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Modeling and Visualizing Bacterial Colony Purification Without the Use of Bacteria or Laboratory Equipment †

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INTRODUCTION

Microbial communities are complex, consisting of numerous species of bacteria, fungi, viruses, and protitsts. To conduct laboratory investigations, it is important to isolate individual members of these communities. Purification is often the first step in any microbial laboratory study and is a component of most undergraduate microbiology curricula (1). Typically, a quadrant streak is presented to students by demonstration of the procedure, followed immediately by a student attempt using live bacteria. Common errors, such as crossing back onto a previously streaked area, or forgetting to sterilize the loop in between streaks, can result in poor dilution and separation of individual bacterial colonies that is not evident until the next laboratory session. Furthermore, learning to isolate purified colonies requires using expensive and biohazardous material, physical laboratory space, and specialized equipment. This can create safety concerns, especially when many novice students are attempting the technique for the first time.

Difficulty in mastering bacterial purification may result from a lack of real-time feedback on the success of the ability to dilute and isolate individual bacteria. Feedback is a valuable tool in aiding the acquisition of new skills (reviewed in 2), and the quadrant streak simulation tool described in this work provides the student with immediate feedback on their ability to isolate a single species of bacteria so they may make adjustments and practice prior to working with live cultures. Practicing skills in a simulated, hands-on environment can lead to greater performance and improved retention of laboratory skills (3–6). Given the natural hazards and constraints of working with living organisms, simulation can be an effective strategy to help students gain confidence and proficiency ahead of time in a safe and less expensive setting (6).

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This paper describes an activity that simulates bacterial purification without the use of biohazardous materials and provides immediate feedback to students regarding the efficacy of their technique.

PROCEDURE

Preparation of simulated bacterial culture

Prior to the laboratory session, the simulated culture is prepared by mixing watercolor paint from tubes with two easily distinguishable colors (red and blue) of extra-fine glitter until the paint is saturated and takes on a "gritty" appearance. Each color of glitter represents a different bacterial species. This activity can be expanded to additional complexity by using three or four distinct colors of glitter. When choosing glitter, finer particles are preferable to larger ones, as larger particles tend to clump, and do not spread well during subsequent steps. In addition, finer particles more accurately reflect the relative size of bacterial cells, and can saturate the paint with more particles. The paint must not dry to a permanent finish and must be water-soluble for this technique to be effective; student-artist quality watercolor or gouache paint is ideal, while acrylics and oil paints will not work. Dark or intense shades of paint work better than lighter shades, as lighter colors make it difficult to distinguish the dilution of each streak. Darker shades retain some color with each streak and are more easily visible, simulating the dilution that occurs in a live culture as the loop is sterilized at each step. The paint-glitter mixture is then spread thickly in a single line on a piece of white semi-gloss cardstock (Fig. 1A) and allowed to dry. This represents the initial streak of the bacterial culture. Students will first moisten this line to begin the simulation. Thin or rough paper should be avoided, as it will fall apart or absorb too much water as the students complete their streaks. A listing of materials and their cost is provided in Appendix 1.

In-class activity

To begin, the instructor describes the quadrant streak, with emphasis on dilution and separation to isolate individual colonies. Then, the technique is demonstrated using a mixed bacterial culture and a nutrient agar plate. During this

demonstration, emphasis is placed on the streaking pattern and sterilization of the inoculating loop between quadrants. The technique is then demonstrated again using the simulation materials: paint-glitter mixture (the "culture"), cardstock ("culture medium"), and a paintbrush or cotton swab (the "loop"). The cotton swab and soft, synthetic paintbrush are equally effective. The line of dried "culture" is first moistened by using the "loop" to simulate the initial streak. The "loop" is passed over the top streak several times with moderate pressure, which wets the paint and loosens the glitter. The "loop", is then "sterilized" by running it under tap water to rinse any glitter and paint from it, and is then used to perform the second streak (Fig. 1B). The brush is rinsed again and the glitter spread for a third streak (Fig. 1C). Students should see the dilution of the paint color, as it becomes lighter and more translucent with each streak, and individual particles or "colonies" of glitter may be visible, both red and blue. Each color of glitter represents a different species of bacteria from the original mixed "culture." A fourth streak may be done following an additional rinsing of the brush, to mimic a true quadrant streaking technique (Fig. 1D). After the instructor demonstrates the simulated quadrant streak, students conduct the simulation before using living bacterial cultures.

