

Antimicrobial Susceptibility Testingⁱ

Introduction

It is not enough to just identify your organism. You also need to know what antimicrobial agents your organism is susceptible to. There are several methods to determine this.

Dilution testing is used to quantitatively determine the minimal concentration (in mg/ml) of antimicrobial agent to inhibit or kill the bacteria. This is done by adding two-fold dilutions of the antimicrobial agent directly to an agar pour, a broth tube, or a micro-broth panel. The lowest level that inhibits the visible growth of the organism is considered the Minimum Inhibitory Concentration (MIC). The agar pour method is considered the reference test procedure in Europe. The broth dilution method is more widely accepted in North America. The E test (AB Biodisk) is a plastic strip with a gradient concentration of antimicrobial agents impregnated in it. The strip is placed directly on the surface of an inoculated plate. The MIC is read from the strip where the growth inhibition intercepts the disk. These strips are relatively expensive.

Many physicians however do not need to know the exact MIC, but just which antibiotics the pathogen is susceptible, intermediate, or resistant to. The Kirby-Bauer agar diffusion method is well documented and is the standardized method for determining antimicrobial susceptibility. White filter paper disks (6 mm in diameter) are impregnated with known amounts of antimicrobial agents. Each disk is coded with the name and concentration of the agent. For example, 10µg of Ampicillin is indicated on the disk by AM-10. The code is listed on the Disk Zone Diffusion Diameter Chart. The impregnated disks are placed on an inoculated Mueller Hinton Agar (MHA) plate. The drug diffuses through the agar. The plates are incubated for 16-24 hours. The agar may be supplemented with blood or you may use blood agar for fastidious organisms. The diameter of the visible zone of inhibition is measured and compared to reference values. There should be sufficient bacteria to form a visible lawn of growth where it is not inhibited by the drug. The results are interpreted qualitatively as resistant, intermediate, or susceptible. The standard protocol must be followed exactly for you, or any clinical lab, to interpret the results reliably.

There may be some inhibition of growth and the organism could still be considered resistant to that antimicrobial agent if the zone diameter is smaller than the reference values listed on the chart. Also note that different antimicrobial agents have different measurements for resistant, intermediate, and susceptible. A zone of inhibition may be considered susceptible for one antimicrobial agent and not for another. For example, in order for ampicillin (AM-10) to be an effective antimicrobial agent, the zone of inhibition for enterics and most streps must be greater than 16mm while for staphs it must be greater than 28mm.

When determining which antimicrobial agents are best for treatment when multiple zones of inhibition are present, be sure to look at the relative zone of inhibition for that particular antimicrobial agent and compare your measurements to that. For example, let's say that your enteric organism has a zone of

inhibition around the Polymixin B disk of 20mm and a zone of inhibition around the Tetracycline disk of 20mm. Because these measurements are larger than the susceptibility zones listed on the Disk Zone Diffusion Diameter Chart, both of these antibiotics would be considered as possibilities for treatment. However, when we look more closely we see that a 20mm zone of inhibition for Tetracycline is only 1mm larger than what is required to be susceptible while a 20mm zone of inhibition for Polymixin B is 8mm larger than the minimum susceptibility measurement needed. In this particular case then, Polymixin B and Tetracycline would both be adequate for treatment, but the Polymixin B would be the best choice.



Pseudomonas aeruginosa on MHA incubated at 37°C for 24 hours.



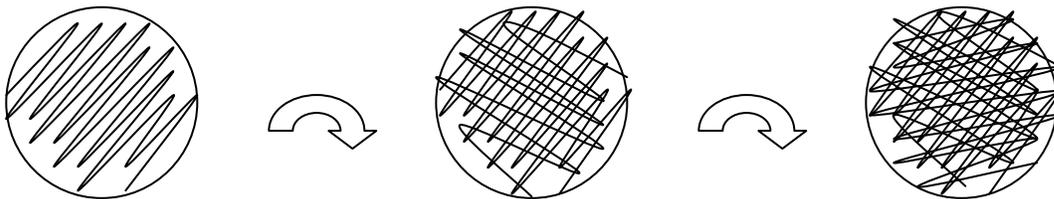
E. coli on MHA incubated at 37°C for 24 hours.

