



Sci-On[®] Biology

Sci-On[™] # S-30

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



Storage:

Store this experiment at room temperature.

EXPERIMENT OBJECTIVE:

Students will learn about microorganisms that exist in our environment through this hands-on activity. In this experiment, they will isolate microbes from water and air sources.

All 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.

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Experiment Components

Storage:

Store this experiment at room temperature.

This experiment is designed for 30 students working in pairs.

Contents

- A 1 Bottle of Ready Pour Agar
- B 3 Sleeves Petri Plates
- C 10 ml Pipets (2)
- D 1 Pack of 1 ml Pipets
- E 20 ml Sterile Water Sample

None of the experiment components have been prepared from human sources.

Experiment Requirements

- Water Samples
- Test Tubes
- Pipet Pump or Bulb
- Hot Plate or Water Bath
- Aluminum Foil or Plastic Wrap
- 10% Bleach Solution

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

Microbes exist everywhere, in our food and water, on and in our bodies, and in the air we breathe. It is difficult to understand how living organisms exist without being able to see them. Microbiological science techniques have made it easier for us to study these tiny creatures with tools such as the microscope. 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.

Microbial growth occurs from a single organism (cell) which multiplies quickly and forms visible masses called colonies. Colonies of different organisms may vary in several ways. Individual colonies can be 1 mm to 5 mm in size, with some organisms characteristically forming small colonies. Their pigments can range from pink, yellow or white. Borders of individual colonies can form unique shapes such as wavy, serrated or smooth. The surface of a colony may form textures which appear mucoid or slimy, smooth with a uniform texture, or rough with a granulated texture.

EXPERIMENTAL PROCEDURES

EXPERIMENT OBJECTIVE

Students will learn about microorganisms that exist in our environment through this hands-on activity. In this experiment, they will isolate microbes from water and air sources..

LABORATORY SAFETY



1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. Although it is unlikely that the microbes isolated in this experiment would be considered pathogenic, extreme caution should be utilized by the students. It is good laboratory practice to follow simple safety guidelines in handling and disposal. At the completion of the experiment:

5. Wipe down the lab bench with a 10% bleach solution, disinfectant or soapy water.
6. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal. Disinfect materials as soon as possible after use in one of the following two 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 media 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.



7. Always wash hands thoroughly with soap and water after handling contaminated materials.

EXPERIMENTAL PROCEDURES**Activity One****Activity One****Each Lab Group should have the following materials:**

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

WATER ANALYSIS

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.

Activity Two**Activity Two****Each Lab Group should have the following materials:**

- Two petri dishes containing solidified media
- Marking pencil and tape

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.

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?

Lesson Plan Outline

If you don't find answers to your questions in this section, call our

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Please have the following information:

- The kit number and title
- Kit lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Mon - Fri
9:00 am to
5:00 pm EST

This lesson plan outline, written by a teacher, can be used as a guideline to fit your specific classroom experience.

I. Purpose

For students to gain an understanding of the abundance and diversity of microorganisms.

II. Experiment Skills

Students will have the opportunity to see the various types of microorganisms she/he comes into contact with daily.

III. Materials

Components which are provided with this kit, and a list of additional requirements to perform this experiment are listed on page 2.

IV. Prerequisite Knowledge

Students should have some understanding of what microorganisms are and how we protect ourselves from them.

V. Focusing Event

Students will collect and describe many different types of microbes.

Suggestions for Lesson Plan Content

1. Microorganisms are tiny unicellular or multicellular organisms that exist in many different environments, including in and around our bodies, our homes, hot mineral springs, and frozen glaciers. Microorganisms include bacteria and fungi (yeasts and molds).
2. Write the following words on the board.

Bacteria Microscopic Fungi Macroscopic
Colony Media Spores

Apply the vocabulary words to the exercise.

- Bacteria are unicellular organisms that can only be observed under high-power microscopes. They divide in multiple planes when they reproduce. When they reproduce on a solid nutrient surface, they form macroscopic masses called colonies.
 - Fungi are microscopic organisms that are more complex than bacteria. They also form colonies on solid media. They sometimes form spores. Spores are reproductive structures that produce single cells. Eventually, the single cells will combine to form a complete fungus. Spores are carried in water and air currents.
3. Isolation of Microorganisms from Water and Air

DAY ONE

- Have sterile water, molten media ready.
- Hand out lab instructions. Student experiment instructions are provided in the experiment starting on page 4.
- 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.

- Closure

Clean up and make sure all plates are properly stored. Answer any student questions.

**Suggestions for Lesson Plan
Content, continued****DAY TWO**

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

DAY THREE

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

Clean up and go over questions.

4. Anticipated Results

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

5. Alternative experiment:

Allow plates from different groups to remain open at different time intervals.

Preparations for Experiment



Wear hot gloves and goggles during all steps that involve heating.



Failure to loosen cap prior to heating or microwaving may cause the Ready Pour™ media bottle to break or explode.

On the day before the lab:

Have students collect water samples. Suggestions for sources include tap water, distilled water, spring water, standing water (from puddle, pond), water from stream or river. Label and dispense 1 ml of each sample into a test tube and cover with foil or plastic wrap. Place these samples in a refrigerator.

On the morning of the lab, prepare the Ready Pour™:

1. Equilibrate a water bath at 55°C for step 5 below.
2. Loosen, but do not remove, the cap on the Ready Pour™ media bottle to allow for the venting of steam during heating.
3. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks
4. Heat the bottle of Ready Pour™ media by one of the methods outlined below. When completely melted, the amber-colored solution should appear free of small particles.

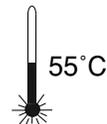
A. Microwave method:

- Heat the bottle on High for two 30 second intervals.
- Using a hot glove, swirl and heat on High for an additional 25 seconds, or until all the Ready Pour™ media is dissolved.
- Using a hot glove, occasionally swirl to expedite melting.

B. Hot plate or burner method:

- Place the bottle in a beaker partially filled with water.
- Heat the beaker to boiling over a hot plate or burner.
- Using a hot glove, occasionally swirl to expedite melting.

5. Cool the melted Ready Pour™ media by placing it in a 55°C water bath to prevent the agar from prematurely solidifying.



**Preparations for Experiment,
continued**

6. Use sterile 10 ml pipet to dispense 5 ml molten media to 30 petri plates, carefully set aside to solidify. Keep plates covered. Place remaining media back into 55°C water bath or on the hot plate.

NOTE: Keep the media warm at 55°C to be used during the Experimental Procedures on page 5.

Quick Reference: Pouring Agar Plates

1. Use a sterile 10 ml pipet with a pipet pump to transfer the 5 ml of media to each petri plate. Pipet carefully to avoid forming bubbles.
2. Rock the petri plate back and forth to obtain full coverage.
3. If the molten media contains bubbles, they can be removed by passing a flame across the surface of the media.
4. Cover the petri plate and allow the media to solidify.

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