own samples because we picked things that we used and were surrounded with every day making it more interesting to research."

"We learned microbes are truly all around us."

"Fun and interactive lab."

"The length of time to conduct experiment was more than enough time and extremely helpful in finalizing our results in a lab write-up."

Would like to change or improve within this module

"We want to sample more sites."

"Didn't like how lab was spread out over time."

"We'd rather fill out a form [for the lab report]."

"We would like to see the microorganisms under the microscope."

"We wanted more guidance on what charts and figures should look like."

Table 1. Student comments specific for this CURE. Cadets were surveyed immediately following lab report submission as to what they enjoyed or wanted to improve within this module. Representative comments reflect the majority of feedback received (n=143 lab groups).

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
This instructor encouraged students to be responsible for their own learning.	53	42	5	0	0
This instructor used effective techniques for learning, both in class and for out-of-class assignments.	47	44	7	1	1
My fellow students contributed to my learning in this course.	32	44	17	7	0
My motivation to learn and to continue learning has increased because of this course.	38	40	28	4	0
After taking this course, I can apply multiple disciplines to solve ill-defined problems.	30	46	20	4	0

Table 2. End of course feedback. Students were surveyed at the end of the course as to their thoughts on learning attributable to their instruction (n=117 students).

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Enclosure 1—CURE Protocol & Specific Instructions

The following document provides specific guidance to students and includes instructor guidelines for grading the assignment, CURE: Microbes Among Us. This is designed for non-majors biology but an optional exercise (Enclosure 2) is available for further characterization of isolates by advanced students.

LEARNING OBJECTIVES:

1. Apply the scientific method and experimental processes to a relevant cadet area.
2. Learn how to perform a quadrant streak.
3. Perform semi-quantitative analysis and morphological qualitative comparisons of cultured samples.
4. Identify antibiotic effectiveness/resistance of cultured organisms.
5. Synthesize, interpret, and communicate results of experimental data in written form (lab report)

PRE/POST-LAB REQUIREMENT

- Read lab introduction and procedure.
- View pre-lab video
- Identify lab groups and three locations that your group will sample.
- Obtain designated materials and perform sampling.
- Bring samples to class/lab IAW Table 1 for plating.
- Return to check samples following Lab 5 to obtain pictures/analyze.

LAB REPORT REQUIREMENT: (50 POINTS TOTAL)

This lab report will consist of a signed cover sheet and typed report.

SAFETY: *No contacts will be worn in lab. You will wear gloves, lab coats, and goggles at all times once in the laboratory. You will be using liquid reagents containing microbes. Once cultured, plates may contain multi-drug resistant or pathogenic species. The plates will be wrapped with parafilm and incubated. You will not open these plates after wrapping. You will wash your hands thoroughly* ***EVERY*** *time you remove your gloves and again after completing the laboratory. Report any safety issues or concerns to your instructor immediately.*

Introduction and Overview: Microbes Among Us

The microbial world is a fascinating combination of organisms, ranging from beneficial to harmful. Everything that you come in contact with is populated by species beyond your visual perception. Considering the environments where Soldiers live, work, and conduct military operations, the concept of field sanitation is more than just an additional duty for platoon leaders. It is particularly important that exposure to microorganisms be limited for Wounded Warriors during casualty transport and treatment to decrease colonization and infection.

As you learned in lesson 1, the scientific method can be broken into several major steps including observation, question, hypothesis, predictions, and experiment. This project is specifically designed to familiarize cadets with an application of the scientific method and to further characterize the microbiome of the West Point cadet life. Think about everything you come in contact with each day. From your toothbrush to the classroom desk or the combatives mat, these surfaces are conducive to colonization by microbes ranging from beneficial to pathogenic (disease causing). In this study, you will generate a question and hypothesis regarding microbial life and test your hypothesis experimentally. From these results, you will perform comparison based on microbial growth levels and morphological observations.

