

## Antimicrobial Susceptibility Testing

### The Disk Diffusion Method

Disks containing a known concentration of an antimicrobial agent such as ampicillin are placed on a plate inoculated with a microorganism. The closer the microorganism grows to the disk, the more resistant it is to the antimicrobial.

### Materials

- *Escherichia coli*, Microbiologics catalog # 0335
- 0.85% saline
- Ampicillin Disks (AM-10)
- Mueller Hinton Agar
- 0.5 McFarland
- Sterile swabs
- Vortex mixer
- A forceps or tweezer for handling disks
- Loop
- Incinerator
- Gloves
- Lab coat
- Marker for labelling plates
- Disinfectant
- Biohazard bags
- Sharps container

### Procedure

1. Grow *E. coli* overnight on an agar plate.
2. Subculture *E. coli* onto a new agar plate.
3. Make a light suspension of the *E. coli* colonies from the subculture in a saline tube.
4. Vortex.
5. Compare the suspension to a 0.5 McFarland Standard. The amount of cloudiness in both tubes should be the same. Add more colonies if necessary and vortex again. When the suspensions look similar in density, the number of bacteria in the saline tube will be about  $1$  to  $4 \times 10^8$  CFU per ml.
6. Dip a swab into the suspension.
7. Rotate the swab against the side of the tube to remove excess fluid.
8. Create a lawn of bacteria on a Mueller Hinton Agar plate by swabbing the suspension in three directions (vertical, horizontal, and diagonal).
9. Allow the plate to dry for about three minutes.
10. Place an antibiotic disk on one Mueller Hinton plate.
11. Incubate plate at  $35^{\circ}\text{C}$  approximately 16 to 20 hours.
12. Look for a zone of no growth around the ampicillin disks.

**Test Variation 1:** This test can be performed on several different species of bacteria at the same time. Compare how each strain reacts to ampicillin by using a ruler to measure the size of the zone around the disk.

**Test Variation 2:** Another variation on the test is to compare how *E. coli* reacts to several different types of antibiotic disks. The disks should not be placed too close to each other.

**Test Variation 3.** A third variation on the test is to dip blank disks in a personal care product such as mouthwash.

## Notes

- Products such as Mueller Hinton Agar, a 0.5 McFarland tube, antibiotics, and blank disks can be purchased from companies like Hardy Diagnostics and BD (Becton Dickinson).
- Detailed information about this test is available online at no cost. See the EUCAST Manual and slide show about *Disk Diffusion Test Methodology*:  
[http://www.eucast.org/ast\\_of\\_bacteria/disk\\_diffusion\\_methodology/](http://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/)



Source: Centers of Disease Control and Prevention –  
*Disk Diffusion Test (also called a Kirby-Bauer Test)*