JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION
DOI: https://doi.org/10.1128/jmbe.v19i1.1351

Tips & Tools

Reproducing Clinically Significant Multi-Organism Cultures to Improve Clinical Microbiology Education and Practice †

Rebecca Barr and Mary Feller Davis*
Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT 84602

INTRODUCTION

The purpose of the Clinical Microbiology course for undergraduate Medical Laboratory Science students is to gain familiarity with medically important bacteria and fungi by performing the full range of clinical laboratory tests in microbiology. Many clinical specimen samples, such as stool, contain numerous organisms, but Clinical Microbiology professors may be limited to single-organism specimens in teaching due to the complexity of creating reproducible mixed cultures. To assist professors in training students in the crucial skills of isolating pathogenic bacteria from normal flora to be properly prepared for work in the hospital laboratory, we present protocols to develop reproducible, mixed cultures for educational training.

This article includes detailed protocols for creating mock stool, urine, wound, and throat cultures for academic laboratory use. These cultures are useful as advanced laboratory activities, after students are confident in skills of processing, plating, and identifying organisms in isolation (Box 1) (1, 2). Ideally, students would have multiple laboratory sessions in a single week for this exercise, to independently follow cultures from sample processing to clinical report; however, instructions are included for preparing the samples and having students evaluate grown cultures for the presence of normal flora and pathogens in a single laboratory session. The protocols specify concentrations and amounts of organisms to combine, as well as how to present samples as though students were receiving unknown patient samples in a clinical setting. Protocols are provided for creating clinical samples with normal flora, contamination (indicating poor sample collection), and pathogens in the presence of normal flora and contamination. The cultures contain two to four organisms and take approximately three days to prepare for student use.

*Corresponding author. Mailing address: 4007 LSB, 701 E University Parkway, Provo, UT 84602. Phone: 801-422-6207.
E-mail: mary_davis@byu.edu.
Received: 3 May 2017, Accepted: 27 November 2017, Published: 16 February 2018
†Supplemental materials available at http://asmscience.org/jmbe

PROCEDURE

Required materials

The general protocol is described here, and specific protocols for wound, stool, urine, and throat samples are provided in the Supplementary Materials. In addition to the relevant organisms, required materials include sterile plastic tubes with screwcap lids, sterile 5-mL pipettes, micropipettes and tips, a spectrophotometer, sterile cuvettes, a CO_2 incubator or appropriate container, sheep blood agar (SBA) plates, additional agar plates appropriate to the site of interest (see Supplementary Materials for suggestions), sterile inoculation loops, and prepared tryptic soy broth (TSB). For mixed cultures in a non-clinical laboratory, other agars, such as trypticase soy agar, may be used, but the colony morphologies may not be distinct between organisms.

Topics to discuss in class prior to the laboratory experience

Patient samples submitted to the clinical laboratory must be evaluated for quality of the specimen as well as the presence of pathogens. Poorly collected specimens, especially skin wounds and urine samples, will show "contamination" by normal skin flora (3–5), and a new sample submission to the laboratory may be required. Other patient specimens, such as stool samples, contain an abundance of normal flora, and the challenge is to identify the presence of

BOX 1.
Topics and skills to be covered prior to using these activities

Proper safety equipment and techniques to work in a BSL2 laboratory
Goals of a clinical microbiology laboratory and the workflow in such laboratories, from collection of patient specimens to reports returned to clinicians after workup is complete
Use of solid agars in the clinical laboratory, including those used for general, selective, and differential growth
Likely pathogens and normal flora at various human body sites
Streaking an agar plate for isolated colonies

a pathogen amongst the normal flora. Only the pathogen(s) should be isolated and identified to report to the clinician. Finally, it is possible to have more than one pathogen in a single patient specimen, with or without normal flora. It is important in these circumstances to isolate and identify all pathogens for the clinician.

While the multi-organism cultures reported here are not as complex as some clinical specimens, they provide realistic, introductory examples to aid students in understanding the difficulties encountered with patient specimens in the clinical laboratory.

PROTOCOLS

Instructor and student protocols specific to specimen sites can be found in the Supplementary Materials; a general workflow is shown in Figure 1. Once the instructor has determined the specimen type (wound, stool, urine, or throat) and the mixed culture type (contamination only, pathogen and contamination, mixed pathogens), the Supplementary Materials can be used to identify organisms for the culture. Each organism is grown separately in TSB and diluted to the specified spectrophotometer readings to estimate the amount of organism present. The organisms can then be combined in appropriate ratios to create mock clinical samples. Two options for presenting the samples to students are included, based on whether the students will interact with the samples over one or multiple laboratory sessions. If one session is used, instructors should culture the organisms using suggested media (see Supplementary Materials) and incubate prior to class. Agar plates should be streaked for isolation to enhance the ability to discern colony types; examples are provided in Figures 2 and 3 (6). During class, students will view the plates and evaluate colonies to determine specimen quality and pathogen presence. General colony characteristics (see Supplementary Materials) and the use of selective and differential media will greatly enhance the ability to discern multiple organisms. If multiple laboratory sessions are used, the instructor will inoculate mock specimens (e.g., swab for a throat sample) with mixed culture broths to provide to students. Students will experience processing specimens, selecting appropriate media, and using appropriate streaking techniques. Students incubate their media, evaluate colony types for specimen quality and pathogen presence, and perform tests to identify the genus and species of any pathogens. It is recommended that a total of four laboratory sessions (1 to 2 hours each) be used for this experience.