This laboratory will be conducted in **five phases** by cadet groups (2 cadets per group) that will be designated in class. A large portion of this laboratory will be conducted outside of scheduled classtime. Cadets will collect samples **prior** to Lab 5. During lab period #5, cadets will plate their samples. Cadets will return to the lab after allowing time for sample incubation and take pictures of their plates for further analysis. The final lab report is due at the beginning of Lab 6 (see Table 1). The five phases and general timeline are as follows:

Phase I: Develop experimental design

Phase II: Perform sampling

Phase III: Plate samples during Lab

Phase IV: Verify colony formation and collect experimental data

Phase V: Generate a lab report

Table 1: General Timeline

Task	R-Hour
Phase I/II: Students sign-up for lab groups and obtain sampling materials from instructors in class	2 Mar 18
Phase II: Students sample sites	5 Mar 18
Phase II: Students return samples to instructors in class	6 Mar 18
Phase III: Students plate samples during assigned lab period	7 Mar 18
Phase IV: Students return to lab to take pictures of plates	8 Mar 18
Phase V: Student submit completed Lab Report to instructor	28 Mar 18

Phase I: Develop Experimental Design

Groups will be assigned in class. Your group will outline your experimental design beginning with asking a specific question to help form your hypothesis and predictions. For example, what locations are you interested in sampling for microbial diversity and why? When determining sampling locations, consider the surfaces that you will assess and how your overall design will enable you to answer your question. In lab, you will place your sample (from a single location) on an agar plate without antibiotics and on an agar plate with antibiotics. Some examples of sampling locations include: a classroom (BH268) doorknob, Arvin wrestling mat, Trophy Point cannon tube, etc.

1. Read through the lab before beginning work. It is important that you understand the materials and methods you are required to use in order to develop a meaningful hypothesis and associated predictions.

2. We will frame the experiment by providing you with the following observation: *There are a variety of microbes living on the surfaces cadets come into contact with every day on West Point.*

3. Considering the observation provided above. Determine a question that your group would like to ask and attempt to answer. For instance, you could ask, "Does the wrestling room have more microbes than the classroom?" Your question can be broad or as specific. Write your question below. You will include this is your final lab report.

Question:

4. Read steps 4 and 5 prior to forming your hypothesis. Because we are imposing limitations on your project, it is important for you to brainstorm ideas and sampling locations that will allow you to answer your question. This will also enable you to make more specific predictions for this project.

A hypothesis is a tentative answer to a question (an explanation) that must be testable and falsifiable. Considering your question and the methods you will be using (three sample sites on West Point, step 5), write your hypothesis below. You will include this hypothesis in your final lab report.

Hypothesis:

5. Identify three surfaces that you will sample for your project in Table 2 below.

Table 2: Sample Locations

Sample	**Surface**	**Specific Location**
Example	Hallway facing doorknob	Ike Barracks, Room 315
1		
2		
3		

6. A good hypothesis will allow you to make testable (and relevant) predictions. Predictions are often if/then statements. Develop and write your experimental predictions below. You must make at least three predictions, but you may include as many as you want. You will include these predictions in your final lab report.

Prediction 1:

Prediction 2:

Prediction 3:

Phase II: Perform Sampling

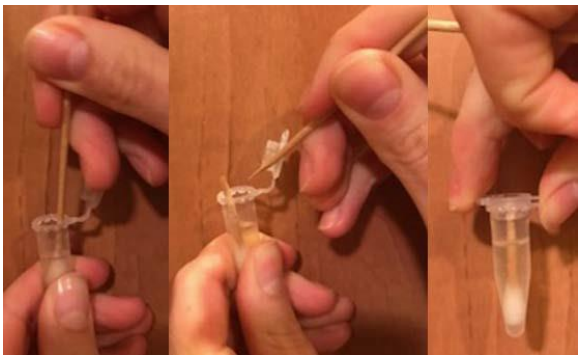
You will receive the sample materials from your instructor prior to your scheduled laboratory.

1. In class, collect and inventory all required materials:
   - 3 Eppendorf tubes filled with sterile solution
   - 3 sterile cotton swabs
   - 1 Ziploc bag

2. Label the Ziploc bag with your names and section prior to leaving the classroom.

3. Label each tube (#1, #2, and #3) IAW Table 2 from Phase I.

4. You will take samples on the date indicated in Table 1. When you are ready to sample your first location, open a sterile cotton swab. If the surface is dry, dip your cotton swab in the Eppendorf liquid to moisten. Swab the surface with several strokes (covering the swab) and place the swab tip in the Eppendorf tube. Break off the top of the swab tip and close the tube tightly (see right). Place the sample in your Ziploc bag.



5. Repeat step 4 for the locations 2 and 3. Record the date/time you sampled the locations in Table 3 below. Describe the conditions (temperature, weather, etc.) at the time of sampling.

Table 3: Sampling Collection Information

Sample	Date/time of Sample	Conditions at Sampling Time
1		
2		
3		

6. IAW Table 1, bring your samples to the specified location (on the specified date) in the Ziploc bag for phase III. Samples must be collected by the date specified in order to allow sufficient time for microbial growth.

