



76-6300

# Environmental Microbiology Kit

Teacher's Manual

## Introduction

This kit introduces students in grades 6 and up to the study of microbes in the immediate environment. It is designed for 5 teams of students who will learn to make plates; grow bacteria, molds, and yeasts from various sources; make plate counts of bacterial colonies; and prepare slides for microscopic examination.

Before starting the exercises, sanitize and disinfect the work area with 70% alcohol or a bleach solution (4 parts water to 1 part household bleach). Allow the area to air dry. Wash and dry your hands thoroughly. Mix agar as directed and pour all 40 plates. To avoid unwanted contamination, students should not open the plates unnecessarily. Some activities in this kit involve collecting airborne microorganisms; try to collect them on purpose, not by accident.

## Materials

The materials supplied should be used with the exercises in this kit only. Carolina Biological disclaims all responsibility for any other uses of these materials.

### *Included in this kit*

- 72 microscope slides
- 4 sterile collection tubes
- 5 sterile pipets
- 40 sterile petri dishes (60 × 15 mm)
- 50 sterile swabs
- 2 sterile splints
- 2 tubes of sterile water
- 100 wooden applicators
- 2 disposal bags
- dropper bottle of crystal violet stain
- nutrient agar bottled medium
- Instructions for Bottled Media* booklet
- student worksheets

*Needed, but not supplied*

microscope, preferably one with an oil immersion lens

pipet bulbs

## Procedures

**Note:** Exercises 1–5 can be completed in one class period and are presented in the order of their difficulty. Consider their increasing challenges when organizing the 5 student teams to complete them.

Each team will use 8 prepared plates. Students should label all samples and corresponding plates to record their sources. Store inoculated plates at room temperature. After 3–5 days, your students should record their results and complete Exercise 6. *Save all inoculated plates at the end of each exercise so they can be examined in Exercise 6.*

Make at least one copy of each exercise and hand them out to the appropriate teams. Distribute a Student Worksheet to every student, upon which they will record their findings. Make an overhead transparency of the Class Summary, located at the end of this manual, upon which you can summarize your students' results. When Exercise 6 has been completed, all plates should be put into the disposal bags and discarded.

## Exercise 1: Microbiology of Air

The air is filled with microorganisms. In this exercise, students will collect and study some of these airborne organisms.

### Procedure

1. Label the 8 plates for this exercise "Air."
2. Select locations around the school where the students of this group can collect airborne microflora (cafeteria, media center, athletic field).
3. Expose each plate to the air for 30 minutes. They may be exposed while gelling.
4. Replace the covers and store the plates at room temperature.
5. Students should record the results after 3–5 days.

**Note:** What appears on the plates after a day or two are not single organisms but populations known as colonies. A colony forms when a microorganism settles into an environment suitable for its growth.

## Exercise 2: Microbiology of School Surfaces

Students will isolate and study the microorganisms of surface dust.

### Procedure

1. Label the 8 plates for this exercise "Surfaces."
2. Collect samples from tabletops, desktops, or floors by rubbing the ends of sterile swabs over these surfaces.
3. Streak each sample on the surface of a plate (see figure 1).
4. Collect and streak samples from other places in the immediate area (halls or cafeteria).
5. Students should record their results after 3–5 days.

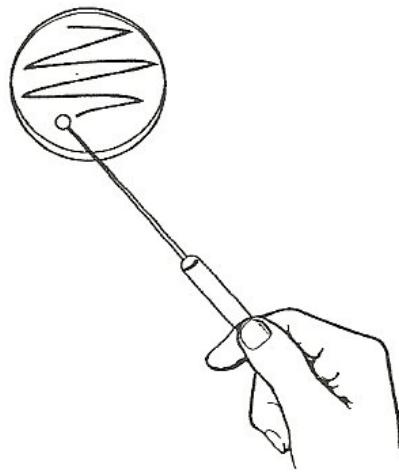


Figure 1

### Exercise 3: Microbiology of the Body

This exercise is designed to show the presence of microorganisms associated with the human body. **Caution:** It is possible that some isolated organisms may cause infection.

#### Procedure

1. Label the 8 plates for this exercise "Body."
2. Each student in this group should touch the inside of his or her cheek with a sterile swab and streak a plate.
3. Other exercises may include cleaning under a fingernail with a sterile swab and streaking a plate, touching a fingertip to the surface of a plate, coughing on a plate, or putting a hair on a plate.
4. Students should record their results after 3–5 days.

## Exercise 4: Microbiology of Soil

Students will attempt to isolate microorganisms from the soil.

