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Tips & Tools

Simulation of MICROBACT Strip Assay Using Colored Liquids to Demonstrate Identification of Unknown Gram-Negative Organisms in Undergraduate Laboratory †

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INTRODUCTION

Microorganisms are a major concern in both clinical infections and food contamination and are a threat to public health, necessitating accurate and rapid identification of isolates to the species level for quick and successful treatment to improve patient care (1–3).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing are the most commonly used methods for identifying unknown microorganisms in clinical, food, and environmental samples (4–10), a crucial prerequisite for quick and accurate intervention. These modern technologies are quicker and more accurate than conventional methods for microbial identification, such as plating on differential agar or other phenotypic-based assays including the API-20E and MB-12E systems (7, 11, 12). A major obstacle to bringing these modern technologies into the undergraduate teaching laboratory is the up-front cost of purchasing the specialized equipment and software, as well as the need for access to computer and database facilities and skilled personnel to operate the equipment and software. These challenges can prevent undergraduate educators from exposing those modern technologies to a large number of inexperienced students in teaching laboratories. Furthermore, in developing countries, costs may limit access to this equipment, which may not be available in all areas (13). Where modern technologies are unavailable, microbiologists in clinical and veterinary diagnostic laboratories and in food industries have to rely on conventional methods and commercial biochemical identification kits to identify bacteria from clinical and environmental sources for disease control and treatment (3, 11, 14).

To overcome the challenges of using modern technologies, we used the Oxoid MICROBACT GNB 12A biochemical identification kit with colored liquids instead of live organisms in our undergraduate teaching laboratory to identify four unknown oxidase-negative and gram-negative rods isolated from four different hypothetical clinical cases. The commercially available MICROBACT GNB 12A, 12E, 12B and 24E kits are used in clinical and routine diagnostic laboratories for the identification of Enterobacteriaceae and other gram-negative bacteria from clinical and other sources (14–17). In recent years, a combination of gene sequencing and biochemical test results precisely identified organisms used in the construction of databases for MALDI-TOF MS (18). Thus, teaching these phenotypic-based assays to undergraduate students, so that they may identify unknown organisms to the species level using biochemical tests, comprises an important intellectual pillar of the microbiology course.

The MICROBACT kit employs a simple procedure with visible color reactions and provides reliable results (12, 19–21); however, when this assay is performed by inexperienced undergraduates, there is an unacceptable rate of misleading biochemical reactions due to bubble creation, cross-contamination from adjacent wells during inoculation, and improper sealing of the strips leading to evaporation of the inoculum. Additionally, the light weight and slim design of the strip makes it easy to mishandle and knock over, leading to contamination of the bench and incubation baskets with live organisms. Finally, two consecutive days are required to complete each of the tests and to obtain accurate results, which can be problematic for scheduling of undergraduate teaching laboratories within a large teaching university delivering a number of science courses. These factors in combination can lead to difficulty in accurate identification of the microorganism, which can ultimately lead to discouragement and disengagement of the students with this exercise.

We have designed a simulated MICROBACT strip assay in a single lab that overcomes the problems associated with using live microorganisms and provides second-year

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biomedical science students with an accurate, visual learning experience that conveys the basic concept of the MICROBACT assay and the use of phenotypic assays to correctly identify unknown bacterial isolates. We used non-toxic and affordable artificial colors (Table 1, Appendix 1) to simulate the effects of actual microorganisms on a MICROBACT 12A identification strip. Use of artificially colored liquids removed the potential hazard of using real organisms, allowing effective facilitation of student's learning in a very safe and economical way, with minimal laboratory skills and reduced instructor intervention. Students record the results from the simulated MICROBACT strip of unknown bacterial isolates (Fig. 1) and discuss the results within groups, which creates a collaborative learning environment in the classroom to reinforce students' learning.

This simulated MICROBACT strip assay can easily be incorporated in other laboratory exercises that involve the use of phenotypic assays for bacterial identification without any exposure to the actual live microorganisms.

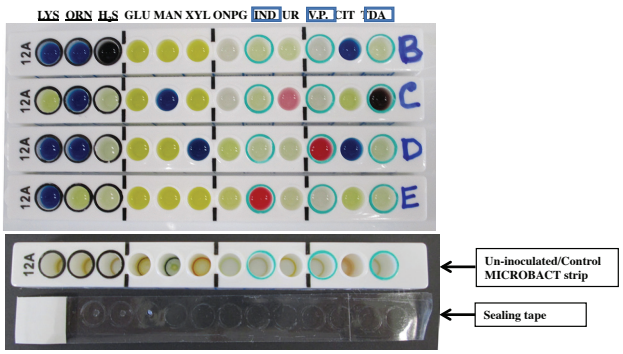

FIGURE 1. Results of simulated MICROBACT strip assay for four sample unknowns: gram-negative bacteria (B, C, D, and E) and an un-inoculated control strip.

