SOURCEBOOK OF LABORATORY ACTIVITIES IN PHYSIOLOGY

Transforming a cookbook undergraduate microbiology laboratory to inquiry based using a semester-long PBL case study

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Nicolaidou V, Nicolaou P, Nicolaou SA. Transforming a cookbook undergraduate microbiology laboratory to inquiry based using a semester-long PBL case study. Adv Physiol Educ 43: 82–92, 2019; doi:10.1152/advan.00167.2018.—Microbial physiology is a basic course taught throughout biomedical science disciplines. Students study the structure, growth, and metabolism of microorganisms and often find it difficult to learn the information, usually because they fail to see the wider applications. The current microbiology laboratory series describes how to transform a “cookbook” undergraduate laboratory to an inquiry-based one by incorporating problem-based learning. The students use a food poisoning case study that develops over a series of seven experiments and take on the role of the microbiology technician who is responsible for coming up with the answer and submitting a report to a clinician. The case provides coherence to the sessions, and the students are given the opportunity to learn about, and practice, common techniques they would encounter in a clinical microbiology laboratory. Those include the aseptic method, cultivation of bacteria, quantification of bacteria in culture, isolation of pure culture, morphological observation by light microscopy, Gram staining, the use of selective and differential media, and the effectiveness of a variety of antimicrobials and antibiotics. This laboratory series has been designed so that it can be implemented in any setting, using simple materials and inexpensive, nonspecialized equipment. The use of “real” biological samples is avoided for safety reasons. Alternatively, commercially available microbial strains are used, as these have been verified to be nonpathogenic.

Through the laboratory sessions, students are called upon to draw and report appropriate conclusions from the analysis of experimental data. Student assessment evaluates the extent to which intended learning outcomes are achieved by learners.

Objectives and Overview

The inherent challenge of any biomedical course is that students are expected to memorize a vast amount of information. This is usually translated into more lectures that promote passive learning, while, by general agreement, the learning of science should be an active process (1, 10, 13). This is where laboratory sessions have the potential to play a key role. Unfortunately, most laboratories are taught using cookbook methodology, and as such precious laboratory time is wasted. The series of activities presented in this paper aim to change the way microbiology laboratories are taught by incorporating a long case study that serves as the link for the entire course. This paper provides an alternative to the way microbiology laboratories are taught.

The objectives of the current laboratory series are to:
1. Transform a traditional cookbook-style microbiology laboratory course to an inquiry-based one using a long-case problem-based learning (PBL).
2. Encourage student learning both within and outside the laboratory.
3. Increase student transversal skills, which include the ability to think critically, take initiative, problem solve, and work collaboratively.

The current laboratory series is both generalizable and modifiable and may be applied to other basic physiology laboratories as well.
Background

Making the change from cookbook to inquiry. Recent educational reforms in the US (1) and Europe (13) have called for a science education that places emphasis on scientific literacy, by making connections between science and everyday life, with the aim of preparing young students to use science as part of their lives (11). Although great steps have been taken in that direction, students in undergraduate biomedical education are exposed to vast amounts of information, and the teaching of microbial physiology is no exception. The information is usually conveyed through lectures that, although necessary, do not encourage deep learning (3).

What supports the assimilation of information are the accompanying laboratory sessions. In an ideal setting, the objectives of the laboratory are to help students 1) apply theory and see it in practice, 2) increase critical thinking skills via application of the scientific process, and 3) heighten interest and motivation (16). The most popular and probably the most heavily criticized style of laboratory teaching is the expository or “cookbook,” where the student is provided with an opening lecture on the topic followed by step-by-step instructions on how to perform an experiment. This method may reduce cost by using less material and teaching time but at the expense of lower-level of information processing, critical thinking skills, troubleshooting, and interpretation of results (5). Furthermore, it was demonstrated to be less effective than inquiry-based approaches (6, 19, 30). Gradually, the disadvantages of the “cookbook” teaching are being acknowledged, and laboratory teaching is starting to shift toward methods that require the student to design an experiment with an unknown outcome, design an experiment with a predetermined outcome, or a carry out a hypothesis-driven laboratory (19, 29).

The teaching style that is proposed in the present paper is problem-based instruction or problem-based learning (PBL). PBL has three key characteristics: 1) it takes the form of small-group discussions (22); 2) it is under the guidance and coordination of a PBL tutor; and 3) the students work on real-life elaborated PBL cases, which they are required to understand and conclude based on scientific knowledge (2). The basic educational principle behind PBL is that it will help enhance critical thinking, self-directed learning (4), and skills involving collaborating with others, such as teamwork and group communication (15). PBL encourages the constructivist approach, where students build their knowledge based on their experiences (9). This makes PBL a student-centered learning approach, where the students take control of their own learning. Since science students are future scientists in the making and are the students who will work in clinical and research laboratories, it is imperative that these students engage their critical thinking skills while performing these activities. So, it appears that PBL may be ideal for preparing these students for their future roles.

An important thing to consider is where learning occurs while the student is performing an experiment. Interestingly, Domin (10), using a small number of students in a single university course, found that PBL-based laboratory learning occurs inside the laboratory during the practical, where students were more cognitively engaged, whereas, for the expository style practical, learning takes place outside of the laboratory after completion of the practical. This is quite interesting, as it appears that learning is not occurring during the laboratory in the traditional laboratory. Undeniably, reflection is an integral part of learning, and, whereas for expository style teaching it can occur after the laboratory, in PBL it can occur both during and after the session. Furthermore, in both a 4-yr and 10-yr long study by Luckie et al. (18, 19), where student-driven inquiry-based teaching was used (incorporates some of the principles of PBL: group work and inquiry), in the laboratory it was demonstrated that this mode of teaching led to deeper learning and better understanding of scientific principles. Furthermore, efforts have been made to make microbiology laboratories more inquiry-driven using cases, additional reading, and team-based techniques (21, 23, 29).

Microbial physiology. Microbial cells are the foundation of all life on earth and have a tremendous impact in nature and human life. The human body is inhabited by around 100 trillion individual organisms (~10× the number of human cells in the body) represented by almost a thousand different species (31). The structure, morphology, and metabolism of microbial cells and the dynamics and control of microbial growth are fundamental aspects of microbial physiology covered by any basic microbiology course. Good knowledge of these aspects as well as practical skills in the microbiology laboratory are essential for characterizing and identifying microorganisms (see Additional Resources below, no. 1, Ref. 26). It is this knowledge and skills learned in theory that students can bring together in practice for this real-life scenario to identify an “unknown” pathogen in a diagnostic sample.

