Protocol

GoldBio Disk Diffusion Assay Protocol

Introduction

At GoldBio, we ensure that 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 the disk diffusion method, a modified version of the Kirby-Bauer 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

- Antibiotic to be tested
- Control Antibiotic (either previously tested product or commercially available disk)
- 1 overnight culture of resistant strain
- 1 overnight culture of susceptible strain
- 6 mm Whatman filter paper disks
- Sterile petri dishes
- 2 Agar plates (with no antibiotic)
- Tweezers
- Ethanol

Method

1. The day prior to your test, pick isolated colonies of your susceptible and resistant strains and grow overnight (16-18 hours) or to stationary phase (generally ~6 hours of growth) in 5 ml broth medium at 35-37°C.

2. The day of the test, dilute your cultures back and grow to mid-log phase (log phase is bacteria's most productive phase, generally ~1-4 hours of growth). Standardize the inoculum size by measuring the absorbance at 600 nm. At the time of the test, absorbance should be 0.5 at 600 nm. If necessary, adjust turbidity with broth.

   Note: Absorbance of 0.5 at 600 nm follows the McFarland standard, which indicates the number of bacteria within a given range to standardize microbial testing.

3. While your cultures grow, make a stock solution of the antibiotic to be tested

and the control antibiotic, if available.

a. Stock solution can be made at a standard concentration, e.g. 100 mg/ml for ampicillin.
b. Filter and sterilize the stock solution (this is recommended for all aqueous solutions).
c. Label the solution with the antibiotic name, concentration, manufacturer/supplier, date created and the lot number.

4. Determine the amount of antibiotic (dependent on the antibiotic) to place on each disk, and make a 1 ml aliquot with that concentration. Make 1 ml solutions for both the antibiotic to be tested and the antibiotic control. For example, in the case of ampicillin, each disk would need to contain 10 µg of Ampicillin. For example, to load 20 µl of solution on each disk, the solution should have a final concentration of 0.5 mg/ml (0.5 µg/µl).

5. In two petri dishes, add 8 pieces of filter paper to one dish and 4 to the other.
   a. Label the dish with 8 pieces of filter paper with the lot number of the antibiotic to be tested.
   b. Label the dish with 4 pieces of filter paper with the lot number of the control antibiotic.

6. Add 20 µl of the antibiotic solution to be tested to each of the 8 filter paper disks. Also add 20 µl of the previously tested, control antibiotic solution to the 4 filter paper disks on the control plate.
   a. Dry the plates, covered on the bench at room temperature for 3 hours, or uncovered in a laminar flow hood for around 30 minutes.
   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 µl of the culture evenly throughout the plate. Allow any extra liquid present to dry.

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 disks 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 Figure 1 at the end of the procedure.

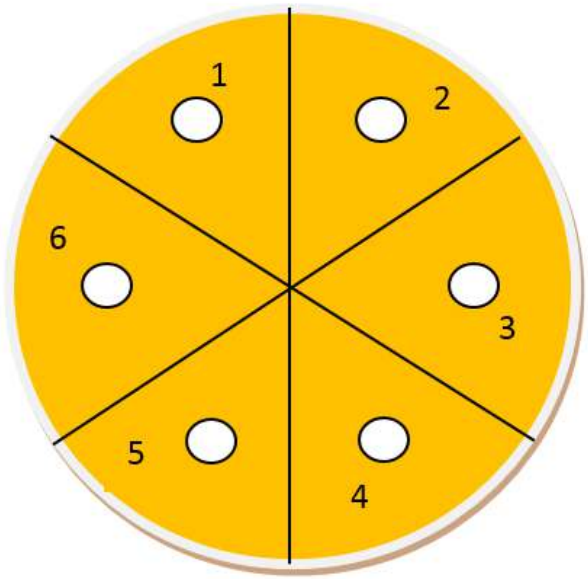

Figure 1. Take care not to move the disk around too much, as this could skew the bacterial growth in the surrounding area.

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 roughly equal to the control for the resistant strain. If using standard strains, compare zones to numbers published by the CLSI.

Tips

- Prepared discs should be stored in sealed containers with dessicant below 8°C.
- Plates should be incubated immediately after application of the discs.
- If colonies grow within the inhibition zone, they should be cultured and identified.

Associated Products

- Antibiotics

References

Ericsson, B. H., Tunevall, G., and Wickman, K. (1960). The Paper Disc Method for Determination of Bacterial Sensitivity to Antibiotics: Relationship Between the Diameter of the Zone of Inhibition and the Minimum Inhibitory Concentration. *Scandinavian Journal of Clinical and Laboratory Investigation, 12(*4), 414-422. Doi:10.3109/00365516009065406.

American Society for Microbiology (ASM). (n.d.). Kirby Bauer Diffusion Susceptibility Test. Retrieved April 18, 2018, from https://www.asm.org/index.php/kirby-bauer.

Swenson, J. M. and Thornsberry, C. (1984). Preparing inoculum for susceptibility testing of anaerobes. *Journal of Clinical Microbiology*, *19*(3), 321-325.