



Overview of Activity

Measuring Antibiotic Resistance (Kirby-Bauer)

1. Summary of Activity

Students use prior microbiological skills to create a lawn, or carpet, of *S. epidermidis* and *E. coli* on nutrient agar plates. They then place antibiotic impregnated filter paper discs on the agar and incubate the plates. The expected learning outcomes are that students will be able to understand how antibiotics affect bacterial growth, and that students will become familiar with a common technique used to measure bacterial resistance, the Kirby-Bauer test.

2. Intended Grade Levels

9-12

3. Recommended Prior Knowledge

Students should be comfortable with sterile technique, have an understanding of what antibiotics are, how they work and against what type of organism they are effective. In addition, they should know the structural differences between Gram-positive and Gram-negative bacteria.

4. Materials list

All of the following quantities are for pairs of students (15 groups in a class of 30 students), unless otherwise noted.

60 TSA 60 mm plates (4 TSA plates/group) or 30 TSA 100mm plates (2 plates/group)

30 sterile swabs

15 fine tipped marking pens

15 bent glass rods

15 dropper bottles of 70% isopropanol (rubbing alcohol)

15 Bunsen burners

15 forceps

soap and matches

1 each per lab table: nutrient broth culture of *S. epidermidis* and *E. coli*.

biohazard bag, parafilm and scissors (one set for the class)

5. Teacher Instructions

- In all labs in this series, the agar tryptic soy agar (TSA) is used. This is only one of several that are suitable. Others, such as nutrient agar or LB agar will also work. All students should practice the skills found in this lab, though material can be shared between pairs or groups of three

students. More than three to a group means too much down time for students and the lab will not be finished in a reasonable amount of time.

- You may wish to tell students that, ordinarily, TSA agar is not used for this test. The Kirby-Bauer test calls for Mueller-Hinton agar as the standard growth medium. Different bacteria may respond differently to different growth media and therefore a single type of agar medium must be used when doing this procedure in a professional setting. TSA is used in this lab to avoid the cost of buying an extra agar medium and should work fine for demonstration purposes (as would any general growth medium, such as nutrient agar or LB agar).
- Preparation Instructions for Mueller-Hinton and LB broth and plates:
 1. **Mueller-Hinton:** 38 g premixed, dehydrated Mueller-Hinton agar; add water to 1 L. Mix above ingredients and autoclave (121 °C, 20 psi, 30 min) or if you don't have an autoclave boil the media, or cook it in a pressure cooker for 1 hour. Allow media to cool to about 55 °C, then pour into plates. Let plates cool overnight. Store plates inverted in the refrigerator.
 2. **LB Agar and LB Broth:** 5 g yeast extract, 10 g Bacto-tryptone, 5 g NaCl, 15 g agar; add water to 1 L. Mix above ingredients and autoclave (121 °C, 20 psi, 30 min) or if you don't have an autoclave boil the media, or cook it in a pressure cooker for 1 hour. Allow media to cool to about 55 °C, then pour into plates. Let plates cool overnight. Store plates inverted in the refrigerator. To make LB broth, follow the recipe, except leave out the agar.
- Students should have hands-on familiarity with sterile technique and techniques for culturing bacteria on agar plates. Lab activities for familiarizing students with these techniques can be found at <http://www.colorado.edu/Outreach/BSI>
- You may wish to familiarize students with techniques for creating a lawn of bacterial growth. Complete coverage of bacteria over the entire plate is essential for this lab activity.
- This lab was written for using 60mm Petri dishes. If you prefer 100 mm dishes, you may wish to modify steps 5 and 6 of the procedure by having the students place all four discs on a single plate. The discs should always be widely separated from one another, but not touching the edges of the dish.
- Always have students dispose of Petri dishes in a biohazard bag.
- If you would like to develop this experiment into an open-ended inquiry, after going over introductory information, have students postulate potential purposes and hypotheses, then create a set of class questions related to possible results of a Kirby-Bauer test. Students could then create and carry out their own procedures. Examples of inquiry questions: 1. Is there a difference in antibiotic inhibition when one uses different strains of bacteria?; 2. Is there a difference in the zones of inhibition if different potencies of drugs are used?; 3. Is there a difference in the zones of inhibition if different agars are used?

6. Materials Price List/Ordering Information

Carolina 1-800-334-5551 www.carolina.com

Item	Ordering Number	Price
Bacterial Spreader	BA-21-5820	\$2.70 each
Inoculation Loop	BA-21-5826	\$1.80 each
<i>Staphylococcus epidermidis</i>	BA-15-5556	\$7.50/tube (agar)
<i>Escherichia coli</i>	BA-15-5065	\$7.50/tube (agar)
TSA plates	BA-82-2022	\$15.25/pack of 10

(100 x 15 mm)		\$13.25/10+ packs
TSA media tubes	BA-82-7322	\$13.75/pack of 10 \$12.60/10+ packs
TSA Dehydrated media	BA-78-8420	\$17.95/100 g
Biohazard Bags 12 x 24 in.	BA-64-7051	\$36/100 bags
Parafilm 2 in. x 250 ft.	BA-71-3044	\$17.95 each
Sterile Cotton Tip applicators	BA-70-3033	\$19/box of 200

Teacher Note: Isopropyl (rubbing) alcohol can be purchased at any grocery store; Q-tips (sterile in the box, or can be sterilized in a test tube, stoppered by nonabsorbent cotton) could be exchanged for the Cotton Tip applicators; glass pipettes or stirring rods can be melted into a hook shape by a Bunsen burner. 60 x 15 mm Standard petri dishes (500/case) can be purchased through Life Science Products (1-800-245-5774) for \$48.70.