CITIZEN SCIENCE
Bard College

Antibacterial Sensitivity: Antimicrobials and Antiseptics Commercial and Natural

An assay commonly used in clinical laboratories to test the potency of antibiotics and drugs is a filter paper disk-agar diffusion procedure commonly known as the Kirby-Bauer test. A filter paper disk-agar diffusion method is also used for determining the potency of antiseptics. In this exercise, you will have an opportunity to determine both antiseptic and antibiotic potency with a modified Kirby-Bauer test.

Discs of filter paper, impregnated with antibiotic solutions in the same range of concentrations achieved in the human body are placed on an agar plate heavily seeded with the test bacteria. When incubated, the bacteria grow in a smooth lawn of confluent growth except in a clear zone around the antibiotic disc. The clear area is called the zone of inhibition. This inhibition zone does not necessarily indicate the degree of microbial susceptibility to the antibiotic, for zone size itself does not indicate if the antibiotic is appropriate for the use in clinical treatment. Some antibiotics are composed of smaller molecules and diffuse faster, producing a larger zone. This test can be performed with different sets of conditions to answer different questions. For our experiment overnight incubation will allow to determine general sensitivity to the antibiotics tested.

Objectives

1. To evaluate the inhibitory activity of antibiotics, antiseptics, and disinfectants on bacteria using a modified Kirby-Bauer test.

2. To test the inhibitory effects of natural items believed to have antibiotic or antiseptic properties.

3. To observe the effects of these agents on known strains of bacteria, as well as on one of your environmental isolates.

Materials
500 µl *E. coli B*
500 µl *E. coli B* with GFP
250 µl LB media in microcentrifuge tube
6 LB plates
Sterile glass beads
Sterile wooden sticks
pipettors with sterile tips
ruler
marker

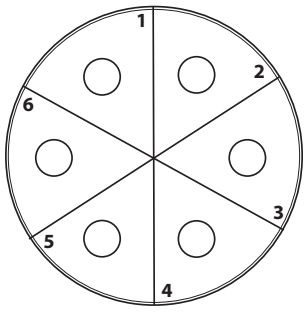
Protocol

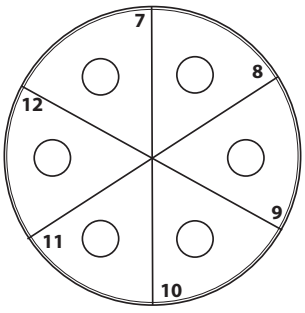
IT IS IMPORTANT THAT YOU DO NOT TOUCH OR CROSS CONTAMINATE THE MATERIALS

1. With a marker, divide the underside of six agar plates into six equal segments and label 3 plates 1 through 6., and 3 plates 7-12. On the periphery of the plate (in small lettering), indicate the bacteria name, amount used and your initials (i.e. E. Coli, 100 µL, initials, GFP, 100 µL, initials or Isolate, 100 µL, initials)

2. Use the following code for the antibiotics and antiseptics:

1. Kanamycin	7. Honey (50%)
2. Ampicillin	8. Lime Juice
3. Gentamicin	9. Cinnamon Oil
4. Hand Sanitizer	10. Ginger Oil
5. Ethanol	11. Thyme Oil
6. Water	12. Peppermint Oil





3. Pipette 100 µL of the *E.coli* culture into the middle of the 2 agar plates labeled *E.coli* 1-6 and *E.coli* 7-12.

4. Shake about 5-10 glass beads onto the plate and replace the lid of the Petri dish.

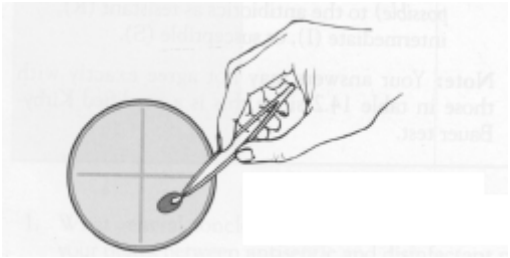
5. Using a gentle random motion, as evenly as possible, let the beads spread the culture across the agar. Avoid a circular motion that will concentrate the bacteria on the outer edges. When sufficient, carefully dump the beads into the used bead container.
   DO NOT REUSE THE BEADS.

6. Repeat steps 3-5 with the *E. coli* with GFP bacteria culture using the 2 plates labeled for *E. coli* with GFP

7. Using a sterile stick, take a colony from your environmental isolate plate and mix with 250 µL of LB broth in a microcentrifuge tube. Pipette 100 µL of the culture into the middle of the agar on each of your plates labeled isolate.

8. Repeat steps 4 and 5 for each of your isolate plates.

9. Once you have prepared all the necessary plates with bacterial cultures, proceed to Commercial Antibiotic Station 1. Using the sterile forceps provided, immerse one of the filter paper discs into the antibiotic solution of Kanamycin.



10. Drain the disc thoroughly on a piece of clean towel or filter paper and place it in the center of section 1 of the one of the three bacterial dishes labeled 1-6. Be careful not to touch the forceps to the agar of the plate.

11. Repeat steps 10, using the same antibiotic/antiseptic for your other two commercial antibiotic plates labeled 1-6.

12. Repeat steps 10-11 for the remaining 5 commercial antibiotic stations.

13. Proceed to Natural Antibiotic Station 2. Using the sterile forceps provided, immerse one of the filter paper discs into the solution of Honey.

14. Drain the disc thoroughly on a piece of clean towel or filter paper and place it in the center of section 7 of the one of the three bacterial dishes labeled 7-12. Be careful not to touch the forceps to the agar of the plate.

15. Repeat step 14, using the same antibiotic/antiseptic for your other two natural antibiotic plates labeled 7-12.

16. Repeat steps 13-14 for the remaining 5 natural antibiotic stations.

17. Invert the dishes and incubate at 37 °C!

18. We will observe and discuss the zones of inhibition next lab period.

REMEMBER TO WASH YOUR HANDS BEFORE LEAVING THE LAB!

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Kirby Bauer Analysis

Today we will analyze the results of our Kirby-Bauer test of E. coli, GFP E. coli and isolate. We have used discs of filter paper, impregnated with antibiotic solutions in the same range of concentrations effective in the human body and placed them on an agar plate heavily seeded with each test bacteria. When incubated overnight, the bacteria grew in a smooth lawn of confluent growth except in a clear zone around the antibiotic disc. Remember, size of the zone of inhibition does not necessarily indicate the degree of microbial susceptibility to the antibiotic, and zone size itself does not indicate if the antibiotic is appropriate for the use in clinical treatment.

Procedure - Day 2

19. Turn over the E. coli commercial antibiotic plate, and with a ruler (calibrated in mm) determine the diameter of the clear zone surrounding each disc.

20. Repeat this process with the GFP E. coli commercial antibiotic plate. You may need to look at the GFP plate under UV light to clearly observe the clear zones.

21. Repeat this process with the environmental isolate commercial antibiotic plate.

22. Repeat steps 19-21 on the 3 natural antibiotic plates.

23. Note all of your observations (i.e. zone of inhibition in mm; shape of the zone; any inconsistencies around the zone) in the chart provided.

24. Finally, draw some conclusions about what you are observing for each antibiotic and antiseptic. Are they inhibitory to bacterial growth? Which seems to be most effective?

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