Several features of bacteria are useful for their identification, including cell size and shape, wall structure, colony morphology, and metabolic and virulence traits. Macroscopically, microorganisms can be observed in cultures, and those that are human pathogens can usually be grown in normal conditions at 37°C, as this is the core temperature of the human body, using basic microbiology media, such as Lysogeny broth (LB) agar. The morphology of bacterial colonies can give some clues as to their identity, but further investigations require the use of microscopy and biochemical characterization. Bacterial cells are typically smaller than eukaryotic cells, ranging in size between 0.2 and 2 μm, and the majority can be observed using the light microscope. They are characterized by prokaryotic cell structure (lacking intracellular membrane system, including a nucleus and organelles) and the existence of cell walls made of peptidoglycan, which provides rigidity, preventing the cells from lysing. As an important step in characterizing and identifying bacteria, microscopic observations reveal a great variety of shapes, including the spherical cocci (singular coccus), elongated rods (bacilli singular bacillus), coccobacilli, vibrios, spirlicetes, and spirilla (singular spirillum). In addition, bacterial cells are usually observed in multicellular associations, such as diplococci, streptococci, staphylococci, tetrad, sarciniae, diplobacilli, and streptobacilli.

Differences in the composition of the peptidoglycan cell wall can be revealed by the Gram staining technique, a cornerstone of bacterial identification and taxonomic division (27). Two major groups of bacteria are distinguished: those classified as Gram-positive (stained purple due to the retention of the crystal violet dye) have cell walls made of a thick layer of peptidoglycan that contains teichoic and lipoteichoic acids, and Gram-negative bacteria (do not retain the crystal violet dye and are instead counterstained red/pink with safranin) that have
cell walls consisting of a thin layer of peptidoglycan surrounded by an outer membrane made of lipopolysaccharide (7). Gram classification is an essential step in the identification of an unknown microorganism and can provide other important clues, such as how susceptible a microorganism may be to certain chemicals/antibiotics. For example, Gram-negative bacteria are more resistant to certain antibiotics than Gram-positive bacteria due to their largely impermeable outer membrane (8).

Different metabolic attributes can also be exploited to identify an unknown microorganism, very commonly by the use of selective and/or differential culture media. A selective medium is a culture medium containing an ingredient that inhibits the growth of microbes other than the desired one. A differential culture medium is a culture medium containing an ingredient that certain microorganisms change in a recognizable way, often a sugar, such as lactose, which is metabolized to produce acids, leading to a color change of the media. Examples of such media are eosin methylene blue (EMB) agar, MacConkey (MAC) agar, and mannitol salt (MSA) agar, all being both selective and differential. EMB agar is selective for Gram-negative bacteria (inhibits the growth of Gram-positive bacteria) and is commonly used for the isolation and differentiation of coliforms and fecal coliforms. It contains the dyes EMB and the carbohydrate lactose, which also allows differentiation of Gram-negative bacteria based on their ability to ferment lactose. Lactose fermenting bacteria can be pathogenic. Inability to ferment lactose is a trait that distinguishes bacteria that are always pathogenic from those that are sometimes pathogenic. Visual distinction between Escherichia coli, other nonpathogenic lactose-fermenting enteric Gram-negative rods, and the Salmonella and Shigella genera is done based on colony color. In EMB agar, E. coli colonies have a characteristic metallic green sheen (17). Similar to EMB, MAC agar contains bile salts and the dye crystal violet, which inhibit the growth of Gram-positive bacteria and select for Gram-negative bacteria. It also contains lactose, which allows differentiation of Gram-negative lactose fermenters and nonfermenters. Those that ferment lactose produce acid end products, which react with the pH indicator neutral red and produce a pink color (see Additional Resources below, no. 2, Ref. 24). MSA agar contains a high concentration of NaCl (~7.5–10%), making it selective for Gram-positive staphylococci, as this level of NaCl is inhibitory to most other bacteria. It is also a differential medium for mannitol-fermenting staphylococci, since it contains the carbohydrate mannitol and the pH indicator phenol red for detecting acid produced by mannitol-fermenting staphylococci. If an organism can ferment mannitol, an acidic by-product is formed that will cause the phenol red in the agar to turn yellow. Staphylococcus aureus produce yellow colonies with yellow zones, whereas other staphylococci produce small pink or red colonies with no color change to the medium (see Additional Resources below, no. 3, Ref. 25).

**Learning Objectives**

After completing this series of activities, the student should be able to:

1. Design a scheme for investigation of unknown samples/suspected pathogens in the microbiology laboratory.
2. Explain the plate count method of counting bacteria and the use of dilutions.
3. Perform serial dilutions and use the plate count method to calculate the number of organisms that are present in the different samples.
4. Perform the aseptic technique.
5. Perform the streak plate method, generate isolated bacterial colonies, and prepare wet-mount and/or smear from a bacterial culture for microscopic observation.
6. Prepare and interpret a bacterial smear using the Gram staining protocol.
7. Interpret the growth of organisms from their samples on a chemically defined medium and complex medium.
8. Interpret the growth of organisms from their samples on a selective medium and a differential medium.
9. Describe the methodology used to determine whether an antibiotic is an effective agent for the patient and interpret the results.
10. Describe the methodology used to determine the sensitivity and/or resistance of the organisms found in the patient and restaurant samples to various antiseptics, disinfectants, and antibiotics and interpret the results.
11. Evaluate and explain which antibiotic is most suitable to be administered to the patient.
12. Evaluate and explain which chemical is most suitable to be used at the restaurant to decontaminate surfaces and avoid a recurrence of food contamination in the premises.

These are the main learning outcomes. For each session, additional learning outcomes have been formulated to further guide student learning. More information may be found in Supplemental Data S1a (available in the data supplement online at the Advances in Physiology website).

**Activity Level**

This activity is suitable for second-year undergraduate students in biomedical science fields who have a basic understanding of cell biology techniques and are enrolled in an introductory microbiology course. This case study could also serve as the basis for the development of shorter and more focused cases, thus extending its applicability to other health professional students, i.e., medical students.

