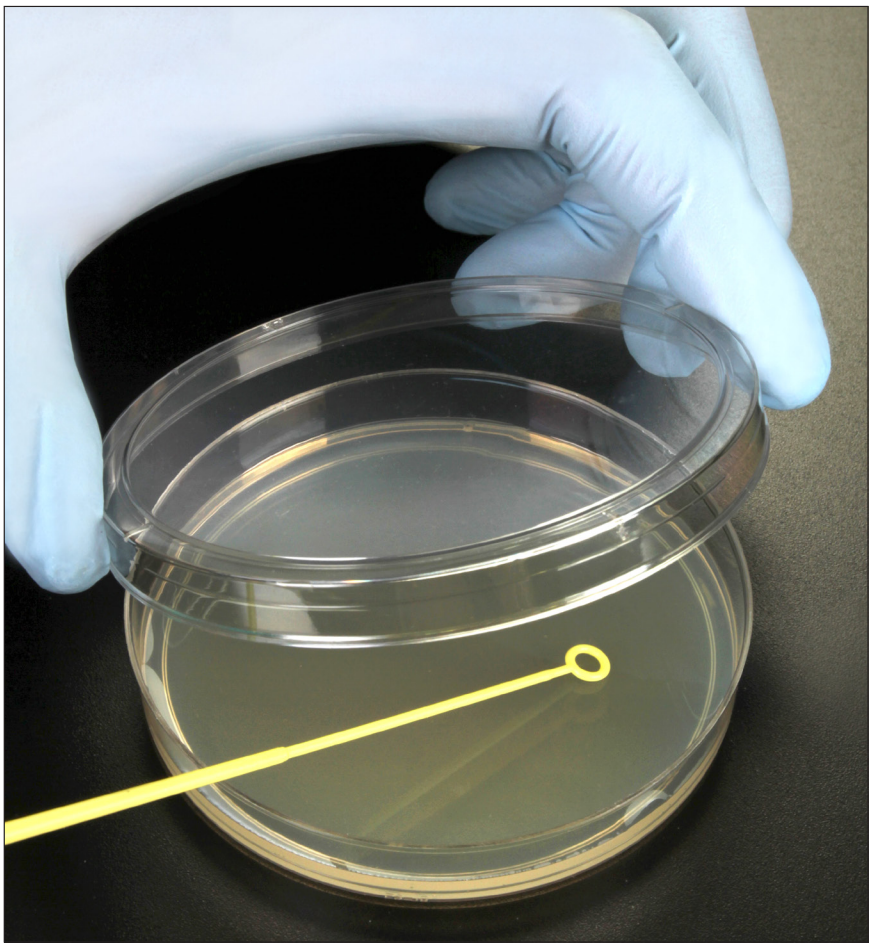
Sterile Technique

TEACHER'S MANUAL AND STUDENT GUIDE



CAROLINA®

World-Class Support for Science & Math

Sterile Technique

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TEACHER'S MANUAL

STUDENT GUIDE*

*Photocopy the Student Guide as needed for use in your classroom.

Sterile Technique

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Overview

Activities introduce students to sterile lab practices for studying microorganisms. Students perform two exercises: the transfer of sterile nutrient broth to a culture tube and the transfer of sterile nutrient broth from a culture tube onto a nutrient agar plate. Students must be able to perform both of these techniques without introducing unwanted microorganism growth if their future experiments with microorganisms are to be successful. Students will demonstrate their abilities by producing a culture tube and agar plate showing no growth of microorganisms after 4–7 days of incubation. The materials provided in this kit are sufficient for 10 groups of 3 students each.

Objectives

Students will

- learn and practice sterile techniques used in microbiology experiments.
- practice techniques for establishing colony growth of bacteria on a petri plate.
- understand the use of a positive control.

Correlation to Science Standards

To view the national and local standards met by this kit, **visit www.carolina.com/correlations**.

Science and Engineering Practices

- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing and interpreting data
- Constructing explanations and designing solutions
- Obtaining, evaluating, and communicating information

Crosscutting Concepts

- Patterns
- Cause and effect
- Scale, proportion, and quantity
- Stability and change

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Time Requirements

Preparation ..... 60 minutes

Activity 1: Pipetting ..... 20 minutes

Activity 2: Streaking Agar Plates ..... 30 minutes

Activity 3: Examining Culture Tubes and Petri Plates ..... 5 minutes per day over 4–7 days

Materials

Included in the kit:

10 5-mL tubes of sterile nutrient broth
6 125-mL bottles of sterile nutrient agar
30 disposable 1-mL sterile pipets
70 sterile inoculating loops
40 sterile petri plates
25 sterile culture tubes
10 nonsterile culture tubes
CSO Digital Resource Card
Teacher's Manual and reproducible Student Guide

Needed, but not supplied:

500 mL 70% ethyl or isopropyl alcohol
10 test tube racks for 17- × 100-mm culture tubes
10 lab markers
disposable gloves
heat-resistant gloves
scissors
paper towels
clear tape
bleach or autoclave
laboratory refrigerator
incubator (optional)

Safety

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE) such as gloves, chemical splash goggles, and lab coats or aprons. Ensure that students understand and adhere to these practices. Know and follow all federal, state, and local regulations as well as school district guidelines for the disposal of laboratory wastes. Students should not eat, drink, or chew gum in the lab and should wash their hands after entering and before exiting the lab.

If using a microwave to melt the agar in this kit, watch the agar carefully to ensure that it does not boil out of the bottle. *Do not microwave the bottle of agar without first loosening the cap, as this could make the bottle explode*. The agar will be very hot once melted. Use heat-resistant gloves to remove the bottle from the microwave.

