EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-30

Edvo-Kit #S-30

How Clean is the Water We Drink and the Air We Breathe?

Experiment Objective:

In this experiment, students will learn about the microorganisms that exist in our environment. Microbes present in air and water samples will be isolated and cultured on nutrient agar plates. Students will identify differences in the isolated bacteria and characterize the appearance of individual colonies.

See page 3 for storage instructions.

1.800.EDVOTEK • Fax 202.370.1501 • www.edvotek.com • info@edvotek.com

Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Experiment Components

Contents		Check (√)
A	Ready Pour Luria Broth Agar	❑
B	3 sleeves Petri Plates	❑
C	10 ml Pipets (2)	❑
D	1 Pack of 1 ml Pipets	❑
E	20 ml Sterile Water sample	❑

Experiment #S-30 is designed for 10 groups.

Storage:
Store entire experiment at room temperature

Requirements

- Water samples
- Test tubes
- Pipet Pump or Bulb
- Hot Plate or Water Bath
- Aluminum Foil or Plastic Wrap
- 10% Bleach solution
- 37° C Incubator (optional)
- Microwave
- Waterbath (55° C)

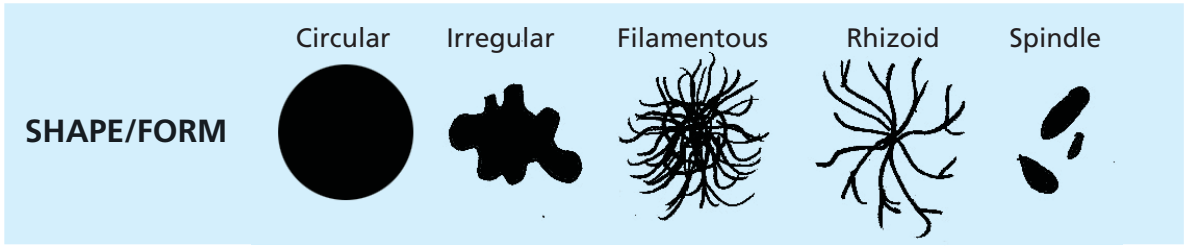
All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

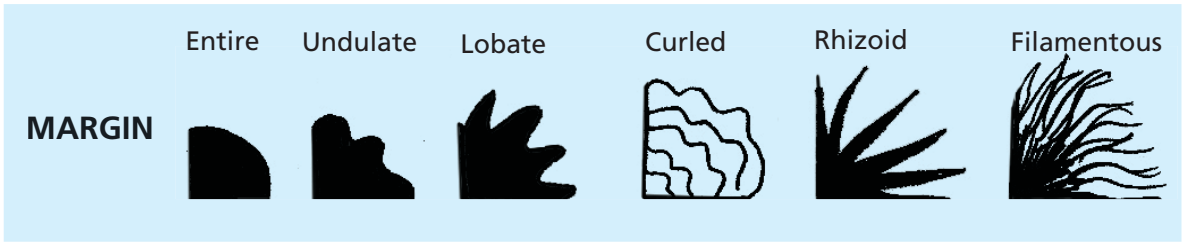
Background Information

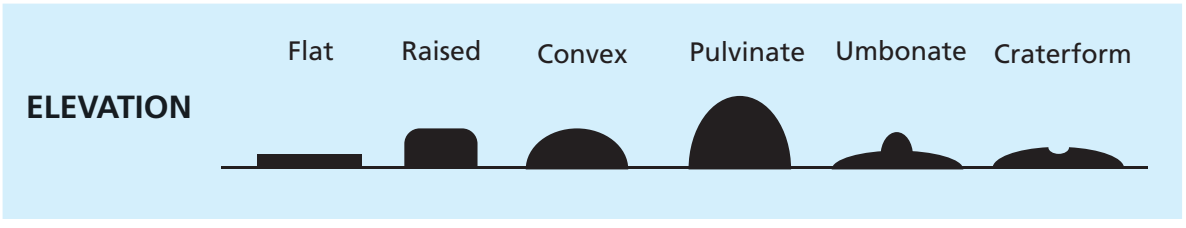
Even though we cannot see microbes, they are everywhere -- in our food and water, on and in our bodies, and in the air we breathe. Techniques in microbiology (the study of microscopic organisms) have allowed us to learn more about these tiny creatures. By using various culture methods, microorganisms can be isolated and pure cultures can be grown. This is useful in the areas of food microbiology, drug manufacturing, disease detection, and many other industrial applications.