**Prerequisite Student Knowledge or Skills**

Before doing this activity, students should have an understanding of the basic principles of cell and molecular biology. Students should know how to perform basic molecular biology techniques, such as pipetting, preparing slides, and using the light microscope. Importantly, before starting any practical work, students should also have received basic training on important safety issues, including the safe and responsible handling of microorganisms, clean up and disposal procedures, and reporting of accidental spills and exposures.

**Time Required**

This series of activities requires seven 2-h sessions. A detailed breakdown of the 2 h per session is indicated in Supplemental Data S1a. The format can be altered depending on scheduling needs.
METHODS

Equipment and Supplies

The majority of supplies required to establish this series of laboratory exercises are basic and easy to procure. These include plastic consumables (petri dishes, 50- and 15-ml conical plastic tubes, single-use plastic sterile pipettes, plastic single-use Pasteur pipettes, plastic sterile spreaders), sterile swabs, forceps, and a pipette gun. Inoculating loops can be plastic single-use or metal that can be sterilized using a Bunsen burner. Bacterial strains used in this laboratory series were Escherichia coli (155065A), Staphylococcus aureus (155554A), Bacillus cereus (154870A), and Pseudomonas aeruginosa (155250A) and can be purchased from Carolina (https://www.carolina.com). Basic microbiological culture media LB agar used throughout was purchased from Oxoid Microbiology Products, Thermo Scientific (https://www.thermofisher.com/us/en/home/industrial/microbiology.html). For the preparation of samples for microscopic observation, microscope slides and coverslips are required, as well as immersion oil, when using the ×100 objective lens of the compound microscope. Gram staining kits used in laboratory exercise 3 can be purchased from Sigma-Aldrich (https://www.sigmaaldrich.com), and used according to manufacturer’s instructions. For laboratory exercise 6, selective/differential microbiology culture media required are MAC agar, EMB agar, and MSA agar also from Oxoid Microbiology Products, Thermo Scientific (https://www.thermofisher.com/us/en/home/industrial/microbiology.html). Finally, for laboratory exercise 7, solutions required are 5% sodium hypochlorite/bleach, 70% ethanol, 3% hydrogen peroxide (http://www.emdmillipore.com/US/en), and Dettol. Filter paper cut in small circles and sterilized can be impregnated by submerging them in the various solutions, before applying them to cultures. Ready-made antibiotic disks impregnated with chloramphenicol, penicillin, tetracycline, kanamycin, and ampicillin can be purchased from Bio-Rad Laboratories (www.bio-rad.com). Equipment used throughout the laboratory exercise series are Bunsen burners, an incubator suitable for microbiology culture, such as the SIF 5000R Laboratory Companion incubator (www.stuart-equipment.com), and compound light microscopes, such as the OPTIKA B-150 (http://www.optikamicroscopes.com/optikamicroscopes/) with ×4, 10, 20, and 100 objective lenses.

To conform with biosafety level 2 guidelines, all laboratory areas should include nonporous floors, bench tops, chairs, and stools, sink for hand washing, eyewash station, and lockable door to the room. Biohazard signage should be posted where cultures are used or stored, on doors to room where microorganisms are handled, as well as on any container used to transport cultures (12). Disinfectants known to kill the organisms handled should be used according to manufacturer’s instructions to disinfect surfaces before and after laboratory sessions. Finally, discarded materials should be collected in suitable and clearly sign-posted waste containers, and proper decontamination and disposal should be arranged using either a properly maintained and validated autoclave or licensed waste removal.

Instructions

Laboratory restructure and student groups. The laboratory component of the microbiology course consists of seven 2 h/wk experimental exercises. Traditionally this laboratory was delivered using the cookbook methodology where the students were provided with all of the information before the experiment. Furthermore, before the delivery of each laboratory, they were given a short lecture and demonstration. This is now transformed by the incorporation of a long PBL case study, which is described in detail below. The number of students in the laboratory should not exceed 20. This is important as the students need to work in small groups. The students should be split into two groups of 10. For smaller laboratories, a single group may be created. The PBL scenario includes five samples, so the group of 10 will be further split to five pairs. Each pair takes ownership of one sample for the duration of the activities and shares the information with their group.

Long-case PBL laboratory design. The laboratory design follows the seven Maastricht steps of PBL listed below (2):
1. Clarifying unfamiliar terms presented in the problem
2. Defining the problem
3. Brainstorming any ideas
4. Generating hypothesis based on information given
5. Formulating learning objectives (LOBs)
6. Performing self-directed learning
7. Discussion of findings

Once the cycle is completed, the case continues the following week, building on information acquired in the previous experiment. The cycle continues until laboratory session 7, when the case finishes. Furthermore, this is adapted to incorporate required experiments, as indicated in Fig. 1.

The long PBL case study weekly format. The long case study spans all seven laboratory sessions that are designed to function as an ongoing investigation, with the students getting to the correct answer at the end, after having completed all of the exercises. For each session, the case is structured as follows:
1. Laboratory learning outcomes: these are provided in the beginning of the laboratory notes and are only available to the tutor (Supplemental Data S1a). The students should come up with these by the end of the session through discussion, brainstorming, and tutor guidance.
2. Tutor notes: for each piece of information provided to the student, additional information is available for the tutor to facilitate the flow. Tutor notes include prompting questions and answers so that anyone, even a nonexpert, could facilitate the group (Supplemental Data S1a).
3. Student information: information on the clinical case and process hints. This section is provided to the students (Supplemental Data S1b).

This is student led but at the same time guided as the tutor steers them in the right direction by asking the right questions. Figure 2 may be used as an overview guide, indicating expected results for each experiment.

The PBL case study and laboratory details. LABORATORY 1: INTRODUCTION TO THE MICROBIOLOGY LABORATORY AND THE CLINICAL CASE STUDY. PBL SESSION. In the first session, students are presented with a case study that gradually unfolds detailing clinical symptoms of a food-borne disease, history, list of foods consumed, and other information necessary to investigate the illness. In food-borne or other infectious disease outbreaks, an epidemiologist does

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Fig. 1. Format of problem-based learning (PBL) used in the microbiology laboratory.
the majority of the investigative work. The students take the role of the laboratory technicians who work closely with the epidemiologists and will conduct the laboratory investigations. The case is provided page by page and gradually unfolds.