Materials

- 1 actively growing broth or streaked plate of a single organism (pure culture)
- Gram stain materials (optional)
- 1 Mueller Hinton Agar (MHA) plate (use BHI plate for all streps)
- 1 jar sterile saline
- 1 sterile test tube
- 1 sterile 5mL pipette (with pipette bulb)
- 1 sterile swab
- 0.5 McFarland test standard
- McFarland reference card
- Spectrophotometer (optional)
- 8 disk dispenser or individual disk dispensers
- Antibiotic disk cartridges
 - Ampicillin (AM-10)
 - Chloramphenicol (C-30)
 - Nalidixic Acid (NA-30)
 - Penicillin G (P-10)
 - Polymixin B (PB-300)
 - Streptomycin (S-10)
 - Tetracycline (Te-30)
 - Trimethoprim (TMP-5)

Procedureⁱⁱ:

1. Perform a Gram Stain to confirm culture purity from your subculture plate.
2. Using a sterile 5mL pipette, add 5mL of sterile saline to a sterile test tube.
 - Alternatively, a tube of sterile water or a tube of sterile tryptic soy broth (TSB) can be used.
3. Using an inoculating loop or needle, select several colonies from your subculture plate and transfer to a tube of sterile saline.
 - Select several colonies so you don't inadvertently pick a non-representative colony.
4. Dilute your organism to obtain a turbidity equivalent to the 0.5 McFarland test standard.
 - Hold your diluted tube and the 0.5 McFarland test standard against the black-lined McFarland reference card to accurately rate the turbidity.
 - This could also be measured in a spectrophotometer (87% transmittance at 686nm).
5. Within 15 minutes of diluting your organism, dip a sterile swab into the properly adjusted inoculum. Lift it slightly out of the suspension and firmly rotate the swab several times against the upper inside wall of the tube to express excess fluid.
 - If your swab is too wet your agar surface will not dry correctly and the antimicrobial agents in the disk will diffuse through the wet surface and not into the agar.
6. Streak the entire surface three times with the swab, turning the plate 60 degrees between streakings (turn the swab too) to obtain an even inoculation.



7. Close the lid and let sit for 3-5 minutes before applying the drug impregnated disks.
8. Apply the disks by means of a dispenser using aseptic technique. Deposit disks so that the centers are at least 24 mm apart; up to 12 disks may be placed on a 150mm plate, 5 disks on a 100 mm plate.
 - We usually apply more than the recommended disk number to conserve plates and our recommendations are not used to treat any patients!
9. Lightly press the disk down with a sterile swab to make contact with the surface.
 - **You don't want to smush it into the agar itself!**
10. Place your plate agar side up (inverted) in a 37°C incubator.
 - Streptococcus organisms should be on BHI instead of MHA plates.
 - Streptococcus organisms should be incubated in an atmosphere enriched with 5-10% CO₂.
11. Examine the plate after 16-24 hours incubation.
12. Measure (in mm) only zones showing complete inhibition by gross visual inspection.
 - Hold the measuring device (ruler or calipers) over the back of the inverted plate over a black non-reflective surface and illuminate from above.
13. Compare the values you obtained with those on the Disk Diffusion Zone Diameter Chart to determine the susceptibility level to the antibiotics used.

14. Report the values as:

- **Resistant** - indicates that clinical efficacy has not been reliable in treatment studies.
- **Intermediate** - implies clinical applicability in body sites where the drug is physiologically concentrated or when a high dosage of the drug can be used.
- **Susceptible** - implies that an infection due to the organism may be treated with the concentration of antimicrobial agent used, unless otherwise contraindicated.

Disk Diffusion Zone Diameter Chart:

Antibiotic	Code	Disk Potency	Zone Diameter (mm)		
			Resistant	Intermediate	Susceptible
Ampicillin					
Enterics	AM-10	10 µg	≤13	14-16	≥17
Staphs			≤28	-	≥29
β-Hemolytic Streps			≤18	19-25	≥26
Other Streps			≤16	-	≥17
Chloramphenicol					
Streps	C-30	30 µg	≤17	18-20	≥21
Enterics & Staphs			≤12	13-17	≥18
Nalidixic Acid					
Staphs, Streps, & Enterics	NA-30	30 µg	≤10	11-12	≥13
Penicillin G					
Staphs	P-10	10 µg	≤28	-	≥29
β-Hemolytic Streps			≤19	20-27	≥28
Other Streps			≤11	12-21	≥22
Enterics			≤11	12-21	≥22
Polymixin B					
Staphs, Streps, & Enterics	PB-300	300 U	≤8	9-11	≥12
Streptomycin					
Staphs, Streps, & Enterics	S-10	10 µg	≤11	12-14	≥15
Tetracycline					
Streps	Te-30	30 µg	≤18	19-22	≥23
Enterics & Staphs			≤14	15-18	≥19
Trimethoprim					
	TMP-5	5 µg	≤10	11-15	≥16

ⁱ The information presented in this lab is from The Manual of Clinical Microbiology, 8th Ed. The procedures are paraphrased from the National Committee for Clinical Laboratory Standards (NCCLS) 2000. Approved Standard. M2-A7.

ⁱⁱ Procedures were taken from HardyDisk® Antimicrobial Sensitivity Test (AST) Disks, 2001.