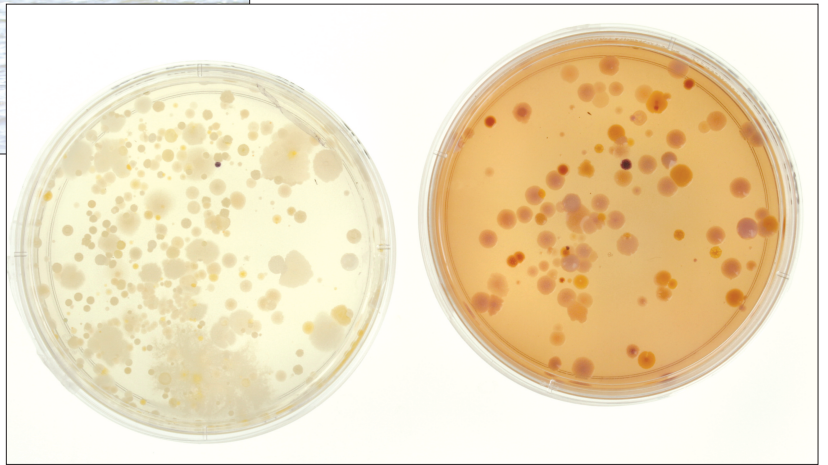


Carolina™ Bacterial Pollution of Water Kit

TEACHER'S MANUAL
AND STUDENT GUIDE



Carolina™ Bacterial Pollution of Water Kit

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TEACHER'S MANUAL

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

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Carolina™ Bacterial Pollution of Water Kit

Overview

Students learn about the importance of clean water in human health and some of the methods of disinfecting water for human use. Students learn about and perform a coliform count, a method commonly used in testing the safety of water for human use. This test also provides practice in sterile technique. Students collect water samples and test them for chlorine and chloramines, chemicals used in water treatment. Within 16 hours of collection, the students test their water samples for bacteria and specifically for coliforms by plating the samples on both nutrient agar and MacConkey agar plates. Water samples can be collected during or outside of class time. Plating is completed within a single 50-minute lab period. The analysis performed 48–72 hours later also occupies a single lab period. In addition, students research to find your state's legal standards for the number of coliform bacteria allowed in water used for various human activities. This kit will accommodate a class of 30 working in groups of 3.

Objectives

Students will

- learn about the importance of clean water for human health.
- perform a simple version of a coliform bacteria count, a common test of the safety of water for human use.
- gain experience plating and culturing bacteria.
- use sterile technique for working with bacteria.
- use a test strip to determine chlorine and chloramine levels in water.

Content Standards

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

Time Requirements

Preparation

Pour plates.	at least 1 day before Part 2	60–90 minutes
Prepare materials for water collection and chlorine/chloramine testing.	day of or day before Part 1	15 minutes
Set up student stations.	day of or day before Part 2	15 minutes

Part 1

Collect water sample and test for chlorine and chloramines.	10–15 minutes
Perform the chlorine and chloramine test at the time the sample is collected. Collect the sample within 16 hours of plating, preferably just before lab.	

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Part 2

Plate water samples.	During lab	15–30 minutes
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Part 3

Analyze plates and discuss.	24–72 hours after Part 1	30–50 minutes
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Have students test their water sample for chlorine and chloramines at the time they collect it. Ideally, they should also plate their sample immediately after they collect it, but they may wait up to 16 hours before plating. As time passes after the sample is collected, some of the bacteria lose their viability. The more time that passes before plating the sample, the more likely it is that the plate counts will under- or misrepresent the bacterial population of the water source.

Materials*Included in the kit:*

- 10 sterile collection tubes
- 32 sterile bulb pipets
- 20 petri dishes (100 × 15 mm)
- 2 bottles nutrient agar
- 2 bottles MacConkey agar
- 10 free chlorine/total chlorine test stripes
- 10 charts for use with the chlorine test strips
- 2 disposable spreaders, pack of 10
- 1 Teacher's Manual and reproducible Student Guide

Needed, but not supplied:

- household bleach to disinfect materials by soaking
- ethanol or isopropanol for spraying work surfaces
- 10 permanent markers
- boiling water bath or hot plate(s) with beaker(s) of water
- water samples collected by students
- Bunsen burner or alcohol burner
- 30 small, sealable, plastic bags at least 7" wide
- clear tape or parafilm
- 10 beakers or similar containers for measuring and holding 250 mL (1 cup)
- paper towels

Storage

All materials can be stored at room temperature. The test strips, and the MacConkey and nutrient agar are marked with expiration dates.

Safety

Use this kit only in accordance with established laboratory safety practices. 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.

