

Testing an Antibiotic Using a Disk Diffusion Assay

Introduction:

At Gold Biotechnology, we make sure each of our products is thoroughly tested to the highest standards. In addition to a typical certificate of analysis (COA), we also test our antibiotics through a modified version of the Kirby-Bauer method, also known as the disk diffusion method. The test compares the antibiotic's ability to prevent bacterial growth to a common set of standards created by the CLSI (Clinical Laboratory Standards Institute), in order to assure its functionality at a certain concentration. As a control, we use a strain sensitive to the antibiotic to determine its effectiveness, and a resistant strain to assure it's the correct substance. Each lot is tested with the following procedure.

Materials needed:

Antibiotic to be tested

Control Antibiotic (either product that has been previously tested, or commercially available disk)

1 overnight culture of resistant strain

1 overnight culture of susceptible strain

6mm Whatman filter paper disks

Sterile petri dishes

2 Agar plates (with no antibiotic)

Tweezers

Ethanol

Procedure:

1. The day before you want to test, pick isolated colonies of your susceptible and resistant strains and grow overnight or to stationary phase.
2. The day of the test, dilute your cultures back and grow to mid-log phase, around an OD of 0.5
3. While your cultures are growing out, make a stock solution of the antibiotic to be tested, and a control antibiotic if available.
 - a. Stock solution can be made at a standard concentration, e.g. 100mg/mL for Ampicillin.
 - b. Filter sterilize the stock solution (recommended for all aqueous solutions).
 - c. Label the solution with the antibiotic name, concentration, manufacturer/supplier, date created, and lot number.
4. Determine the amount of antibiotic to place on each disk, and make a 1mL aliquot with that concentration.
 - a. Example- for Ampicillin testing, each disk contains 10µg of Ampicillin. We want to load 20µL of solution on each disk, so we need a solution with a final concentration of 0.5mg/mL (=0.5µg/µL).
 - b. Make 1mL solutions for both the antibiotic to be tested, and the antibiotic control.

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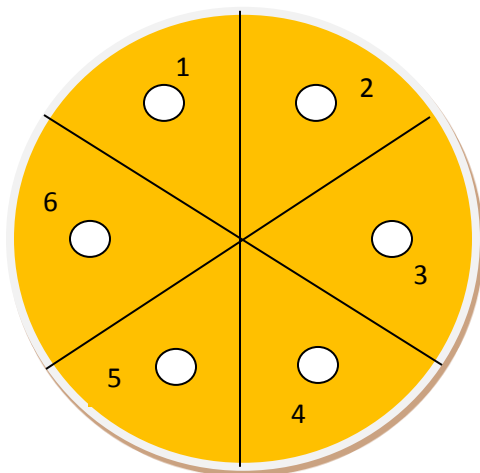
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5. In two petri dishes, add 8 pieces of filter paper to one and 4 to the other.
 - a. Label the dish with 8 pieces with the lot number of the antibiotic to be tested.
 - b. Label the dish with 4 pieces with the lot number of the control antibiotic.
6. Using a pipet, add 20 μ L of the solution to be tested to each of the 8 filter paper disks. Also add 20 μ L of the previously tested solution to the 4 filter paper disks.
 - a. Let these dry covered on the bench at room temp for 3 hours, or uncovered in a laminar flow hood for around 30min.
 - b. Make sure the disks are fully dried before placing on the plates.
7. While the disks are drying, label each agar plate with the corresponding culture and divide into 6 sections. Label each Section 1-6.
8. Once your cultures are ready, spread 150 μ L evenly throughout the plate. Allow any extra liquid to dry on the plate.
9. Once the disks are dry, flame-sterilize a pair of tweezers. Then place one blank disk in section 1 of each plate. Gently place the disk on top of the agar and lightly press it down with the tweezers.
10. Flaming the tweezers between disks, place the disk's containing the antibiotic in sections 2, 3, 5, and 6. In section 4, place the disk containing the control antibiotic. Your plates should look like this at the end of this procedure.



Take care not to move the disk around too much, as this could skew the bacterial growth in the surrounding area.

11. Incubate the plates inverted overnight or until cells have grown out completely.
12. The next day, measure the area of inhibited bacterial growth with a ruler. The antibiotic being tested should match or slightly exceed the control for the susceptible strain, and be roughly equal to the control for the resistant strain. If using standard strains, compare zones to numbers published by the CLSI.