Cleanup and Disposal

If microorganism growth occurs in any of the tubes or on any of the plates, autoclave them. If an autoclave is not available, soak the contaminated materials in a solution of one part bleach to nine parts water. Keep the container covered to contain the odor of the bleach solution. Keep the materials immersed in the bleach solution for a minimum of 24 hours. Pour the bleach–water into a laboratory sink and rinse generously with tap water. Dispose of the materials in accordance with federal, state, and local regulations.

Preparation

Preparing Agar Plates (at least 24 hours before Activity 2)

1. The agar provided in this kit can be melted using a boiling water bath or a lab microwave.

   Boiling water bath: Slightly loosen the caps on the bottles of nutrient agar, and then place the bottles in the boiling water bath to melt the agar. Make sure the water level is even with the agar level. Melting requires 30–40 minutes in boiling water. Swirl the agar inside the bottles to be sure that it has melted completely. Use a heat-resistant glove to remove the bottles. **Note:** Water must be kept at a constant boil to melt the agar in 30–40 minutes. Otherwise, it will take significantly longer.

   Microwave: Loosen the cap on the bottle and place the bottle in the microwave for 1-minute intervals, swirling the agar between each interval to ensure even melting, until the agar is completely melted. The bottles of melted agar will be hot. Use appropriate personal protective equipment. Watch the agar carefully while it is in the microwave to ensure that it does not boil out of the bottle.
   Microwaving the bottle without first loosening the cap can cause the container to explode.

2. Cool the agar in the bottles to 60°C by allowing the water bath to cool to that temperature, or by removing the bottles from the microwave and letting them cool for several minutes. The bottles should feel comfortably warm to the touch.
3. Disinfect work surfaces with 70% isopropanol or ethanol before preparing the plates.
4. Carefully cut the top of the sleeves containing sterile petri plates and save the sleeves for storage. Remove the plates from the sleeves, being careful to keep the lids in place.

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5. Label the bottoms of 20 petri plates "S" for sterile. Label the bottoms of 10 petri plates "NS" for nonsterile. Place the closed petri plates top-side-up in a row on the edge of a disinfected lab bench away from drafts.
6. Pour the melted agar from the bottles into the petri plates. Lift the lid of each plate only high enough to pour a layer of agar sufficient to cover the plate bottom approximately 3 mm deep. Do not set the lid on the lab bench. Replace the lid on the petri dish.
7. Allow the agar to solidify and cool. Return the plates labeled "S" to one of the plastic storage sleeves or wrap them with plastic wrap. Return the plates labeled "NS" to the other plastic storage sleeve or wrap them with plastic wrap.
8. Store the plates upside-down in a lab refrigerator until needed. This will prevent condensation from dripping onto the agar.

General Preparation

1. Review the SDS provided with this kit. Make certain you have appropriate personal protective equipment for every student in your class.
2. Review the content of the Teacher's Manual and the Student Guide. Familiarize yourself with the activity instructions, required materials, and assessments.
3. Photocopy the Student Guide for each student.
4. Gather the materials that are needed but not supplied.
5. Remove 10 of the sterile inoculating loops from their packaging, making them nonsterile.
   Note: Sterile items are in factory-sealed packages. Items in zip-top bags, bubble wrap, or plastic wrap are not sterile.
6. Remove 10 of the sterile pipets from their packaging, making them nonsterile.
7. Label the 10 nonsterile culture tubes "NS."
8. Set up 10 workstations for Activity 1 and stock each with the following materials:

2 sterile pipets	test tube rack
1 nonsterile pipet	70% alcohol
1 nonsterile culture tube	paper towel
5-mL tube of nutrient broth	lab marker

9. Choose a location for a central materials station for students to obtain gloves and sterile culture tubes.
   Note: To maintain sterility of the sterile culture tubes, make sure they are not opened until needed. Students should not retrieve these items until their lab stations have been disinfected with 70% alcohol.
10. Decide where and how the culture tubes and petri plates will be incubated. Incubation at 37°C will provide the fastest results. If an incubator is not

available, the plates may be left at room temperature. If the class uses room-temperature incubation, monitor the plates for a longer period of time.
Note: For best results, cultures created in Activity 1 should be incubated for a minimum of 24 hours at 37°C or 48 hours at room temperature before Activity 2 is begun.

11. Set up 10 workstations for Activity 2 and stock each with the following materials:

6 sterile inoculating loops	paper towel
nonsterile inoculating loop	tape
70% alcohol	lab marker

Procedure

1. Divide the class into groups of three students each. Assign each group to a workstation.
2. If necessary, teach students how to remove caps from bottles and culture tubes using the pinky finger, how to pipet liquids, and how to streak plates for colony growth. If needed, students can practice these techniques using the culture tubes, pipets, and inoculating loops that will be designated as "nonsterile."
3. Review all safety protocols with the students before they begin. Describe the procedure for groups to follow in collecting materials from the central materials station.
4. Distribute the Student Guide to each student. Have students read the Background information and then answer the Pre-laboratory Questions. Review students' answers to the Pre-laboratory Questions as a whole-class discussion.
5. Have students conduct Activities 1A and 1B according to the instructions in the Student Guide. Monitor the class to ensure that students are using safe laboratory techniques and wearing appropriate PPE.
Note: For best results, cultures created in Activity 1 should be incubated for a minimum of 24 hours at 37°C or 48 hours at room temperature before beginning Activity 2.
6. Have students conduct Activities 2A and 2B according to the instructions in the Student Guide. Monitor the class to ensure that students are using safe laboratory techniques and wearing appropriate PPE.
7. After each group has disinfected their workspace, provide them with two nutrient agar plates marked "S" and one nutrient agar plate marked "NS."
8. After the lab, instruct students how to clean up their workspace and return materials.
9. After students have completed the lab activities, have them answer the Questions in the Student Guide. Review the students' answers to the questions as a class discussion.

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