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# Microorganisms In Our Environment

Teacher's Guidebook

(Cat. # BE-106)



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## **MATERIALS INCLUDED**

This kit has enough materials and reagents for 30 students (six groups of five students.)

- 1 vial Bac: E.C. K-12 Agar Stab
- 1 vial LB Broth
- 2 bottles LB Agar (Premade)
- 8 Petri Dishes
- 1 Inoculating Loop
- 30 Cotton Swabs

## **SPECIAL HANDLING INSTRUCTIONS**

- All reagents can be stored at room temperature

## **ADDITIONAL EQUIPMENT**

- Water bath or Beaker and Thermometer.
- Incubator (optional)

## **TIME REQUIRED**

- **Day 1:** 2 hours

## **OBJECTIVES**

- Grow microorganisms found in our environment.
- Introduction to aseptic techniques.
- Introduction to microorganisms.

## **BACKGROUND**

Microorganisms, or microbes, are tiny organisms, often single celled that are invisible to the naked eye. Microbes are found everywhere, they are on and in our bodies, and in the food and water we drink and in the air we breathe. It is often difficult to understand and learn about organisms that are too small to visualize, however microbiological techniques allow scientists to grow microbes to a concentration that makes them visible. Microbes include bacteria, fungi and viruses.

Using specialized growth media, known as LB agar, a single bacterium can multiply rapidly forming a colony of identical bacteria, which is visible to the naked eye.

The aim of this kit is to provide students with sterile plates that contain the solid growth media. They can then collect samples from their classroom and school and dab them onto the growth media and visualize the presence of microbes in the samples.

## PRE EXPERIMENT SET UP

### **Preparation of LB Agar Plates.**



*Wear heat protective gloves throughout the agar melting and pouring procedure*

*Make Agar plates the day before the experiment.*

1. Loosen the cap of the bottles containing the LB Agar.
2. Place the bottle in a large container, such as a beaker or saucepan and add water to the container up to the level of the agar.
3. Heat the water until it begins to boil. Simmer for 1 hour and swirl the agar bottle every 5 minutes, until all the agar has melted.
4. Turn off the heat.
5. Remove the bottle from the water bath. Allow the agar to cool to the point it can be held comfortably in your hand, this takes 20-40 minutes.
6. Using aseptic techniques (figure 1), pour a ~0.5cm/ ¼” layer of agar into each Petri dish. This is approximately 15-20ml.



Figure 1

7. Replace the lids. Do NOT move the plates until the agar is completely set. This takes 20-30 minutes.
8. Once completely set, store the Petri dish upside down (figure 2) in a refrigerator until needed. For long-term storage, wrap in a plastic bag or store in an airtight container.



Figure 2

**Prepare Positive Control Plates (Use aseptic techniques)**



*The K-12 Agar stab provided with this kit is a bacteria sample that has been specifically selected as the most innocuous materials. The bacteria are widely used in teaching and research and are non-pathogenic to humans.*

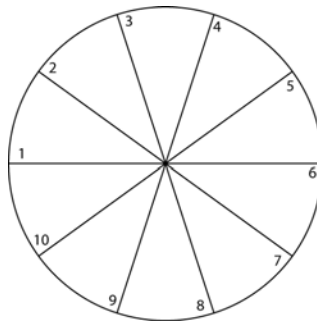
1. *This can be done as a demonstration:* The morning of the experiment transfer ~0.8ml LB broth to the bacterial agar stab and incubate at 37°C for 30 minutes. Vigorously shake or vortex.
2. Dip the inoculating loop into the bacterial suspension and spread over the surface of one of the LB agar plates in a zigzag manner. Try to use all available space. Incubate the positive control plate with the student plates.
3. The final Petri dish is to be left with the lid off in the classroom until it is time to incubate the plates. This plate is designed to capture microbes in the air.

## MATERIALS FOR EACH GROUP

- 1 Petri Dish with Growth Media
- 5 Cotton Swabs

## PROCEDURE

1. Do not open the Petri dish until required to do so.
2. Label the Petri dish with your group name.
3. The Petri dish needs to be divided into 10 sections. Five sections will be used for samples collected from around the classroom and five sections will compare the number of microbes on each student's finger. Lay the Petri dish face down on the template below and with a ruler mark the *bottom* of the dish as per the template.



4. Assign one section from sections 1-5 to each student in the group and record their name in the appropriate section in the results section.  
*If the group has less than 5 students, some students can be assigned 2 sections.*
5. Taking it in turns, lift the lid of the Petri dish, holding it above the agar to prevent unnecessary contamination. Gently place your index finger into the center of the section. Do NOT push to hard, you only need to touch the surface.  
*If a student has two sections, touch the first as described, then wash your hands and touch the second section.*
6. For the second set of experiments, assign a section from sections 6-10 to each student in the group and record their name in the appropriate section of the results section.
7. The cotton swabs in the bag are sterile; only remove from the bag when required. Do not handle the cotton end. Take it in turns and remove a cotton swab from the bag, wipe the cotton swab over a surface of choice and gently press onto your Petri dish section. Record the section swabbed in the result sections.  
*Examples of surfaces to test include workbench, shelves, pens, bathroom surfaces, keyboards and mice, floor, walls, light switches. If liquids are to be tested, such as*

*tap water, ensure only a tiny amount of liquid is dabbed onto the Petri dish surfaces to prevent it running into other sections.*

8. Once all the sections have been touched with the test samples, place the Petri dishes, *upside down*, in a warm place. Preferentially in a 37°C incubator, but any warmer than normal area of the laboratory will work.
9. Examine the plates the following day and record your results in the next section.