Phase III: Plate Samples

During your designated lab period, bring your inoculated samples to the lab.

1. All group members will wear gloves.

2. Retrieve your samples and obtain the following materials:

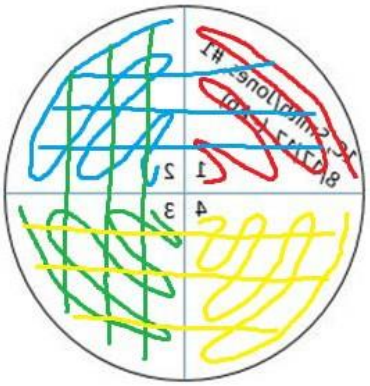
- 24 sterile streaking loops
- 3 agar plates without antibiotic
- 3 agar plates with antibiotic
- 6 pieces of parafilm (2 inches long)
- 2 rubber bands

3. Mark the **bottom of the plate** as outlined in the figure below with a sharpie. Ensure you mark the bottom (agar side) and not the lid. For each sample, you will mark two plates: one with antibiotic and one without antibiotic. Divide the plate into quadrants. Label the top of the agar plate with your section, group names, sample location number, and date. (e.g. 1C, Smith/Jones, #1, 3MAR17,-AB). Ensure that the plate is clearly labeled as with antibiotic (+AB) or without antibiotic (-AB).

4. Using a dilution streak technique, perform quadrant streaking for each sample as described below.

a. Obtain the agar plate labeled for location 1 without antibiotic (-AB). Place the plate, agar side down on the bench and remove the lid.

b. Vigorously shake the Eppendorf tube to ensure the microbes are suspended in the solution. Dip a sterile loop into the solution obtaining a thin layer of solution across the loop (like a bubble wand).



c. Place the loop to the outer edge of your plate in quadrant 1 and streak towards the center of the plate (shown in red). Take care to glide along the surface and not to gouge the agar. Spread it in a zig-zag pattern along the plate. Discard the sterile loop.

d. Rotate the plate a quarter of a turn and gently drag a **new** sterile loop through quadrant 1 into quadrant 2 three times (shown in blue). Then, place the loop

to the outer edge of quadrant 2 and streak towards the center of the plate in a zig-zag manner. Discard the sterile loop.

e. Rotate the plate a quarter of a turn and gently drag a **new** sterile loop through quadrant 2 into quadrant 3 three times (shown in green). Then, place the loop to the outer edge of quadrant 3 and streak towards the center of the plate in a zig-zag manner. Discard the sterile loop.

f. Rotate the plate a quarter of a turn and gently drag a **new** sterile loop through quadrant 3 into quadrant 4 three times (shown in yellow). Then, place the loop to the outer edge of quadrant 4 and streak towards the center of the plate in a zig-zag manner. Discard the sterile loop.

g. Replace the lid on the plate.

h. Repeat this procedure for location 1 on a plate with antibiotic and locations 2 and 3 with and without antibiotic. It is extremely important to use the exact same technique on every single quadrant and on every single plate so that you may make fair comparisons during your analysis.

5. Take each plate and stretch/wrap the edges with 2 squares of parafilm.

6. Stack your plates and secure them together with rubber bands.

7. Leave your plates upside down (agar side on top) in the designated incubator for your instructor/section. Note, the streaks from the first quadrant will likely yield heavy growth while the second will yield less dense growth. Ideally, the third or fourth quadrants will yield single colonies, which you will be able to examine more closely (see picture below).

8. IAW Table 1, you will need to return to the lab daily to check the progress of your plates (read phase IV for more informtion). If you or your lab partner are not able to make it during this time, coordinate with your instructor for access.

9. A semi-quantitative method of classifying microbial growth is determining the quadrant in which the most diluted streak of microbes can be observed. For example, in quadrant 1 there is heavy growth, while there is extremely sparse growth in quadrant 4 in the figure to the right. However, because there is growth in quadrant 4, the microbial growth level of this sample is classified as 4+. If the growth stopped in quadrant 3, it would be classified as 3+. Make predictions on the microbial growth level you expect in each of your agar plates in Table 4 below.