1. The case begins with the clinical presentation of a patient visiting her physician with abdominal cramps and diarrhea.
2. The first task of the students is to list the key information. That is, the group identifies any pieces of information from the patient presentation that may be of potential relevance to the problem. One person acts as the scribe and lists the information on the whiteboard. In this first step, students are listing only, not speculating about causes.
3. Subsequently, a list of hypotheses to account for the patient’s problem, giving a possible explanation, is drawn. The group is asked to brainstorm about possible underlying problems and justify why they are considering each hypothesis. They are also asked to nominate the three most likely hypotheses.
4. In the next part, the group is asked what questions they would ask the patient to help choose between the three most likely hypotheses. Questions may initially be open questions to elicit further key information, but the group is asked to also formulate questions that will help distinguish between the hypotheses and consider how such questions will be useful, i.e., depending on the answer if they favor one or the other hypothesis. A variety of explanations could be given by the students, and at this point they are encouraged to brainstorm widely.
5. Once the process is completed, another piece of information is provided. Students find some answers to the questions they formulated as information about the patient’s history is provided, and they are asked to list this new information. This will support or discount hypotheses and may even lead to the formulation of new ones. Students find the definitive answers in the last piece of information regarding the preliminary diagnosis and treatment of the patient.
6. At this stage, principles of microbiology and working in a laboratory are highlighted, as well as the experimental process required to study an unknown microorganism. In attempting to elaborate on these topics, students will identify things that they know, as well as areas in which they feel they need to know more. Students are asked to record such gaps in knowledge as possible LOBs. A LOB is a concept, area, or item of knowledge that is not already known/understood sufficiently (or at all), and that is required to completely explain the hypothesis or some aspect of the case. The students are expected to formulate LOBs on basic principles of working in a microbiology laboratory, i.e., laboratory safety and rules, the importance of aseptic technique.

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**Fig. 2. Flow chart indicating expected results at each experiment.** Samples are as follows: A, patient sample E. coli Gram-negative rod; B, meat storage E. coli Gram-negative rod; C1, cutting board coccus S. aureus Gram-positive coccus; C2, cutting board rod P. aeruginosa Gram-negative rod; D, food preparation surface S. aureus Gram-positive coccus; E, sink contains no detectable levels of microorganisms. EMB, eosin methylene blue agar; lac, lactose fermenter; LB, Lysogeny broth; MAC, MacConkey agar; MSA, mannitol salt agar.
when sampling and growing microorganisms, the concept that organisms are everywhere, etc. Students will need to research the requirements for growing microorganisms in the laboratory, i.e., appropriate media, temperature, etc. Finally, they need to come up with the experiments required to identify an unknown microorganism in the laboratory and report back at the second laboratory exercise.

**PRACTICAL WORK AND LINK TO THE CASE.** Before starting the practical session, the instructor should reiterate important safety rules and best practices for the microbiology laboratory and inform students of the potential risks (see also Safety Considerations below). In the brief practical session that follows, students are asked to sample their environment and inoculate LB agar plates and liquid cultures, simulating sampling from the restaurant where the food poisoning took place. It should be noted that sometimes sanitarians and/or epidemiologists instead of laboratorians do restaurant/food-handling environmental sampling, and the samples are quickly sent to the laboratory for bacteriological testing. It is useful, however, to get the students to do this to serve as an introduction to culturing microorganisms and to allow them to appreciate the ubiquity, diversity, and abundance of environmental microbes. As students are not able to see the result of their sampling immediately, a short text giving the next information for the case is presented and discussed. Based on this, students should suggest that the aim is to determine the number of cells in liquid culture which is the topic of laboratory 2. To achieve that, students may be asked to consider that some samples have no growth, whereas others have a lot, i.e., samples that have no growth may point to the fact that the surface/location from which they were taken was cleaned well, a sample that has high growth could be the source of contamination, etc. They have to also consider whether all cells growing in cultures are pathogens and what levels of bacterial load are dangerous. Students have to research all of the LOBs that were documented and be ready to report back at the following laboratory session, as this information will form the basis for the experimental design of the exercise and the progression of the case. This is a process that has to be repeated at each subsequent laboratory exercise.

**STRUCTURE OF SUBSEQUENT LABORATORIES.** After the first laboratory exercise, which is dedicated almost entirely to introducing the clinical case scenario, all subsequent laboratory exercises are structured as follows:

1. Observation of results of the previous laboratory exercise and summary of the data up to that point
2. Report back on LOBs established during the previous laboratory exercise
3. Discussion and establishment of the experimental procedure by the students based on their LOBs; report back findings
4. Experimental procedure (practical)
5. Presentation of the next part of the case study and setting of new LOBs

**LABORATORY 2: QUANTIFICATION OF MICROORGANISMS.** In the second laboratory, the students are expected to learn how to quantify microorganisms, and the session was designed as follows:

1. PBL case report back: Students review the information provided so far regarding the case. Environmental sampling cultures prepared during the previous session are sealed and only observed, since the pathogenicity of microorganisms is unknown. They can, however, demonstrate the ubiquity, diversity, and abundance of environmental microbes.
2. Students report back on the LOBs, and through discussion they decide on the experimental design and proceed with the experiment. Every effort is made to link the case to their new learning.
3. Next, students perform serial dilutions of different samples labeled accordingly to fit the case scenario, i.e., “patient sample,” “food preparation area X,” “foodstuff X,” etc. There can be as many samples as there are groups of students. In this laboratory, the students are divided into five groups. Each group can perform the tests for a different sample, i.e., A, patient sample; B, meat storage; C, cutting board; D, food preparation surface; E, sink. Unknown samples to be used are provided to the students. For simplicity, all samples can be *E. coli* (students should not be aware of this) as macroscopically all samples look the same.

