Scientific Method: Is This Broth Culture Pure?

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Abstract

This simple exercise is designed to allow students to explore the fundamental steps of the scientific method and at the same time reinforce basic microbiological laboratory techniques (wet mount, Gram staining, streak plating) and concepts (cellular versus colonial morphology). Each student is supplied with a nutrient broth culture containing at least two morphologically similar types of bacteria (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*). After preliminary microscopic examinations of the organisms' cellular morphology (wet mount, Gram stain), the student is asked to hypothesize whether the "culture is pure" and subsequently perform an experiment to confirm this hypothesis (streak plate—colonial morphology).

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Most textbook descriptions of the scientific method involve a flow diagram indicating the various stages scientists should follow in the pursuit of successful experimentation. Any specific example that is presented is usually highly complex and convoluted, requiring months of experimentation or expensive equipment. The experiment presented here, on the other hand, is very straightforward and relatively easy as it employs techniques any introductory microbiology student will usually be exposed to early in the semester. It also allows the students to creatively combine their newly acquired skills with an important theoretical concept and thus stress a more holistic approach to the study of microbiology.

PROCEDURE

Materials.
First week (per student or per group)
Standard microbiology laboratory equipment (Bunsen burner, loop, etc.)
Nutrient broth culture containing a mixture of bacteria (24 h, 37ºC)
Microscope
Microscope slides, coverslips, etc.
Stains for simple and Gram staining
Nutrient agar or other nonselective agar plates (for streak plating, 2 per student for replica plating)
Incubator

Second week (per student or per group)
Incubated plates with colonies
Standard microbiology laboratory equipment (Bunsen burner, loop, etc.)
Magnifying glasses or stereo microscopes (to examine features of colonial morphology)
Microscope
Microscope slides, coverslips, etc.
Gram stain (Students should examine cellular morphology of each colonial type.)

Instructor Version.
Week one. During the first week, each student (or student group) is supplied with a nutrient broth culture and asked to hypothesize whether the culture is "pure" or "mixed". (No additional instructions are given at this point; however, it is expected that students have had a lecture about the scientific method.) Surprising as it may sound to the instructor, this relatively simple request usually generates a fair bit of discussion and consultation between the lab partners and other groups in the class. However, the final consensus reached by all students is that preliminary microscopic examinations (i.e., a wet mount and a Gram stain) of the culture are required before they are able to formulate a hypothesis. If a student opts to perform a simple stain, she/he soon realizes that it is of limited value, as it does not add significantly to information obtained by the wet mount.

Note: In order to make this exercise more challenging, I purposely select a mixed culture of two organisms with a similar cellular morphology (e.g., a slow and a rapid motile rod or a motile and a nonmotile rod), but with a distinctly different colonial morphology on a nonselective culture medium (nutrient agar or Trypticase soy agar). The difference in motility forces the student to differentiate between "true motility" and "Brownian movement". If the instructor wishes to use three bacteria, I suggest that one of them be of distinctly different morphology, otherwise the exercise becomes pretty difficult.

Based on his/her interpretation of the microscopic observations, the student formulates a hypothesis, then decides on the necessary experiment(s) he/she can use to test this hypothesis. Although there are several procedures available, most students opt to prepare a streak plate in order to obtain isolated colonies and thus the best results to either support or disprove their hypothesis. Students decide how the plates should be incubated (e.g., aerobically or anaerobically, 20ºC or 37ºC). This last step, however, depends on how much basic technique they have been exposed to prior to the experiment.

Week two. During the second week, students examine their plates to determine whether the results support the original hypothesis or a new hypothesis is needed. They then write a short report summarizing the exercise.

Although this exercise may appear rather simplistic to the experienced instructor, it is an excellent way to:

- illustrate basic concepts of the scientific method,
- reinforce basic microbiological laboratory techniques and concepts,
- stress a holistic approach to microbiology (e.g., relationship between cellular and colonial morphology),
- promote critical thinking, and
- encourage collaboration and discussion.

Safety Issues. None

SUPPLEMENTARY MATERIALS

Additional Hints and Tricks
1. This activity is designed to be taught at the early stages of an introductory microbiology course after students have learned:

- aseptic technique,
- streak plating and colonial morphology,
- parts and functions of a microscope,
- preparation of wet mounts and cellular morphology, and
- preparation of heat-fixed smears and Gram staining.

The student should thus be able to identify morphological differences between individual cells and discrete colonies.

2. Several combinations of bacteria work well at this stage. Use a 24- to 48-hour nutrient broth culture (37ºC) containing any of the following combinations as the "unknown mix" and nutrient agar or Tryticase soy agar for streak plating.

Ratio of organisms in unknown mixture	Incubation temp (ºC)	Streak plate results
Serratia marcescens—1 part *Escherichia coli*—1 part	25	*S. marcescens*—pink colonies *E. coli*—creamy-colored colonies
Serratia marcescens—1 part *Micrococcus luteus*—3 parts	37	*S. marcescens*—cream-colored colonies *M. luteus*—bright yellow colonies
Escherichia coli—1 part *Micrococcus luteus*—10 parts	37	*E. coli*—creamy-colored colonies *M. luteus*—bright yellow colonies

3. The degree of difficulty of this exercise depends upon the specific organisms and the combination of organisms used. For example, a mixture of *E. coli* and *S. marcescens* is more challenging than a mixture of *E. coli* and *P. aeruginosa*, not only because of their similar cellular morphology but also because of their similar colonial morphology, especially if the student selects 37ºC as the incubation temperature. Note: if the incubation temperature is lowered to 25ºC, colonies of *S. marcescens* will demonstrate a distinctive pink coloration whereas *E. coli* does not, so the two colonial types can be clearly differentiated.