PROCEDURE

Practical exercise and learning objectives

The demonstration of simulated MICROBACT strip assay was scheduled to be completed within 15 minutes, as part of a two-hour microbiology laboratory (with a class size of 120 students). This exercise was part of a larger experiment involving the identification of several unknown clinical isolates from relevant case scenarios using different types of biochemical assays and differential media (see Fig. 1, Appendix 3). The learning outcomes of this short exercise are as follows:

1. Understand the concept of Oxoid MICROBACT strip assay, become familiar with the procedure, and learn to interpret the results of MICROBACT 12A kit assay
2. Learn to identify a range of unknown gram-negative organisms using biochemical properties/phenotypic assays
3. Learn about safe handling of BSL2 (biosafety level 2) cultures through recording the results of a simulated MICROBACT strip assay

Materials and methods

Groups of four students were provided with four MICROBACT 12A strips labeled B, C, D, and E showing simulations of different biochemical reactions (corresponding to four unknown isolates), along with a control (uninoculated) strip (Fig. 1). A biohazard bin, paper towels, and disinfectant were also available to clean up accidental spillage. Full instructions were provided in each student's laboratory manual (Appendix 2). Demonstrators and lecturers were available to answer questions. The methodology to simulate MICROBACT 12A strips without using bacteria is summarized in Figure 2, and detailed instructor notes are available in Appendix 1.

Determining student learning

Students were instructed to observe and interpret the results of the MICROBACT strip assay for each unknown isolate (Fig. 1) and to identify and record the isolate using the MICROBACT identification package as recommended by the manufacturer (Thermo Fisher Scientific; see Table 2, Appendix 1) and outlined in Appendix 3. Students were instructed to correlate results from this element of the laboratory with other tests performed (Fig. 1, Appendix 3) in a previous lab and to revisit for any unexpectedly contradictory tests.

Safety issues

In accordance with The University of Auckland's health and safety regulations, all students were instructed to wear a closed lab coat, closed shoes with covered toes, gloves, and safety glasses before commencing any work within the teaching laboratory and to follow laboratory safety guidelines. Students had already received training about laboratory safety, microbiology techniques, and laboratory techniques during their previous microbiology laboratory sessions and lectures (please see Prerequisite student knowledge, Appendix 1). Students are not informed that MICROBACT strips are simulations with non-hazardous artificial colors; students handled all MICROBACT strips according to the ASM biosafety guidelines for BSL2 organisms (https://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf).

DISCUSSION

The use of artificially colored liquids (instead of live organisms) to simulate MICROBACT strip assay does not produce biological wastes requiring specific disposal protocols and allows the MICROBACT strips to be reused for many years, which is cost-effective for large classrooms. The simulation also requires minimal skills for preparation

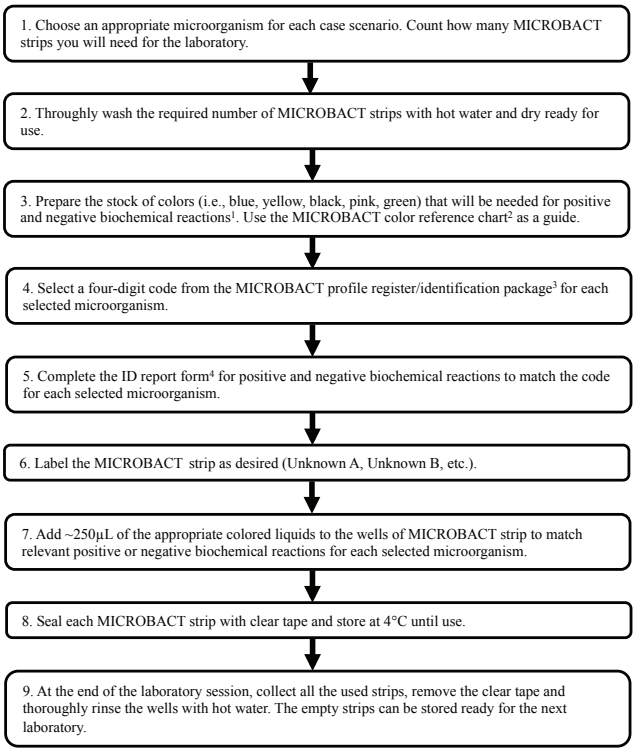

FIGURE 2. Schematic illustration of simulating MICROBACT strip assay for demonstration to identify unknown gram-negative isolates. Notes: [1]See Table 1, Appendix 1. [2]See Table 1, Appendix 2 (along with MICROBACT color reference chart that comes with the kit). [3]See Appendix 1. [4]See Table 2, Appendix 2, and Table 1, Appendix 3.

and removes the time-intensive incubation steps (Appendix 1). The simulated MICROBACT strip assay allows the biochemical identification tests to be taught without any risk to students from handling hazardous microorganisms, which would be suitable for an introductory microbiology course taken by less-experienced undergraduate students, while still allowing students to practice identifying numerous gram-negative bacteria, thus aiding the acquisition of new skills and enhancing critical-thinking skills.

SUPPLEMENTAL MATERIALS

Appendix 1: Teacher's instructions
Appendix 2: Student's instructions—student laboratory protocol
Appendix 3: Instructor's model answers

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The authors declare that there are no conflicts of interest.

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