**Note:** Samples provided to students can have different turbidity to underline the importance of accurately determining the number of cells in the culture. In addition, this serves to reiterate that bacterial load cannot be evaluated by eye, as a solution appearing clear to the eye does not mean it is free of bacteria. Samples should be prepared in advance to have different turbidity. This may be done roughly. McFarland standards (barium sulfate or latex particles suspensions) are also useful for approximate bacterial density and estimating colony forming units (CFUs) (0.5 McFarland standard corresponds to $1.5 \times 10^5$ CFU/ml). Samples A, B, and C should be more turbid as a first clue for the potential source of contamination; however, students may not pick up on that at this point. One sample can be very dilute and not appear particularly cloudy, so it can be subsequently discounted as a source of contamination. In this case sample E, the sink contained almost no bacteria. Based on the turbidity of the sample, each team can be asked to consider what type of dilution is most appropriate, i.e., more turbid samples to be diluted more times. In the end, each team can choose what to do i.e., teams A, B, and C with more turbid samples could do $4 \times 100$ times dilution series, whereas teams D and E with less turbid samples could do $4 \times 10$ times dilution series. The volume of inoculum should be $100 \mu$l so that students are able to spread it well on the agar plates.

4. **PBL case:** Following the practical session, the next piece of information regarding the case is given to the students. At this point, the students will not have the values from the quantification of samples, so the scenario tells them that more than one sample contains high numbers of bacteria and could be the source of contamination. It is also important to keep reminding the students that quantification cannot give any information regarding the types of bacteria contained in the samples, and that any sample probably contains more than one type of microorganism. Students have to formulate the LOBs regarding the aseptic technique as well as the importance of obtaining a pure culture and how this is done.

5. The students are expected to perform independent study and prepare a report explaining their findings at the end of each laboratory exercise as part of their assessment.

**LABORATORY 3: PURE CULTURE AND ASEPTIC TECHNIQUES.** In the third laboratory, the students will be expected to develop their aseptic techniques, and the session was designed as follows:

1. **PBL case report back:** Students review the information provided so far regarding the case.

**Notes:** It is important to discuss what worked or not and why. Students should discuss whether the aseptic technique was successful in enabling them to get countable plates and accurately quantify their samples. If no plate was countable (between 30 and 300 clearly separated colonies that can be counted), discuss why and provide values for calculations (Supplemental Data S1a). These values should be relevant to how turbid each sample was. To calculate the concentration of bacteria expressed as CFU, students have to consider the number of colonies counted or provided, the dilution at which these colonies were observed, and the volume of inoculum. [CFU (number of bacteria/ml) = (number of colonies/volume of inoculum in ml) \times dilution factor]. Students should be able to comment on what they would have done differently and how the results affect what to do next, i.e., *sample E* gave no colonies so it can be disregarded as a source of contamination. This also provides the opportunity to question the students’ perception of the significance of the numbers of bacteria, i.e., whether high bacterial
load is significant or insignificant, or whether it is on its own an indication of risk.

2. Students report back on the LOBs, decide on the experimental design, and proceed with the experiment. Every effort is made to link the case to their new learning.

Notes: Students must be able to give the definition of the pure culture, the importance/purpose of achieving it, as well as be able to describe how to do it using aseptic technique. A common experimental design must be agreed on by all, based on what the students report back.

3. The practical of laboratory 3 focuses on aseptic technique and the streak plate method for obtaining pure cultures. Students should be able to explain aseptic technique manipulations and practice them by this stage. If resources allow, each student should have the opportunity to streak more than one plate to practice this important technique. They can also use an online simulation (see Additional Resources, below, no. 4, Ref. 28).

Streaked plates should be incubated inverted at 37°C overnight and subsequently stored also at 4°C until observation.

Notes: At least two samples have to be E. coli (the patient sample and the source of contamination). The rest can be S. aureus or a mixture of both organisms. Since bacterial colonies of the two bacteria growing on LB agar look different macroscopically, students should be able to comment on this during the next laboratory exercise. They will hence be able to report that two different types of bacteria are present in the same sample. Additionally, they can comment as to which sample resembles the patient sample. For this exercise, samples were as follows: A, patient sample E. coli; B, meat storage E. coli; C, cutting board E. coli and S. aureus; D, food preparation surface S. aureus; and E, sink not studied further.

4. PBL case: Following the practical session, the next piece of information regarding the case is provided. As students are not able to observe the results of the streaking immediately, pictures are provided to further the case. Students should be able to discuss that different morphology suggests different organisms, but the same morphology does not suggest the same organism. Additionally, they should be able to compare the organisms from the different areas of the restaurant and food samples to the patient sample, since observation of the colony morphology does not reveal any differences. Isolation of single colonies allows the study of pure cultures with smears and microscopy.

LABORATORY 4: LIGHT MICROSCOPE. At the start of laboratory 4, students observe the plates they had prepared in laboratory 3 and discuss what they found.

Notes: It is important to ask whether they consider that the streaking method worked, allowing the isolation of single colonies and hence pure cultures. If not, they should be able to discuss possible reasons and elaborate on what they could have done differently to improve their technique. In addition, they should be asked to make some conclusions as to the organisms growing in their plates and potentially the source of contamination. Students should be able to observe the difference in colony morphology between E. coli and S. aureus, if the streaking was done well, and the fact that sample C contains two different types of colonies, hence at least two different types of bacteria. This provides another opportunity to reiterate the importance of isolating and studying pure cultures. In case the students’ plates are not very good, the instructor can present some plates that were previously prepared.

1. PBL case report back: As always, students are asked to summarize the case in light of the new data, and a few minutes are dedicated to address some of the theory.

Notes: The practical of laboratory 4 focuses on the description of the parts of the microscope and its proper use, as well as preparation of smears and microscopic observation.

2. Students use the plates from the previous exercise to prepare smears. Each student can prepare as many samples as he/she wants. Students should be able to prepare at least one smear.

Notes: Since the students will use the plates from the previous session, samples will be either E. coli (the patient sample and the source of contamination) or S. aureus. The former is rod-shaped, whereas the latter is a coccus, so students should be able to observe the difference. In sample C, students should be able to see two different morphologies.

3. Each team should report on the morphology of its sample, i.e., bacillus or coccus, and document all results in a table.

4. PBL case: Following the practical session, the next piece of information from the case is given to the students. Students should have spent the previous hour trying to observe the samples under the microscope and should have complained at how difficult it is. They should be encouraged to ponder why that was the case. They will most probably say that this is because bacteria are very small, but it is important to mention that it is also because they are see-through, so staining them to increase contrast would make them easier to see.