### Procedure

1. Label the 8 plates for this exercise "Soil."
2. Collect 2 pea-size soil samples with sterile wooden splints and add 1 sample to each vial of sterile water. Use a different splint for each collection. Label each vial and 4 corresponding plates.
3. Shake the vials to obtain uniform suspensions.
4. With sterile swabs, streak each sample on 3 plates using the following procedure:
  - Dip the end of a swab about one-fourth inch into the water-soil suspension. Lift the cover of a plate as little as possible and carefully streak the surface of half the plate as shown in figure 2.
  - Using the end of another sterile swab, cross through the end of the first streak, then streak at right angles across the other half of the plate as shown in figure 3. To avoid contamination, lift the plate cover as little as possible during the process.

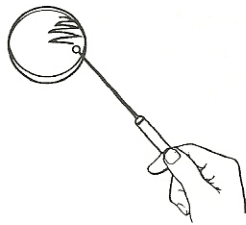


Figure 2



Figure 3

This streaking method should result in colonies. Streaking is another method of dilution and isolation, and is suitable when there are large numbers of organisms in a sample.

5. For each sample, cover the surface of a plate with water-soil suspension.
6. Students should record their results after 3–5 days.



## Exercise 5: Microbiology of Water

Students will collect water samples, inoculate plates from the samples, and determine bacterial count by the plate-count method. Not all bacteria in the samples will grow on any one medium, so an absolute count will not be possible. Some samples may contain innumerable microorganisms, making a count impossible.

### Procedure

1. Label the 8 plates for this exercise "Water."
2. Determine where the students should collect the water (drinking fountains, aquarium, stream, pond, or puddle). **Note:** The people collecting the water can contaminate the samples. They must avoid touching the mouth of the collecting tube while taking a sample.
3. Label each of the 4 water samples. Mark 2 plates to correspond to each sample.
4. One student should practice pipetting with the extra pipet, while others collect samples. When pipetting, the student should not draw water into his or her mouth and should use the index finger to control the flow of water into the pipet.
5. Lift the cover slightly and pipet 1 mL of the corresponding sample onto a plate as shown in figure 4. Distribute the sample evenly on the surface of the plate and let the plate absorb it.

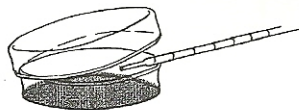


Figure 4

6. Students should record their results after 3–5 days.
7. Determine the number of bacteria in the water sample by counting the number of colonies on the 2 plates and taking an average. If a plate has 24 colonies and 1 mL of water was used, there are 24 bacteria per mL of sample. Bacteria counts are usually reported as the number per mL.



## Exercise 6: Examination of Microorganisms

The students examine microorganisms grown during the previous exercises. Use the Class Summary to record the findings of all the teams. You may reproduce the summary in whatever manner works best for your class—overhead transparency, on a chalkboard, or photocopies.

### Procedure

#### *Gross Examination*

Students should observe and record characteristics of colonies on the plates. Colonies of bacteria and yeasts do not have the thread-like structures (mycelium) and powdery texture common to molds. Identification is best determined by microscopic examination.

#### *Slide Preparation*

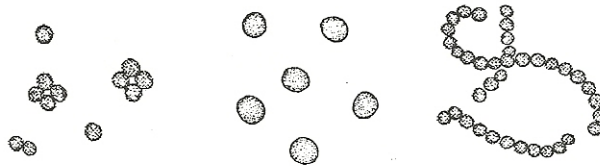
1. Place a drop of tap water on a slide.
2. Touch the wooden applicator to a selected colony, then touch the applicator to the drop of water on the slide. Stir to mix the bacteria with the water and spread the mixture thin with the applicator.
3. Let the slide air dry.
4. Affix bacteria to the slide by passing it quickly over the flame five times.
5. Flood the slide surface with stain.
6. After 1 minute, wash the slide gently with tap water.
7. Stand the slide on edge to dry.

#### *Microscopic Examination*

Examine the slide under a microscope, preferably one with an oil immersion lens.

Bacteria appear in several shapes—spheres, rods, and spirals.

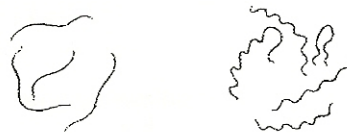
### Spheres



### Rods



### Spirals



Yeasts have budding structures and are obviously different from bacteria.



Molds are branched and will frequently have specialized fruiting structures.



## Class Summary

Exercise	Plate No.	No. of Colonies	Kind of Organisms
1. Air	1_____	_____	_____
	2_____	_____	_____
	3_____	_____	_____
	4_____	_____	_____
	5_____	_____	_____
	6_____	_____	_____
	7_____	_____	_____
	8_____	_____	_____
2. Surfaces	1_____	_____	_____
	2_____	_____	_____
	3_____	_____	_____
	4_____	_____	_____
	5_____	_____	_____
	6_____	_____	_____
	7_____	_____	_____
	8_____	_____	_____
3. Body	1_____	_____	_____
	2_____	_____	_____
	3_____	_____	_____
	4_____	_____	_____
	5_____	_____	_____
	6_____	_____	_____
	7_____	_____	_____
	8_____	_____	_____