We have found that it is easiest for students to learn the principle of "isolating only the pathogen" when given many examples. It is important to stress the importance of understanding whether a clinical sample contains only normal flora (which should not be worked up or reported to the clinician) or gross contamination from poor sample collection (which may require a new sample submission), as well as how to identify a pathogen among normal flora and how to identify two distinct pathogens in the same specimen.

SAFETY ISSUES

Organisms used in these mixed cultures include BSL1 and 2 organisms. All work should be performed in BSL2 laboratories, with appropriate personal protective equipment (https://www.asm.org/index.php/guidelines/safety-guidelines). Prior to working in a BSL2 environment, students should also be trained in BSL1 procedures. All ASM biosafety guidelines were followed during the creation and use of these protocols.

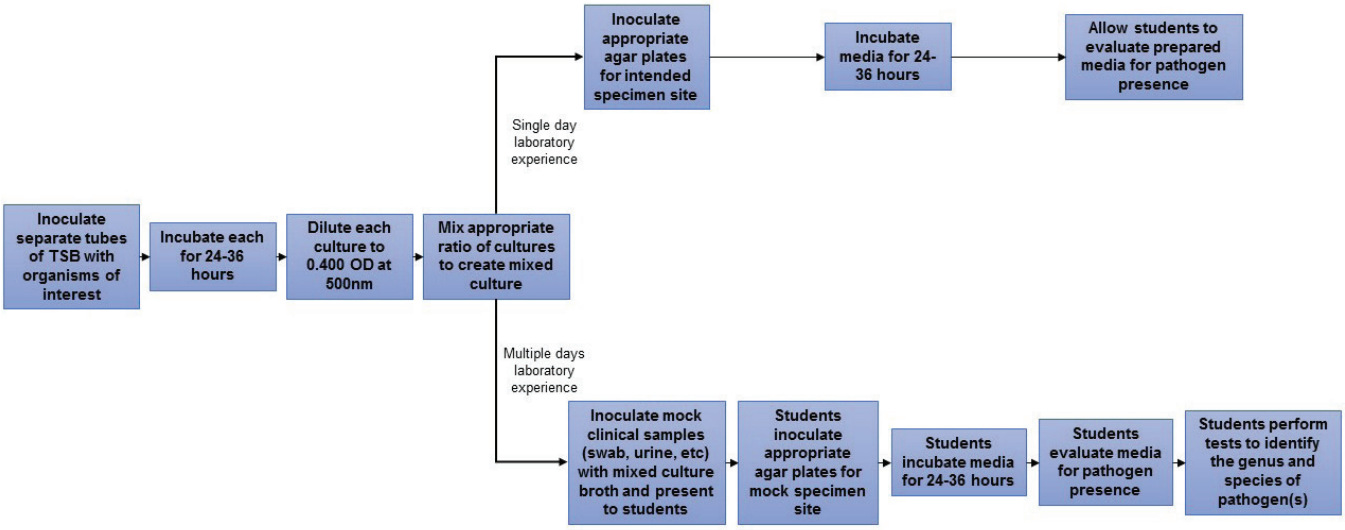

FIGURE 1. An overview of the general protocol.

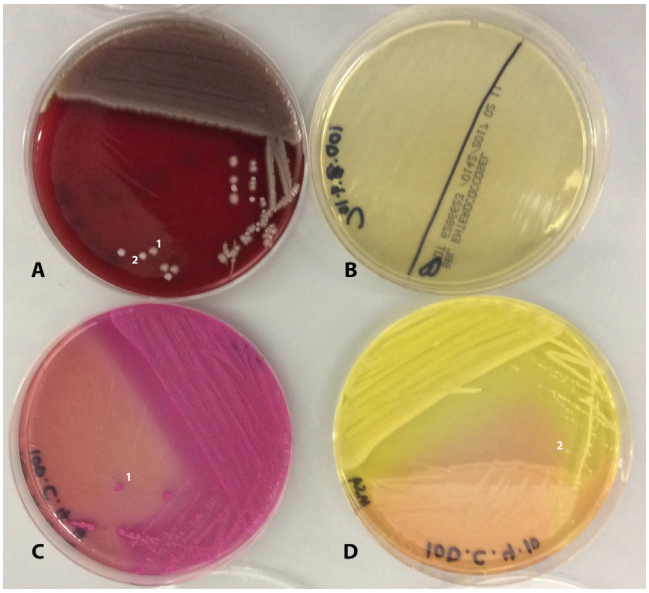

FIGURE 2. Mixed pathogen wound culture with *Escherichia coli* (1) and *Staphylococcus aureus* (2). A) Sheep blood agar (SBA): both organisms grow on this enriched media. *E. coli* colonies tend to be slightly larger and grayer, and *S. aureus* colonies tend to be smaller and whiter. B) Bile esculin agar (BEA): neither organism grows on this selective agar. C) MacConkey agar (MAC): only *E. coli* grows on this Gram-negative selective media. Due to lactose fermentation by *E. coli*, pink colonies are observed. D) Mannitol salt agar (MSA): only *S. aureus* grows on this selective agar. Due to mannitol fermentation by *S. aureus*, yellow colonies are observed.