LABORATORY 5: GRAM-STAINING OF BACTERIAL SAMPLES. In the fifth laboratory session, the students are expected to learn about and perform the Gram-staining, and the session was designed as follows:

1. PBL case report back: Based on their microscopy observations, students summarize the case, adding the new data to their cumulative table. Based on the LOBs report back, students discuss what they found, and a few minutes are dedicated to address some of the theory. A common methodology to be followed for the current exercise is agreed on. For this laboratory session, the Gram-staining protocol is performed. Students should be able to clearly describe all of the reagents and steps of the Gram-staining protocol, mentioning also the bibliographic sources from which they drew the information. They can also practice using an online simulation (see Additional Resources, below, no. 4, Ref. 28).

Notes: The practical of laboratory 4 focuses on the preparation of smears of the different samples, staining using the Gram-staining protocol, and microscopic observation. Students will be told that liquid pure cultures were prepared from their samples, and they will use these for Gram staining.

2. Students will already know how to prepare smears and fix them with the Bunsen burner from the previous exercise. Students will do this in groups as done previously: A, patient sample E. coli Gram-negative rod; B, meat storage E. coli Gram-negative rod; C1, cutting board coccus S. aureus Gram-positive coccus; C2, cutting board rod P. aeruginosa Gram-negative rod; D, food preparation surface S. aureus Gram-positive coccus. Students are not told which organism is contained in each culture. E. coli is a Gram-negative rod, S. aureus is a Gram-positive coccus, and P. aeruginosa is a Gram-negative rod. Bacillus cereus can be used as an example of a Gram-positive rod to compare with E. coli.

3. Students should be able to observe, take pictures, and report on their assigned samples. All results should be reported in a table. They should be able to comment on which sample has the same characteristics as the patient sample and hence comment on which samples may and may not be the source of contamination.

4. PBL case: Following the practical session, the students will have concluded that more than one sample (B and C2), is Gram-negative bacilli, similar to the patient sample A. LOBs will focus on other tests that can be useful to further compare the characteristics of the microorganisms in the patient and restaurant samples, i.e., the use of various media to study metabolic characteristics. Students may also be asked to refer back to the original list of methods they had proposed in the first laboratory report back. What other tests can tell bacteria apart?
LABORATORY 6: CHEMICALLY DEFINED, COMPLEX, SELECTIVE AND DIFFERENTIAL MEDIA. In this session, the students are expected to differentiate microorganisms using selective and differential media. The session was designed as follows:

1. PBL case report back: Based on the Gram classification of samples and morphology, students will now be able to draw more conclusions as to the potential source of contamination responsible for the case of food poisoning.

2. A cumulative table containing all information can be drawn on the board, and a few minutes can be dedicated to discussing what students know so far, which sample is which, etc. For example, they may point out that samples C1 and D cannot be the source of contamination as they do not share Gram-staining characteristics and morphology with sample A, the patient sample (Gram-positive cocci vs. Gram-negative rods). In turn, samples B and C2 are both Gram-negative rods, the same as the patient sample A. Based on report back, students will propose how to further proceed, i.e., the use of other tests to differentiate between the samples in this case, using different types of media. Students should be able to report back on the different types of selective/differential media, what they contain, and how organisms grow on them. A common laboratory method to follow for the current exercise is agreed on by the students.

Notes: To actually confirm the identity of the microorganisms, biochemical or genetic tests would have to be performed, and students may suggest such tests. However, the focus of this case study is to compare the samples between them and identify the one that is the likely source of contamination.

3. For the practical of laboratory 6, students are provided with the samples as follows: A, patient sample E. coli Gram-negative rod; B, meat storage E. coli Gram-negative rod; C1, cutting board coccus S. aureus Gram-positive coccus; C2, cutting board rod P. aeruginosa Gram-negative rod; D, food preparation surface S. aureus Gram-positive coccus. Students are not told which organism is contained in each culture. They are also provided with petri dishes with different types of media: LB agar, MAC agar, EMB agar, and MSA agar. Students do this in groups as before: they divide their plates into two halves and plate their sample with the patient sample in each half so that they can compare all samples against the patient’s side by side. It needs to be emphasized to students to be careful when plating, so that one culture does not drip into the other, so only little inoculum should be taken.

4. PBL case. Following the practical session, some more information is given to the students; however, this does not reveal the identity of the source of contamination. Students should be able to conclude that once they can observe the results of the selective/differential media cultures. Instead, they are told that the physician responsible for the patient’s treatment has asked the microbiology laboratory to run a test using the patient’s sample and to suggest the most appropriate therapeutic agent. At the same time, they are asked to recommend to the restaurant appropriate chemicals to be used to clean all areas to contain the spread of the pathogenic bacteria and to prevent future contamination.

Notes: At this point, students can refer to knowledge from their lectures, i.e., definitions of levels of control, physical and chemical methods of control, etc., so it is important to link back to acquired knowledge. It is also important to clearly differentiate between agents that can be used to clean a surface and those that can be administered to a patient i.e., disinfectant/antiseptic vs. antibiotic.

LABORATORY 7: ANTISEPTICS, DISINFECTANTS, AND ANTIBIOTICS. In the seventh and final laboratory, the students put the final pieces of the puzzle together, and the session was designed as follows:

1. PBL case report back: At the start of laboratory 7, students observe the plates they had prepared in laboratory 6 and discuss what they found. They can comment on how successfully they plated their samples and how the different media worked or not. Most importantly, based on the appearance of the colonies on the different media, they should be able to identify the sample that represents the source of contamination.

Notes: More specifically, even though both are Gram-negative rods, the E. coli in sample B can be differentiated from the P. aeruginosa in sample C2 based on the ability of the former to ferment lactose and produce strong acid end-products, giving rise to dark, blue-black colonies with a metallic green sheen on EMB agar. On the other hand, P. aeruginosa gives light gray colonies; the absence of color is indicative of its inability to ferment lactose. Comparing also with the appearance of the colonies in sample A (patient sample), students should be able to conclude that the source of contamination is hence sample B, meat storage. Growth on other media can also be discussed, i.e., on MSA agar samples C1 and D, but not samples A, B, or C2 grow, as this media allows only growth of Gram-positive staphylococci. In case the students’ plates are not very good, the instructor can present some plates that were prepared by them. Students have to be able to discuss possible reasons and elaborate on what they could do differently to improve their technique.