Self-assessment occurs when students immediately see their results and can determine whether they are achieving single particle separation of the glitter, analogous to colony purification. They should be able to distinguish the red "species" of glitter from the blue in a background of very pale paint. The paint should reflect the correct streaking pattern, and should appear more dilute with each streak. Students may choose to repeat this activity if their first attempt does not achieve these goals, making adjustments to their technique. Furthermore, by viewing the students' results, the instructor can provide feedback and advice to improve their technique before culturing live bacteria. Examples of improper isolations are provided in Appendix 2.

SAFETY ISSUES

None.

CONCLUSION

The quadrant streak simulation described in this paper offers an immediate visualization of a common microbiological technique. It allows students to assess their dilution and separation technique and instructors to immediately provide specific recommendations for improvement, which could lead to earlier mastery and understanding of the quadrant streak.

SUPPLEMENTAL MATERIALS

Appendix 1: Materials used
Appendix 2: Examples of improperly conducted isolations

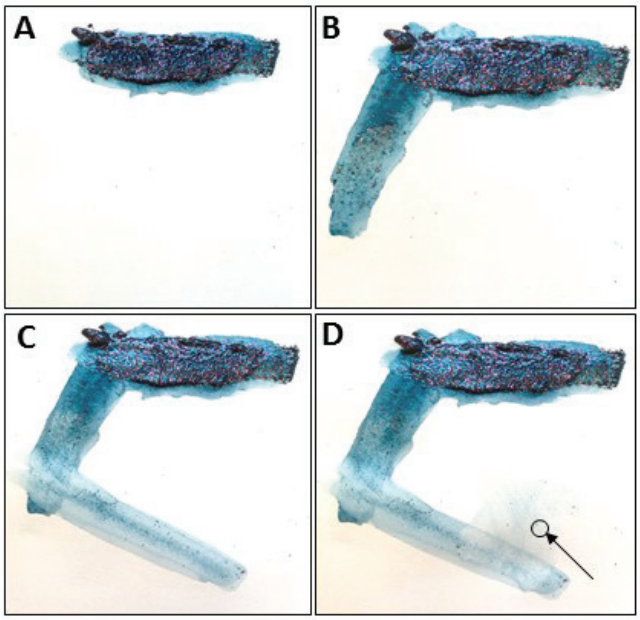

FIGURE 1. The visual simulation tool for the quadrant streak. (A) The initial material the student receives with paint and glitter is "reactivated" by running a wet paintbrush over it several times. (B–D) Appearance of the tool following correct second, third, and fourth streaks. The arrow and circle indicate a single "colony" isolated.

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REFERENCES

1. Merkel S, Reynolds J, Siegesmund A, Smith A, Chang A, Hung K, Smith H. 2014. ASM recommended curriculum guidelines for undergraduate microbiology education. American Society for Microbiology.
2. Shute VJ. 2008. Focus on formative feedback. Source Rev Educ Res 78228173:153–189.
3. Kolozsvari NO, Feldman LS, Vassiliou MC, Demyttenaere S, Hoover ML. 2011. Sim one, do one, teach one: considerations in designing training curricula for surgical simulation. J Surg Educ 68:421–427.
4. Svoboda J, Passmore C. 2013. The strategies of modeling in biology education. Sci Educ 22:119–142.
5. Makransky G, Thisgaard MW, Gadegaard H. 2016. Virtual simulations as preparation for lab exercises: assessing learning of key laboratory skills in microbiology and improvement of essential non-cognitive skills. PLOS One 11:e0155895.
6. Herrmann-Werner A, Nikendei C, Keifenheim K, Bosse HM, Lund F, Wagner R, Celebi N, Zipfel S, Weyrich P. 2013. "Best practice" skills lab training vs. a "see one, do one" approach in undergraduate medical education: an RCT on students' long-term ability to perform procedural clinical skills. PLOS One 8:1–13.