When using the Bunsen burner or alcohol burner, keep combustible materials, including hair and clothing, away from flame. Do not leave open flames unattended. Know the location of and how to use your nearest fire extinguisher. Melted medium can be extremely hot and will stick to skin and other surfaces. Avoid thermal burns by avoiding contact with melted materials. Use thermal gloves or a hot pad to handle heated agar bottles.

When working with bacteria, it is crucial to use sterile technique and proper laboratory practices to reduce contamination. Ensure that students wear personal protective equipment (PPE) such as gloves, safety glasses and goggles, and lab coats or aprons. Students should not eat, drink, or chew gum in the lab. They should wash their hands upon entering and before exiting the lab and after collecting any samples other than tap water.

Treat all microorganisms as potential pathogens. Keep inoculated plates shut with tape or Parafilm. Sterilize or disinfect equipment, materials, and work areas after use. Either autoclave waste materials (40 minutes at 121°C and 20 lb pressure) or disinfect them by covering them in a solution of 1 part chlorine bleach to 9 parts water and soaking them for 2 hours.

Background

Students will use both nutrient agar and MacConkey agar plates. Nutrient agar is a general medium that supports the growth of a broad range of bacteria.

MacConkey agar supports the growth of most Gram-negative bacteria and is used to identify bacteria that are part of the coliform bacterial group. The coliform bacteria group consists of Gram-negative organisms that ferment lactose.

MacConkey agar contains bile salts and crystal violet as well as neutral red, a pH indicator that turns red at pH values below 6.8. The bile salts and the crystal violet in MacConkey medium inhibit the growth of most Gram-positive bacteria and thus select for Gram-negative bacteria. Colonies of bacteria that ferment lactose turn pink or red on MacConkey agar because they produce acid. As the pH of the medium surrounding the lactose-fermenting bacteria drops, the bacteria take up the neutral red. Note that some Gram-negative bacteria that are more selective about their growth conditions will also not grow on MacConkey agar.

You may want to remind students that the nutrient agar and MacConkey agar will detect only those organisms able to grow on either of those media. Thus, there may be other bacteria in the water sample that remain undetected by this assay.

The test strips used in the lab measure both free chlorine and total chlorine. Free chlorine is the amount of hypochlorous acid and hypochlorite ion in the water and results when chlorine is added to the water system. Chlorine is added to water either as chlorine gas, sodium hypochlorite, or dry calcium hypochlorite.

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Combined chlorine is composed of chloramines, the chemicals that result when free chlorine combines with ammonia or organic nitrogen present in the water. Total chlorine includes both free chlorine and combined chlorine.

On the charts for the test strip the results are given in both ppm (parts per million) and mg/L (milligrams per liter). Because these test strips are generally used to test aqueous solutions, and 1 g of water at 4°C is equal to 1 mL, the chart assumes that ppm is equal to mg/L. At 4°C, 1 g = 1 mL, so 1,000,000 mg = 1 L. Thus, 1 mg/L would be the same as 1 ppm.

Reference:

MacConkey Agar Plates Protocols

ASM MicrobeLibrary

<http://www.microbelibrary.org/component/resource/laboratory-test/2855-macconkey-agar-plates-protocols>

Preparation

1. Pour 10 nutrient agar plates and 10 MacConkey agar plates. Follow this procedure for each set:
 - a. To melt the medium, slightly loosen the cap(s) and set the bottle(s) of medium in a beaker of water on a hot plate or in a water bath and bring the water to a boil. Make sure the water level is even with the level of the medium in the bottle(s). Leave the bottle(s) in the boiling water until the medium has completely melted. This will take approximately 30 minutes from the time the water has boiled. Do not place cold bottles into boiling water.
 - b. Allow the medium to cool to 55°C, either by allowing the boiling water to cool to that temperature or by letting the bottle(s) sit for several minutes at room temperature. At around 55°C, the bottle(s) should feel comfortably hot.
 - c. While you are melting and cooling the medium, prepare a work surface away from any draft or breeze. Wipe the surface with a disinfectant such as 10% bleach or 70% alcohol.
 - d. Wash your hands thoroughly. Unpack the petri dishes, being careful not to open the lids and thus introduce contamination. Align the sterile plates along the edge of your work surface.
 - e. Once the medium has cooled, light a Bunsen burner. Remove the cap and flame the mouth of the bottle of medium. As you pour the plates, lift the lid of each petri dish just enough to pour in the melted medium. Pour to a depth of about 5 mm per plate. Each bottle will pour approximately five plates. After pouring each plate, replace the lid on the petri dish immediately to prevent contamination.

Note: Once you start pouring, keep the bottle at an angle during the entire process. Never set the bottle down or hold it upright once you start pouring as that increases the potential for contamination.