

Life Sciences 11 Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Testing the** **Effectiveness of a Variety of Antimicrobial Agents**  Partner: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

A common method of testing an antimicrobial agent’s effectiveness is to use the **Kirby-Bauer Disk Method** (as outline in the procedure section of this lab handout). In order to interpret the results obtained using this technique, **a zone of inhibition** is measured. The **zone of inhibition** is essentially the area on a nutrient agar plate that is **free of any bacterial growth** as indicated by a “clearing” where bacteria did not grow. The zone of inhibition can be obtained in millimeters by using a ruler to measure one edge of the clearing to the next (taking the diameter of the clearing).

The size of the zone of inhibition can then be used to gage the effectiveness of the antimicrobial agent at stopping or controlling bacterial growth. If **NO** zone of inhibition is visible the bacterial species being tested is **RESISTANT** to the antimicrobial agent and hence, growth is **NOT** **prohibited**. If the bacterial species is **SUSCEPTIBLE** to the antimicrobial agent, then such an agent kills or inhibits / slows the growth of this particular bacterial species.

**Purpose:** To determine the effectiveness of a variety of antimicrobial agents such as **disinfectants, hand sanitizers, traditional methods, and antibiotics** killing or inhibiting the growth of bacteria,

**Materials:** 1 nutrient agar plate, *bacteria from previous lab,* inoculating loops, Bunsen burner, tweezers, control disk and 3 antimicrobial disks, Various antimicrobial agents, ruler

**Question:** Which antimicrobial will produce the largest zone of inhibition of our swabbed bacterial?

**Prediction: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Procedure:**

1. It is important to use sterile technique whenever possible: Wash hands before and after lab. Only open plate when adding something. Do not reach over open plate. Sterilize forceps in between steps. See demonstration from teacher.
2. Get a new agar plate and inoculate the whole plate with E. coli (or one of your colonies): Sterilize inoculating loop in Bunsen burner about 10 seconds. Give it a few seconds to cool down or you will kill any bacteria you collect.
3. Lightly swipe the top of the *E. coli* (or one of your colonies) with your loop to collect some bacteria sample.
4. Streak the ENTIRE plate with a tight zig-zagging pattern across. See diagram.
5. With a sharpie divide the plate into four quadrants like we have done before.
6. Label one quadrant as control. Label other three quadrants according to the antimicrobials you want to test.
7. Add disks to plate: **Sterilize** your forceps with Bunsen burner **before** and **after** each use. Do not let forceps touch anything else. With sterile forceps, collect a disk. If it is a premade antibiotic disk, place it directly on the agar in the middle of the corresponding quadrant. For other antimicrobials, dip disk in antimicrobial solution, shake disk to remove excess liquid, place in middle of corresponding quadrant. Control: dip disk in sterile distilled water with forceps and place in center of quadrant.
8. When finished, place plate back in incubator.

**Day 2:**

1. **WITHOUT REMOVING THE LID**, use **a ruler to measure each zone of inhibition**. Place the ruler at one edge of the clearing and measure the distance to the opposite edge of the clearing. **Repeat for each disk (control + 3 antimicrobials)** and record your results in DataTable 1 under the results section.

\* ***If there is no zone of inhibition visible to/apparent record the zone of inhibition as “less than 7mm” (this is the diameter of each disk).***

1. **Draw the bacterial growth & zones of inhibition in diagram below.** \* *Be sure to label the quadrants in your diagram with the corresponding antimicrobial agents*. *Don’t forget to draw in pencil.*
2. Disinfect your work area and return all equipment.

**Observations:**

1. Control

2)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Table 1: Zones of Inhibition Observed Using a Variety of Antimicrobial Agent**

|  |  |  |  |
| --- | --- | --- | --- |
| **Antimicrobial** | **Zone of Inhibition (mm)** | **Description of Growth** | **Ranking of Effectiveness 1=most effective**  |
| **Control** |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Discussion Questions:**

1. Identify the independent (manipulated) variable \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. Identify the dependent Variable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. Why is having a control important in this lab?
4. Which antimicrobial was the most effective and which was the least effective? What is the evidence? Did this match your predictions? What category was your most effective antimicrobial from? *Make sure to refer to the zones of inhibitions in your answer.*
5. What surprised you the most in this activity? Explain.
6. EVALUATE: What were some sources of error during the experiment and how can you expand/improve this lab in the future?
7. Describe two ways that bacteria can cause disease.
8. Use what you know about bacteria and antibiotics to explain the *dangerous implications* of the overuse of antibiotics in modern society.

GOING FURTHER: Other than cedar oil, what other traditional methods have been used by Indigenous Peoples to protect against illness?

1. Briefly explain how antibiotics work. Name a specific antibiotic and a specific disease it can be used to treat.

|  |  |  |  |
| --- | --- | --- | --- |
| **Curricular Competency**  | **Emerging** | **Developing** | **Proficient** |
| **Analyzing Data -** Use knowledge of scientific concepts to draw conclusions that are consistent with evidence | Finds patterns in data and makes a limited claim about relationships based on these patterns | Identifies and explains the evidence used to support claims about relationships | Fully and effectively explains and gives detailed reasoning for claims about relationships using specific evidence  |
| **Evaluating** - Describe specific ways to improve their investigation methods and the quality of the data | Identifies a source of error and explains how it could have been improved. | Identifies multiple sources of error and explains the effect on the data and how to improve | Explains multiple sources of error and suggests appropriate methods to improve investigations |
| **Evaluating** - Consider social, ethical, and environmental implications of the findings from their own and others’ investigations | Identifies a social, ethical, and/or environmental aspect of the findings | Considers and explains social, ethical, and/or environmental aspects of the findings | Connects social, ethical, and/or environmental aspects of the findings and their implications |

Did your results indicate that the bacteria were **resistant** to any of the antimicrobials? How could you tell?