Large, modern water treatment facilities have been able to supply us with safe drinking water by removing harmful disease-causing bacteria and other microbes. Such water supplies are not sterile and, in fact, do contain small amounts of bacteria. Other water sources (streams, ponds) contain varying levels of microorganisms dependent on environmental influences.

Scientists can use various culture methods to establish pure cultures of microorganisms. When these cultures are plated on a solid surface, the microbes form visible masses of cells called colonies. Each colony starts from a single organism (cell). Colony morphology is an important tool for scientists to differentiate between species of microorganisms. Different species of bacteria will produce unique colonies – the shape, color, texture and size of colonies can vary between microbial species. Individual colonies range from 1 mm to 5 mm in size. The edges of individual colonies can appear wavy, serrated, or smooth. The textures of the colony may appear mucoid (slimy or gummy), smooth (shiny, uniform texture) or rough (granulated texture, matte surface). Some microbes produce pigments, which results in colonies in a wide range of colors (including pink, yellow, and white). This technique allows scientists to make preliminary species identifications that are confirmed using the Polymerase Chain Reaction (PCR) or other diagnostic techniques.





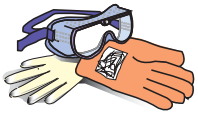


Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will learn about the microorganisms that exist in our environment. Microbes present in air and water samples will be isolated and cultured on nutrient agar plates. Students will identify differences in the isolated bacteria and characterize the appearance of individual colonies.

LABORATORY SAFETY



Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.
6. The bacteria used in this experiment are not considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
      - Autoclave at 121° C for 20 minutes. Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
      - Soak in 10% bleach solution. Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
   C. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Activity One - Water Analysis

For Activity One, each student group should receive:

- Two empty petri dishes
- Water sample
- Marking pencil and tape

Wear gloves and safety goggles

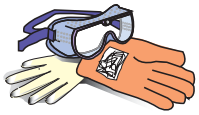
1. Label one empty petri dish with your lab group and the source of your water sample.
2. Pour 1 ml of collected water sample into the empty dish.
3. Label the second empty petri dish with your lab group and the word "control".
4. Your teacher will pour 1 ml of sterile water into the control dish. Replace lid. **DO NOT TOUCH THE WATER!**
5. Have your teacher add 5 ml of hot molten media to your sample and control dish. Replace lid.
6. Very slowly and gently swirl to mix the media and water. Be careful not to spill the agar over the edge of the plate.
7. Cover the plates and allow the mixtures to solidify.
8. Incubate 1-3 days at room temperature or in a 37° C incubation oven for 24 hours.
9. Observe plates and answer study questions.

Activity Two - Air Analysis

1. Label the two media plates with your name or lab group. Keep one closed, tape and label "control".
2. Indicate on the cover of the second plate the area selected by your teacher to place your plate (e.g., floor, near doorway, desk, near an air vent, window).
3. Set your plate at the designated area and remove the lid.
4. Allow plate to remain opened overnight, replacing lid the next morning.
5. Incubate all plates upside down at room temperature for 1-3 days or in a 37° C incubation oven overnight (if no growth occurs, continue incubation).
6. Observe plates and answer study questions.

For Activity Two, each student group should receive:

- Two petri dishes containing solidified medium
- Marking pencil and tape
- Solidified Medium



Wear gloves and safety goggles

Activity Two - Alternative Experiment:
Allow plates from different groups to remain open for different time intervals.