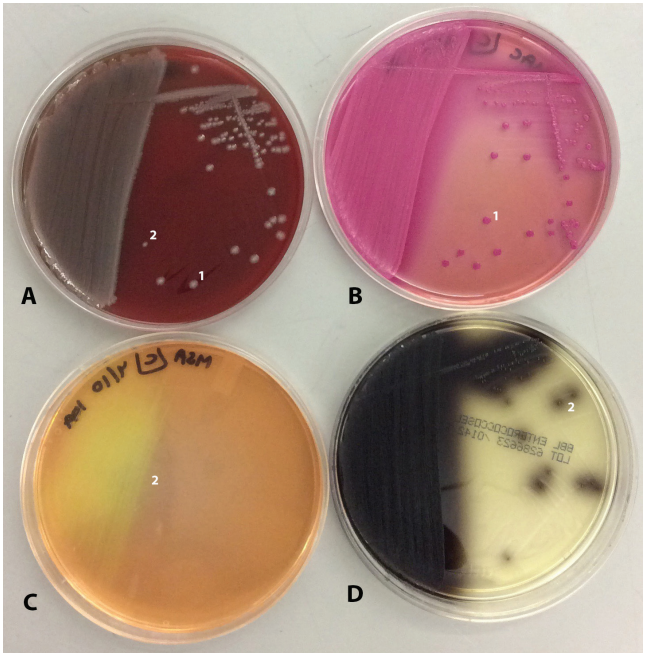

FIGURE 3. Mixed pathogen wound culture with *Citrobacter freundii* (1) and *Enterococcus faecalis* (2). A) Sheep blood agar (SBA): both organisms grow on this enriched media, with *C. freundii* as a larger colony and *E. faecalis* as a small colony. B) MacConkey agar (MAC): only *C. freundii* grows on this Gram-negative selective media. Due to lactose fermentation by *C. freundii*, pink colonies are observed. C) Mannitol salt agar (MSA): inhibited growth of *E. faecalis* can be seen in the first quadrant as small, yellow growth. D) Bile esculin agar: only *E. faecalis* grows on this selective agar. Due to hydrolysis of esculin by *E. faecalis*, black colonies are observed.

CONCLUSION

The addition of mock clinical specimens into the clinical microbiology laboratory adds another dimension to students' learning. For students who will work in a hospital laboratory, it is crucial to be able to distinguish pathogens from normal flora. Ideally, students would be exposed to multi-organism cultures over multiple weeks of laboratory instruction, but the addition of a single laboratory experience can greatly enhance learning. We have used these protocols over two semesters in our Clinical Microbiology course with positive feedback from the students and greater understanding and discussion during lectures about mixed clinical cultures.

SUPPLEMENTAL MATERIALS

Appendix 1: Wound culture protocol
Appendix 2: Stool culture protocol
Appendix 3: Urine culture protocol
Appendix 4: Throat culture protocol
Appendix 5: Organism ATCC numbers

ACKNOWLEDGMENTS

Development of the mixed culture protocols was supported by a Teaching Enhancement Grant from the BYU College of Life Sciences awarded to MFD. The authors would like to thank the BYU Department of Microbiology and Molecular Biology stockroom for their assistance. The authors declare that there are no conflicts of interest.

REFERENCES

1. Kiser K, Payne W, Taff T. 2011. Clinical laboratory microbiology: a practical approach. Pearson, London.
2. Tille P. 2013. Bailey & Scott's diagnostic microbiology. 13th edition. Mosby, St. Louis, MO.
3. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Bourbeau P, Carroll KC, Kehl SC, Dunne WM, Robinson-Dunn B, Schwartzman JD, Chapin KC, Snyder JW, Forbes BA, Patel R, Rosenblatt JE, Pritt BS. 2013. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 57(4):e22–e121.
4. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, NISC Comparative Sequencing Program, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA. 2009. Topographical and temporal diversity of the human skin microbiome. Science 324(5931):1190–1192.

5. Leal SM, Jones M, Gilligan PH. 2016. Clinical significance of commensal gram-positive rods routinely isolated from patient samples. J Clin Microbiol 54(12):2928–2936.

6. Sanders ER. 11 May 2012. Aseptic laboratory techniques: plating methods. J Vis Exp JoVE http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4846335/