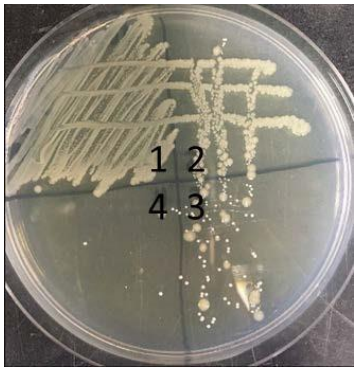


Table 4: Microbial Growth Level Predictions		
Sample	**Without antibiotic growth level**	**With antibiotic growth level**
1		
2		
3		

Explain why you chose the growth level for each sample. You may include this in your lab report introduction.

Phase IV: Verify Colony Formation and Collect Experimental Data

IAW Table 1, you will need to return to the lab to check the progress of your plates. If you or your lab partner are not able to make it during this time, coordinate with your instructor for access. We recommend you take pictures of each plate every lab visit to track their progress. **At no time will you open the agar plates**.

1. Put on gloves and retrieve your plates. Lay them out on the bench (agar side on top). Ensure you are able to clearly see the label in your pictures. If you cannot see the label, make and place a label next to each plate clearly indicating the location sampled and condition (with or without antibiotic).

2. After you remove your gloves (saving them), use your cell phone to take a pictures of each sample. It may be helpful to take multiple pictures (individual and comparison) on both dark and light backgrounds. Use your judgement. You may want to use a ruler located in the lab drawers to place next to your plates for a reference. You will use some of these photos in your final report. Replace your gloves prior to handling your plates again.

3. If your plates have yielded sufficient microbial growth, you will fill in the information below. Be sure to be thorough and detailed as you will only have your pictures after plate disposal. If your plates do not have sufficient growth, return them to the incubator and coordinate a time to return to the lab with your instructor. Ask your instructor if you are unsure if your growth is sufficient.

4. Determine the microbial growth level for each plate (refer to Phase III as a reference). Record your results in Table 5 below. Take notes on microbial growth levels. Point counts (number of colonies present) are not required, but may be beneficial based on your specific plates.

6. Describe the morphology of the colonies appearing on the plates below. Note color difference; shape differences (circular, filamentous, irregular); elevation (raised, convex, flat); and margins (uniform, undulate, filiform, lobate).

Table 5: Microbial Growth Level & Morphology

Sample	Without antibiotic growth level	With antibiotic growth level
1		
2		
3		

Phase V: Generate a Lab Report

This section outlines the final lab report you will turn in to your instructor. This report will be typed in a paragraph format (no bullet points) and be turned in with a signed cover page. As always, use complete sentences for full credit.
*Guidelines shown below for grading lab reports. Consider and use discretion when necessary.

Part 1. Introduction

- (**3 Pts**) Write a brief introductory paragraph about microbes. Include your references.
  - 3 Pts=outstanding/great, 2 Pts=satisfactory, 1=substandard/clear lack of effort,
- (**1 Pts**) State your question. AON (All or Nothing)
- (**2 Pts**) State your hypothesis. AON
- (**3 Pts**) State your predictions based on your hypothesis. Ensure you include why your group thinks one site or another would exhibit more microbial growth. You may reference your original predictions and Table 4. 1 Pt per prediction

Part 2. Outline your methods

- (**2 Pts**) Describe your sampling methods (i.e. how you sampled locations and your treatment of the samples prior to plating). 2=Good, 1=Satisfactory, 0=substandard
- (**4 Pts**) Describe your sampling locations and why you sampled these areas. Include time, date, and conditions when you took your samples.
  - 1 Pt for each location/reason; 1 Pt for time/day/conditions (AON)
- (**2 Pts**) Describe your plating and incubation protocol.
  - 2=Good, 1=Satisfactory, 0=substandard
- (**3 Pts**) Describe your assessment methods (i.e. describe how you assessed microbial growth and morphology).
  - 3 Pts=outstanding/great, 2 Pts=satisfactory, 1=substandard/clear lack of effort,

Part 3. Summarize your results

- In this section you will clearly outline your results. You will draw conclusions in the discussion section. At a minimum you will make a clearly outline the following:
  - (**3 Pts**) Growth and morphology at each location (with and without AB)
  - (**3 Pts**) Growth and morphology between locations (with AB and without AB)
  - (**3 Pts**) Growth and morphology between AB and non-AB plates (disregard location)
  - 3 Pts=outstanding/great, 2 Pts =satisfactory, 1=substandard/clear lack of effort,
- Interwoven in your results summaries above, you will reference and include the following figures (be sure to include figure captions):
  - (**3 pts**) Include side-by-side comparison photographs of your plates for each location sampled (with and without antibiotics).
    - -1 for each missing photo, no more than -3
  - (**3 pts**) Generate a chart/graph showing microbial growth level for each location and condition. -1 for each missing plate, no more than -3
  - (**3 pts**) Prepare a chart/graph (not a table) depicting the differences in growth level between plates with and without antibiotics.
    - Cadets will likely represent this chart in different manners; 3 Pts=good, logical comparison that depicts differences, 2 Pts=satisfactory attempt to address, 1=substandard, 0=nonsensical/not shown