**RESULTS, ANALYSIS & ASSESSMENT**

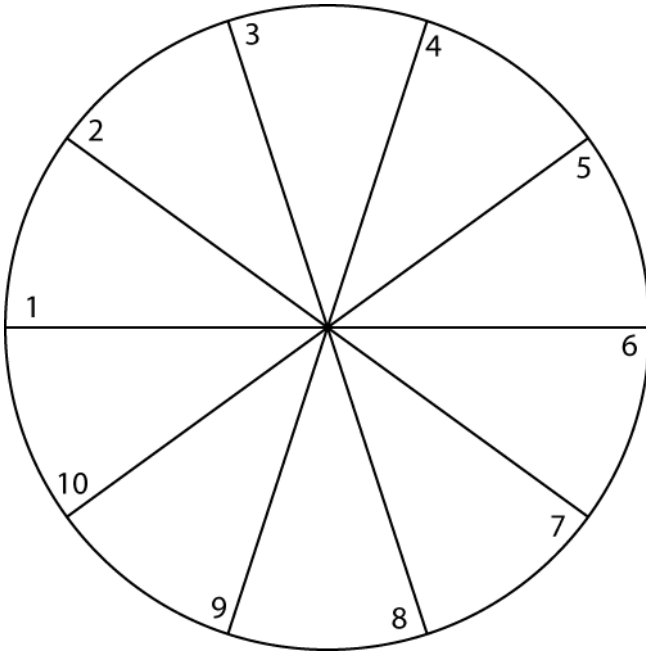
Student Fingerprint section number:

- 1. \_\_\_\_\_
- 2. \_\_\_\_\_
- 3. \_\_\_\_\_
- 4. \_\_\_\_\_
- 5. \_\_\_\_\_

Student Sample section number:

- 6. \_\_\_\_\_  
\_\_\_\_\_
- 7. \_\_\_\_\_  
\_\_\_\_\_
- 8. \_\_\_\_\_  
\_\_\_\_\_
- 9. \_\_\_\_\_  
\_\_\_\_\_
- 10. \_\_\_\_\_  
\_\_\_\_\_

Sketch your results in the diagram below:



Discuss your results below. Describe the amount of growth seen and compare the amount of microbes in each section. Draw conclusions from your results, for example who has the dirtiest hands?

*Discussions will vary depending on the samples collected.*

Examine the Positive control Petri dish and compare to your Petri dish. The control plate is just bacteria, do you plates only contain bacteria? Discuss your observations.

*Student Petri dishes will have bacteria but may have Fungi growing as well.*

Examine the Petri dish that was exposed to the air. Discuss your observations.

*Depending on the environment, there should be some microbial growth on the plate, which is indicative of airborne microbes.*

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## OBJECTIVES

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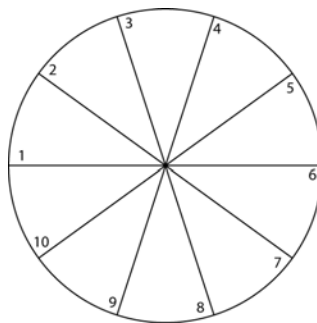
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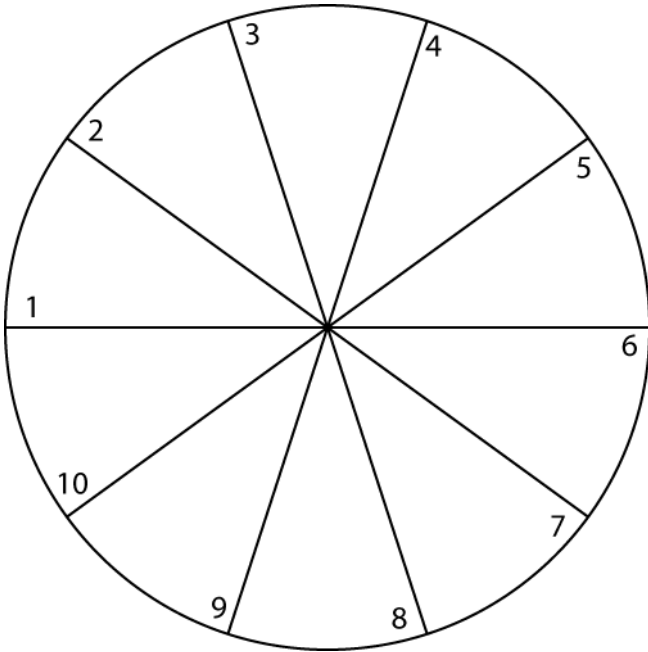
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