2. Having recognized the source of contamination, laboratory 7 concludes with testing the sensitivity of the bacteria to various disinfectants and antibiotics. Students should be able to report back on the methodology for testing disinfectants and antibiotics, i.e., how to perform and evaluate the result of an antibiogram to report on the susceptibility of bacteria to certain disinfectants/antibiotics. A common laboratory method to follow for the current exercise is agreed on by the students.

3. For the practical of laboratory 7, students are provided with a collection of antiseptics and disinfectants, including 5% sodium hypochlorite/bleach, 70% ethanol, 3% hydrogen peroxide, Dettol, as well as antibiotics disks (chloramphenicol, penicillin, tetracycline, kanamycin, and ampicillin), which will be used to set up antibiograms on LB agar petri dishes inoculated with the samples. Each plate can be divided into four parts so that four chemicals can be tested per plate. The focus is on the “patient sample” (sample A) and “source of contamination” sample (which students will have now concluded to be sample B), both cultures of E. coli. Figure 3 shows the expected zones of inhibition in bacterial cultures of samples A, B, and C1.

Notes: Samples can be divided among the students, and more than one person can test the same sample so that replicate values can be obtained and calculation of mean values and standard deviation can be possible. However, all samples can be available for students to use if they desire. The other organisms can be used to observe, for example, the difference in susceptibility to penicillin between Gram-negative (i.e., A, patient sample E. coli) and Gram-positive bacteria (i.e., C1, cutting board coccus S. aureus). Students should return after 24 h to measure the zones of inhibition (the width in millimeters of the clear zones around filter disks where no growth of bacteria is found). The formation of a clear zone is not enough to confirm susceptibility of the organisms to the antibiotic, as the size of the zone can be the function of many variables, including the density of the inoculum, depth of the medium, and diffusibility of the antibiotic. Students need to consult the Inhibition Zone Diameters Interpretative chart to determine the size of the inhibition zone at which each microorganism is considered resistant, intermediate, or sensitive (available in Supplemental Data S1a).

4. Having completed all laboratory sessions, students have to prepare a final report describing their investigations and conclusions with regards to the identification of the source of contam-
When using microorganisms, best practices must be followed to minimize the risks to educators, students, and the community. Students should be made aware of the relevant risks and safety precautions. Furthermore, students are required to demonstrate competency in handling microorganisms safely and responsibly. Students should also be required to sign safety agreements stating that they have been informed about safety precautions.

Because of the microorganisms used in this case study, biosafety level 2 guidelines must be followed. Personal protection, including safety goggles, closed-toe shoes, gloves, and laboratory coats should be used. Only cultures from authorized, reputable sources should be used, and ideally fresh stock obtained annually. All laboratory areas should include nonporous floors, bench tops, chairs, and stools, sink for hand washing, eye-wash station, and lockable door to the room. Personal belongings should be stored separate from the work areas. Hands should be washed after entering and before exiting the laboratory, long hair should be tied back, and touching the face or handling personal items should be avoided. Food or drinks should not be brought into the microbiology laboratory. Biohazard signage should be posted where cultures as used or stored, on doors to room where microorganisms are handled, as well as on any containers used to transport cultures. Disinfectants known to kill the organisms handled should be used according to manufacturer’s instructions to disinfect surfaces before and after laboratory sessions. Disposable loops are preferable than Bunsen burners; however, if the latter are used, this should always be supervised. Finally, discarded materials should be collected in suitable and clearly sign-posted waste containers, and proper decontamination and disposal should be arranged using either a properly maintained and validated autoclave or licensed waste removal. For more information please see Emmert et al. (12).

RESULTS

Expected Results

This series of experiments enables the student to go through the entire process from environmental sampling to identifying the bacteria responsible for an infection. The process takes seven laboratories, and the students are given five samples to identify the culprit, i.e., the infected sample and the microorganism that caused the infection. The mock samples are as follows: A, patient sample (E. coli Gram-negative rod); B, meat storage (E. coli Gram-negative rod); C1, cutting board coccus (S. aureus Gram-positive coccus); C2, cutting board (P. aeruginosa Gram-negative rod); D, food preparation surface (S. aureus Gram-positive coccus); E, sink (contains no detectable levels of microorganisms). Figure 2 is a flowchart indicating the expected results for each experiment.

The data set expected from this set of experiments is quite large, so they are included as Supplemental Data S2. Typical results obtained from these experiments are shown there. Also, the case study (Supplemental Data S1a) includes details as to the results expected for each experiment.

Misconceptions. MISCONCEPTION 1. Before taking the Microbiology course, students may believe that microbes are dangerous, and contact with them should be avoided at all costs. Over the course of these laboratory exercises, they learn that microbes can be found everywhere, on our body, on everyday items, in a bottle of water that appears clear to the eye, and in the food we eat, and that actually only a small fraction is pathogenic. Some, of course, may be responsible for serious disease. In addition, students learn that, when trying to culture and study specific microorganisms, it is very easy to contaminate them with other microorganisms that exist in the environment; hence, the aseptic technique is very important.

MISCONCEPTION 2. Students may have used the microscope to observe different samples before, but it may be the first time they get to observe bacteria. Some may believe that bacteria

Fig. 3. Zones of inhibition in bacterial cultures of samples A, B, and C1. Columns from left to right are as follows. Sample A (patient sample) was tested with antibiotics penicillin, chloramphenicol, tetracycline, kanamycin, and ampicillin by three different students on different Lysogeny broth (LB) agar plates. Antibiotics chloramphenicol, kanamycin, and tetracycline were the most effective. Sample B (meat storage), identified as the source of contamination, was tested with antiseptics/disinfectants 70% ethanol, 5% sodium hypochlorite/bleach, 3% hydrogen peroxide, and Dettol, also repeated by three different students. Dettol and 3% hydrogen peroxide were effective in inhibiting bacterial growth. Sample C1 (Gram-positive cocci from the cutting board sample) was also tested with all antibiotics and antiseptics/disinfectants so that students could compare the susceptibility of Gram-positive and Gram-negative bacteria. Bacteria from sample C1 were more susceptible to the antibiotics tested compared with sample A, as demonstrated by the larger zones of inhibition. Susceptibility to the antiseptics/disinfectants was comparable to sample B. In addition, they should include their suggestions for the most effective antibiotics to be administered to the patient and chemicals to be used at the restaurant to prevent future outbreaks.