Study Questions

1. What are bacteria?
2. What are fungi or molds?
3. What kinds of growth did you observe?
4. What is a colony?
5. Why do these microorganisms grow so well on the media but we don't see them in the air or water?
6. Was most of the growth on the surface of the media or did some grow inside the media? Why?
7. How many bacterial colonies can you count on your plate?
8. How would you explain the difference in the amount of growth found in the different water sources?
9. How would you explain the differences in the amount of growth found in the air in different locations?
10. What would you have done differently if you had to repeat this experiment?
11. Does this mean that bacteria coexists with us?

Instructor's Guide

ISOLATION OF MICROORGANISMS FROM WATER AND AIR

Day One

- Have sterile water, molten media ready.
- Hand out lab instructions.
- Go over instructions and emphasize the following:
  Caution students that they must not touch the sterile water. The molten media is very hot and they must swirl the mixture very slowly so as not to burn themselves. Remind them how to label dishes.
- Clean up and make sure all plates are properly stored. Answer any student questions.

Day Two

- Have students place covers on petri dishes (Activity Two)
- Begin incubation of dishes.
- Discuss Study Questions 1 and 2.

Day Three

- Have students view results and answer Study Questions 3-11.

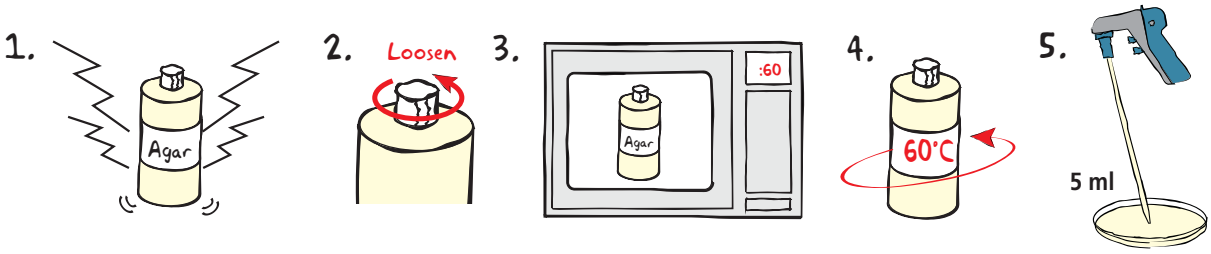
Anticipated Results

Students will observe differences in quality and quantity of microbes isolated. There should not be any growth on any controls.

Pre-Lab Preparations:

POUR AGAR BASE PLATES

If plates are prepared 2-7 days before use, they must be stored in a sealed plastic bag at 4° C.



1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.
2. **LOOSEN**, but **DO NOT REMOVE**, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. **CAUTION:** Failure to loosen the cap prior to heating may cause the bottle to break or explode.
3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).
4. **COOL** the ReadyPour™ Agar to 60° C with careful swirling to promote even dissipation of heat.
5. **POUR** 5 ml of the cooled ReadyPour™ Agar into each of the large petri dishes using a 10 ml pipet and pipet pump. Place remaining media into 55° C water bath for use in Activity 1.
6. **COVER** and **WAIT** at least twenty minutes for the LB-agar plates to solidify. For optimal results, leave plates at room temperature overnight.
7. **STORE** plates at room temperature for no more than two days. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out.

Wear Hot Gloves and Goggles during all steps involving heating.

NOTE for Step 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

NOTE: If plates are prepared more than two days before use, they should be stored inverted in a plastic bag in the refrigerator (4° C). Remove the plates from the refrigerator and warm in a 37° C incubator for 60 minutes before use.

If not performing Activity 1 on the same day as pouring plates, the ReadyPour™ agar may be allowed to solidify. On the day of the experiment, melt as detailed in above protocol, cool to 60° C, and place in 55° C waterbath until needed.

Quick Reference: Pouring LB Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Please refer to the kit insert for the Answers to Study Questions