Perfecting Your Spread Plate Technique

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INTRODUCTION

The Spread Plate Technique, in conjunction with serial dilutions, is a valuable research tool. The transition from “knowing” to successfully “doing” has a steep learning curve. In order to provide meaningful results it is vital that students learn the “art” of making uniform spread plates. The technique has to be repeatable and the results reliable.

There are three areas that merit attention. First, a student must make accurate dilutions using pipettes. Second, one must respect the necessary “short” time interval between agar inoculation and spreading. Third, one must apply a balanced spread technique using a glass hockey stick (plate spreader) to spread the inoculum evenly on the agar surface.

PROCEDURE

It is recommended that all students follow basic lab safety protocols: long hair tied back away from the face, use of basic PPE (personal protective equipment), wearing of closed toe shoes, long pants, nitrile gloves, safety goggles, and a lab coat. Long sleeves and loose clothing are inappropriate for working in the microbiology lab.

The materials needed for a group of four students include: red food coloring, green food coloring, pipetters set to dispense 10 μl, 100 μl, and 300 μl, six agar plates per pair, Saran Wrap, one glass hockey stick per pair, lazy Susan/turntable, one 250 ml beaker containing 50 ml 95% ethanol, one 1L Pyrex beaker, one 500 ml polypropylene beaker containing 150 ml of 10% bleach solution for discarded pipette tips, six agar plates per set to dispense 10 μl, 100 μl, and 300 μl, six agar plates per pair, Saran Wrap, one glass hockey stick per pair, lazy Susan/turntable, one 250 ml beaker containing 50 ml 95% ethanol, an empty one-liter Pyrex beaker, one 500 ml polypropylene beaker containing 150 ml of 10% bleach solution for discarded pipette tips, and one Bunsen burner. Agar type is not critical. The simplest form would be 15 g of agar granules/L of water, autoclaved and poured into plates. Tryptic soy agar, nutrient agar, and Mueller-Hinton agar plates have all been used with comparable results. I use whatever I have available that is “past its expiration date” or “leftover” from a previous lab.

Students start by pipetting five sets of 10 μl, 100 μl, and 300 μl aliquots of red food coloring on Saran Wrap. Students are cautioned to avoid aspirating air/bubbles in the pipette. Aspiration must be done slowly so there is no splatter on the pipette. The release and “blow out” need to be controlled so that the dye stays in one droplet.

Next, students progress to making the spread plates. One student will make the aliquots on each plate and another student will spread the dye on the plate. Two hockey sticks are kept in a 250 ml beaker of ethanol. The hockey stick “blades” should be covered with approximately 50 ml of 95% ethanol. Student A places the first plate on the turntable and removes the lid from the plate. Ten μl of red dye are placed in the center of the agar. Student B removes one hockey stick from the beaker and allows excess ethanol to drain into the beaker. Then, the hockey stick should briefly be placed in a Bunsen burner flame and removed so as to ignite the alcohol but not heat the glass. The alcohol will burn off to sterilize the hockey stick surface. Student B’s hand should always be higher than the hockey stick. The hockey stick is “air cooled.” The sterilized hockey stick is used to spread the dye across the agar surface. It is important that the hockey stick be held level with the agar surface. The bend in the glass should be close to the outer edge of the plate. The open end of the hockey stick should be toward the center of the plate. Very slight pressure should be exerted toward the center. Student A should gently turn the turntable to spread the dye evenly over the agar surface. The lid should be replaced over the agar. After spreading, the hockey stick should be flamed, cooled, and replaced in the beaker of ethanol. If the hockey stick is not sufficiently cooled, the alcohol in the beaker may “burn.” Should this happen, the 1L beaker should be inverted over the flaming beaker to shut out oxygen and quench the fire. It is important that no one panic at this time.

Students should evaluate the dispersion of dye. Time delays result in an obviously darker area in the agar where dye was first applied. Too much pressure on the bend of the hockey stick will distribute dye around the periphery of the agar plate. Too much pressure on the open end toward the center of the plate will keep the dye in the middle of the plate. Students learn the art of balancing the hockey stick so that the dye spreads evenly across the plate without “puddling” in the middle or being spun to the periphery.
Students exchange roles and repeat the process using the green dye on the same plate. The process is repeated on the remaining plates: pipet the 200 μl aliquot onto the second agar plate; spread and evaluate. Next, pipet the 300 μl aliquot on the third plate; spread and evaluate. This conserves agar plates and still allows students to evaluate the dye dispersion pattern. The entire process can be repeated so that each student can complete 2 sets of spread plates.

CONCLUSION

Taking time to learn and understand the fundamental principles that define great spread plate technique is invaluable. In this example, dye is used to accentuate the visible volume of each aliquot and the dispersion pattern on the agar. With the utilization of a second dye, each agar plate can be used twice. The immediate feedback and personal evaluation of these techniques provide an active learning experience that students remember. Students can better understand and apply what they have learned. Students read the procedure, do the procedure, see immediate results, make appropriate changes in technique, and evaluate their results. Students learn from each other, develop confidence, and grow as a team.

Subsequently, serial dilutions of bacterial cultures and food samples are completed in lab. Results are demonstrably improved. Student confidence in their technique is higher and the plate counts are repeatable.

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