A summary and proposed breakdown of activities and time dedicated is shown in Supplemental Data S1a.

Troubleshooting

Although students “come up” with their own experiments, in reality, this is guided learning, and as such it occurs in a controlled and predictable environment. With regards to expected results, it is often likely that these may not be optimal due to students’ lack of experience. Common problems that may arise are mistakes in serial dilutions and spreading of bacteria, which may not allow for the counting of individual colonies in laboratory 2. In this case, colony count values can be provided to the students so that they are able to do the calculations of bacterial load in cultures (Supplemental Data S1a). In laboratory 3, streaking and obtaining a pure culture can also be difficult for students to achieve. While it is a good idea for the facilitator to prepare cultures that can be used for demonstration, students will need to continue to practice this most important technique for success. In laboratory 5, fixed microscopy samples of Gram-stained bacteria can be used in case students fail to achieve good staining. However, it is recommended that the staining be repeated, as proper Gram staining is a critical technique to master in bacteriology. All issues may be ameliorated with planning, good guidance, and repetition, if necessary.

Safety Considerations

When using microorganisms, best practices must be followed to minimize the risks to educators, students, and the community. Students should be made aware of the relevant risks and safety precautions.
are too small to be observed using the light microscope. Even though bacteria are generally smaller than eukaryotic cells, the majority can be observed in the light microscope. Students may also be able to observe bacteria move in wet mounts prepared from fresh cultures.

**Misconception 3.** A common misconception among students is that a single antibiotic may kill all bacteria. This is clearly not the case, and, through this laboratory series, the students will be able to reject this misconception through practical experience.

**Evaluation of Student Work**

The assessment strategy used in this series of experiments is based on the use of a number of assessment tools aimed at evaluating student knowledge, application of knowledge, and practical skills. Specifically, student achievement was assessed via three means, namely 1) end-of-term written examination, 2) longitudinal assessment of practical work, and 3) laboratory reports. The students’ final grade was determined based on their score in the end-of-term written examination (40%), longitudinal skills assessment (20%), and laboratory reports (40%). Each type of assessment is described briefly below.

1. **End-of-term written exam:** The written exam consisted of a combination of true or false (T/F) items and short-answer questions. Short-answer questions are free-text questions, where students need to produce a short answer. Indicative questions are shown in Supplemental Data S3.

2. **Laboratory reports:** Scholars have raised concerns that conventional written tests may not be able to fully assess the learning outcomes expected in the laboratory learning environment (16). Laboratory reports may represent a more valid form of assessment because it assesses an authentic skill, i.e., the writing of scientific reports, which is a skill that future scientists will need to develop and use later on. A common pitfall of laboratory reports is that they may not assess a student’s thinking and understanding, but rather they may be a regurgitation of the experimental procedures followed (20). As such, the sections required for the laboratory report were carefully constructed to assess student understanding of the study design, specific techniques utilized, results, explanation of findings, and limitations. To ensure that all students are assessed consistently, a detailed marking scheme and relative weight of each section was developed and applied to the marking of each laboratory report (Supplemental Data S3).

3. **Longitudinal assessment of practical work:** Assessment of practical work is often a neglected part of assessment in the laboratory setting, while it forms an integral part of the learning process. Continuous assessment by observation, using prescribed assessment criteria, may provide a dynamic form of assessment longitudinally (14, 16). Specifically, each student was assessed at the end of each laboratory session using a marking scheme that we developed to assess the students’ preparation for the session, understanding and critical evaluation of the experimental design and results, laboratory skills, record-keeping, and communication skills (Supplemental Data S3).

**Inquiry applications.** The current laboratory series presents with a number of inquiry applications, as listed below:

1. This is inquiry-based learning as it incorporates key characteristics, such as active learning, use of a case study, critical thinking, and facilitation by a tutor.

2. Although, the questions to be explored are predetermined by the tutor, they are not provided to the students. As such, the students “come up” with their own questions to address. In the process, the tutor guides them to the desired LOBs, as well as allows them to investigate additional information they decide to look up as a group.

3. For each session, the students generate a laboratory design LOB. That is, they are encouraged to come up with their own experimental design. Before the laboratory commences, the students discuss and decide as a group the best experimental design. Different techniques may be allowed, if feasible, but students usually agree on one technique.

4. The experimental design and data analysis rely heavily on the student group. However, because this is an introductory course, and a lot of the techniques are unfamiliar to the students, the tutor acts as a “demonstrator,” working in the clinical laboratory with them, and demonstrates new techniques.

**Wider educational applications.** This type of laboratory may be applied to any type of laboratory that has a series of experiments that build on each other. As such, they can be used as follows:

1. In advanced microbiology laboratories, where students are required to investigate microorganisms using DNA analysis. So once again they can use the preliminary identification techniques such as Gram staining and selective/differential media and then proceed to DNA extraction and isolation and PCR.

2. Common biochemistry laboratories where the students work with a single protein for an entire semester in a similar fashion to this laboratory and use biochemical techniques to make and identify the protein.

3. As a component of pharmacology practicals, to gain better understanding of drug mechanisms and selectivity.

**Additional Resources**

The following resources may be useful for the academic delivering the laboratory series, as well as the students:

1. Basic Practical Microbiology, A Manual (26).

2. MacConkey Agar—Composition, Principle, Uses, Preparation, and Colony Morphology (24).

3. Mannitol Salt Agar for the Isolation of Staphylococcus Aureus (25).

4. Plate Steaking for Isolation and Gram Staining Identification (28).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

V.N., P.N., and S.A.N. performed experiments; V.N., P.N., and S.A.N. analyzed data; V.N., P.N., and S.A.N. interpreted results of experiments; V.N. and S.A.N. prepared figures; V.N., P.N., and S.A.N. drafted manuscript; V.N., P.N., and S.A.N. edited and revised manuscript; V.N., P.N., and S.A.N. approved final version of manuscript; S.A.N. conception and design of research.
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