4. I have occasionally provided students with a pure culture of a motile rod (e.g., *Pseudomonas aeruginosa*); since not all of the cells are always in motion, motility becomes only one criterion the student can use to base her/his decision on. Also, when Gram stained, *P. aeruginosa* has a tendency to have a "beaded" appearance, which many students will identify as "chains of cocci".

To assay motility, inoculate motility agar (semisolid 0.4% nutrient agar in tubes) by stabbing the inoculating needle approximately three-quarters of its depth. Incubate for 48 hours and look for growth. Motile organism growth will be a turbid region extending from the stab while nonmotile bacteria growth will only be along the stab line. Positive control: *Proteus vulgaris*. Negative control: *Staph. epidermidis.*

5. Although I have used gram-negative rods to illustrate the procedures of this exercise, there is no reason why gram-positive rods or cocci could not be used. For example, a mixture of *Staphylococcus aureus* and *S. epidermidis* would be equally challenging. The beauty of this experiment is that it allows the instructor's imagination to run wild.

6. For the more advanced student who has knowledge of selective and/or differential culture media, the instructor might want to try the following combinations of organisms or media.

Organisms	Culture medium	Ratio	Colonial morphology
S. aureus *S. epidermidis*	manitol salt agar	1:1	Yellow colonies, medium changes to yellow Whitish colonies, no change to medium
E. coli *E. aerogenes*	MacConkey agar	1:1	Deep red or fuchsia colonies, white halo around colony Deep red or fuchsia colonies, no halo
E. coli *P. aeruginosa*	MacConkey agar	1:1	Deep red or fuchsia colonies, white halo around colony Greyish or white colonies
E. coli *E. aerogenes*	Endo agar or eosin-methylene blue (EMB) agar	1:1	Deep red or fuchsia colonies with a metallic sheen Deep red or fuchsia colonies, no sheen

7. No matter what organisms are used, the exercise results in a lot of constructive discussion and interactions between student partners or groups and the instructor.

Sample Application

Most of the instructions for this exercise have been described in the procedure. In order to proceed with this exercise, the students should be familiar with the various laboratory techniques and with the concepts and steps of the scientific method.

The instructions students receive are as follows:
"Using the steps as defined by the scientific method, determine if the supplied nutrient broth culture is pure or mixed."

Below is a brief (unamended) report as submitted by one of my former microbiology students which clearly illustrates that this simple exercise has helped to put the various steps of the scientific method into perspective.
Student Report

User Feedback
"I used the 'Is this broth pure?' exercise in lab earlier this semester. It went very well. The students like the fact that they were using the skills they had aquired to answer a question. I liked the fact that they were involved in using the scientific method with a microbiological question. It also saved me a ton of time to have the idea developed and ready to go. The tips on what combinations of microbes to use were particularly helpful. Thanks!"
- Beverly J. Brown, Nazareth College of Rochester, Biology Department, Rochester, NY

Curriculum Resources

The Scientific Method
Is this culture pure?
by
Daryl Perry*
(Thursday Microbio Lab)

The step by step process of the Scientific Method is crucial to guide the experimenter through the unknowns of Science! In this experiment the problem presented was "Is this culture pure?" The experiment was conducted by systematic questioning, beginning with a hypothesis, which was followed by collection of data to test the hypothesis, recording and analyzing observations, concluding in a decision to continue testing or reformulate the hypothesis.

The problem in the experiment was whether or not the culture was pure. In order to make an educated guess, some preliminary observations need to be made. Wet mounts and Gram stains were made in an attempt to answer the question (Fig 1a, b)**. It would be highly unscientific to conclude that the culture was pure on the basis of the wet mount, showing motile rods. Not totally satisfied with the conclusion drawn from the wet mount slide, a Gram stain was produced. The Gram stain predicted the bacteria as gram-negative rods. This data was still insufficient to conclude that the culture was pure. However, a hypothesis needed to be made. It was hypothesized that the culture was pure, and more testing was required.

In order to further understand the mixture, a streak plate was performed (Fig.2)**. The results of the streak plate suggested that the culture was in fact not pure. A byproduct of *P. aeruginosa* results in a green metallic appearance of the agar. The umbonate red and cream appearance of *S. marcescens* was also found on the plate.

Now it was time to analyze the information and it was found that the hypothesis was not supported. A new hypothesis was needed.

The brilliance of the Scientific Method keeps scientists guessing. Even though a hypothesis is supported by one test, it is not sufficient to accept the data and come to a conclusion. Not only are different tests required, but repeated tests are mandatory. Error of the experimenter and inefficiency by any means can affect data so nothing can be blindly accepted.

* Permission was obtained from D.P. to include his report in this submission.
** Figures, consisting of observations comparing cellular and colonial morphology of the organisms, have been omitted in this submission.