Part 4. Discussion

- (**4 pts**) Generate a paragraph discussing your findings across the locations and varying conditions.
    - 4 Pts=Outstanding, 3 Pts=satisfactory, 2=substandard, 1=clear lack of effort
- (**4 pts**) Interpret your data as it relates to your original hypothesis and predictions. Do the data support or refute your hypothesis? Be specific and reference your results section.
    - 4 Pts=Outstanding, 3 Pts =satisfactory, 2=substandard, 1=clear lack of effort
- (**2 pts**) Based on your results, how would you adjust your hypothesis? State your revised hypothesis. 1 Pt for addressing results, 1 Pt for revised hypothesis
- (**2 pts**) Describe a future experiment to further test your revised hypothesis. AON

Part 5. Bonus

- (**3 pts**) What did your group learned from this exercise? Bonus points are awarded based on the thoughtfulness of your answer.
    - 3-Good insights, 2-Satisfactory, 1-Poor, 0-none
- (**2 pts**) Provide at least one item that you liked about this experiment and one item that you would change for future iterations. Being specific allows us to improve future labs.
    - 1 Pt for like, 1 Pt for change

Part 6. (Optional Extension)

- The identification of unknown microbial samples can be accomplished by procedures described within Enclosure 2.

Enclosure 2—Analytical Extension Protocol

This document provides a brief overview and protocol for advanced students to complement the CURE: Microbes Among Us to further characterize and identify isolates. When used in conjunction with Enclosure 1, this accomplishes these additional objectives:

LEARNING OBJECTIVES:

6. Utilize analytical tools to apply chemical properties to unknown specimens.

7. Synthesize and interpret characteristic spectra to uniquely identify microorganisms.

PRE-LAB REQUIREMENTS:

- Read introduction and procedures.
- Acknowledge safe handling & receive information on safe handling of potentially pathogenic organisms.
- Obtain prepared agar plates with unknown specimens.
- Annotate the source of materials (date, sample location) and morphological characteristics.
- Familiarize with the MALDI-TOF-MS theory and operation and the SARAMIS database on the Shimadzu Axima-iD^{Plus} platform.

SAFETY: *Note, cultures may contain highly concentrated unknown pathogens. Personal protective gear should be worn to minimize contact with unknown specimens following sufficient safety training with proper storage and handling of pathogens in a BSL2 lab.*

Introduction and Overview: Characterizing Unknown Microbes

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) may be used to efficiently characterize and uniquely identify microbes such as bacteria, yeast, and fungi (Croxatto, *et al.*, 2012). Briefly, a sample is combined with a matrix on a MALDI sample plate. Once the sample is dry, the plate is loaded into the instrument. The sample is then hit with laser pulses to desorb and ionize the matrix and sample (Counterman, *et al.*, 2003). Because the sample is ionized through a charge transfer from the matrix, this method is considered to be a soft ionization technique and typically results in generation of singly charged ions. The resulting ions are separated in a flight tube based on mass (*m*) and charge (*z*) prior to detection (Croxatto, *et al.*, 2012). Spectra are reported as signal intensity as a function of mass to charge ratio (*m/z*) and, in the case of microbial samples, result primarily from the presence of ribosomal proteins. This unique mass spectral fingerprint is then matched against a database for identification (Cherkaoui, *et al.*, 2010).

Procedure

The following procedure for use in the analysis and identification of microbial samples is adapted from training documents obtained from the Shimadzu Corporation (Shimadzu Corp, Axima-iD^{Plus} Sample Preparation; and *E. coli* Calibration). Individual colonies, required for further characterization, are obtained from the agar growth plates prepared and described in Enclosure 1.

1. Prepare matrix by combining ~40 mg of α-cyano-4-hydroxycinnamic acid (CHCA) with 1 mL of 33/33/33 acetonitrile/ethanol/water (all HPLC grade) containing 3% trifluoroacetic acid (TFA). Use a vortex to thoroughly mix. Prepared matrix may be stored at room temperature and used for up to one week after preparation.

Figure 1. Fleximass Polymeric Sample Plate. Rows B and J show recently deposited microbial samples combined with CHCA matrix. The three sample wells between columns 2 and 3 are for the DH5α-E™ standard.

2. Using a pipette tip or inoculation loop, transfer a small amount of cell material from a single colony on an agar plate to a fleximass polymeric MALDI sample plate as shown in Figure 1. Immediately deposit 1 µL of prepared matrix and allow sample to dry.

3. Repeat step 2, but after transferring cell material to sample plate, add 0.5 µL of 25% formic acid and leave until almost dry. Then, deposit 1 µL of prepared matrix and allow sample to dry.

4. Repeat steps 2 and 3 to yield desired number of sample replicates.

5. Combine 1 µL of ElectroMAX™ DH5α-E™ competent cells with 49 µL of prepared matrix. Vortex to combine then deposit 1 µL in the standard sample wells located between columns 2 and 3 on the sample plate.

6. Using the TargetManager and Auto Experiment, configure the Shimadzu Axima-iD^{Plus} platform to analyze the samples with the Confidence MALDI-TOF MS and identify the samples by comparison the Spectral Archive And Microbial Identification System (SARAMIS) database.

Results

The unknown inoculate is identified by comparing the resulting spectrum to the SARAMIS database on the Shimadzu Axima-iD^{Plus} platform. Figure 2 illustrates a typical spectrum collected using the sample preparation and procedure described above. A known sample of *Acinetobacter baumannii* was cultured on an agar plate and compared across the SuperSpectra in the SARAMIS database. The reference SuperSpectra are generated by combining individual reference spectra and assigning a peak weight to specific peaks to yield a score that is presented as a percent confidence. A score greater than 70% is considered high confidence (Cherkaoui, *et al.*, 2010). For the spectrum of *Acinetobacter baumannii* shown in Figure 2, the sample was identified with an accurate match with 90.80% confidence. From these

results, advanced students are able to identify isolates and further characterize microbial content of sampled areas.

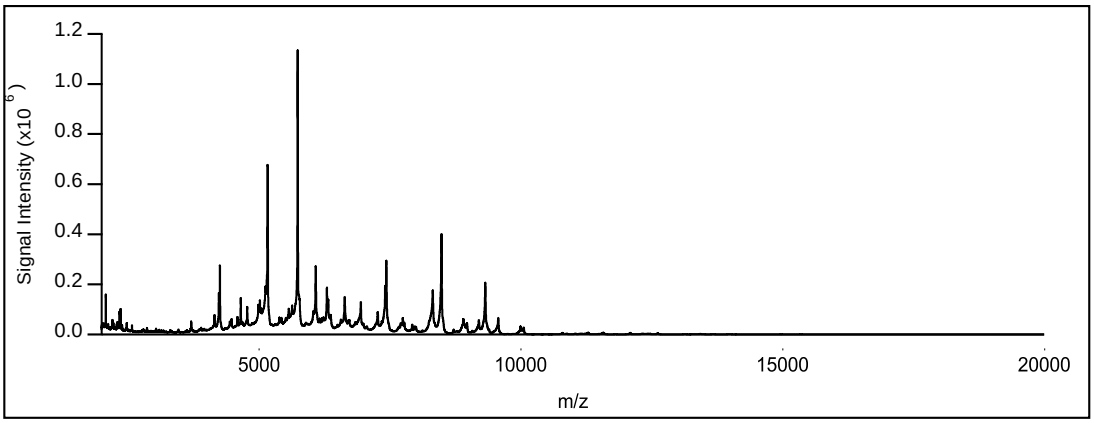


Figure 2. Representative MALDI-TOF MS Spectrum. Signal intensity plotted as a function of *m/z* for a sample of *Acinetobacter baumannii*. The spectrum was accurately matched with 90.80%

REFERENCES

Cherkaoui, A., Hibbs, J., Emonet, S., Manuela, T., Girard, M., Francois, P., Schrenzel, J. (2010). Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. *Journal of Clinical Microbiology*, 48(4), 1169-1175.

Counterman, A. E., Thompson, M. S., Clemmer, D. E. (2003). Identifying a protein by MALDI-TOF mass spectrometry. *Journal of Chemical Education*, 80(2), 177-180.

Croxatto, A., Prod'hom, G., Greub, G. (2012). Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiology Reviews*, 36, 380-407.

Shimadzu Corp. Axima-iD^{Plus} Sample Preparation. *Sample Preparation Advice, DOC 549*.

Shimadzu Corp. *E. coli* Calibration. *Training document.*