

Antibiotic Susceptibility Testing (ABST/AST)

Goal

Offer guidance to physicians in selecting effective antibacterial therapy for a pathogen in a specific body site.

Performed on bacteria isolated from clinical specimens if the bacteria's susceptibility to particular antimicrobial agents is uncertain.

Susceptibilities NOT performed on bacteria that are predictably susceptible to antimicrobials.

Ex. *Streptococcus pyogenes*

Factors to Consider When Determining Whether Testing is Warranted

Body site of infection

- Susceptibility not performed on bacteria isolated from body site where they are normal flora.
- Ex. Susceptibility for *E. coli* is NOT performed when isolated from stool (normal commensal), but is performed when isolated from blood (not normally present at this site)

Presence of other bacteria and quality of specimen

- Ex. Two or more organisms grown in a urine specimen.

Host status

- Immunocompromised patients
- Allergies to usual antimicrobials

Selecting Antimicrobial Agents for Testing and Reporting

Clinical & Laboratory Standards Institute (CLSI): provides

- Standards, methods, QC parameters, and interpretive criteria for sensitivity testing

- If necessary, can alter the breakpoints of the SIR (susceptible, intermediate, resistant) based on emerging resistance
- There are approximately 50 antibacterial agents
- Following CLSI recommendations each laboratory should have a battery of antibiotics ordinarily used for testing
- Drug formulary decided by medical staff, pharmacists, and medical technologists

Selection of Test Batteries

- Generally, labs choose 10-15 antibiotics to test susceptibility for Gram positive organisms and another 10-15 for Gram negative organisms
- Too many choices can confuse physicians and be too expensive
- Primary objective
 - Use the least toxic, most cost-effective, and most clinically appropriate agents
 - Refrain from more costly, broader-spectrum agents

Example of Drug Formulary

Drug	Enterococcus	Staphylococcus spp.
Ampicillin	X	
Cefazolin		X
Clindamycin		
Erythromycin		X
Linezolid	X	X

Oxacillin		X
Penicillin G	X	X
Rifampin		X
Streptomycin-2000	X	
Tetracycline	X	X
Trimeth/ Sulfa		X
Vancomycin	X	X

Example of Drug Formulary

Drug	Enterobacteriaceae	Pseudomonas aeruginosa
Ampicillin	X	
Piperacillin/ Tazo.	X	X
Cefepime	X	X
Imipenem	X	X
Gentamycin	X	X
Tobramycin	X	X
Ciprofoxacin	X	X
Levofloxacin	X	X
Nitrofurantoin	X	

Trimethoprim/Sulfa	X	
--------------------	---	--

Methods of Performing AST

Disk diffusion method: conventional method

MIC (minimum inhibitory concentration method):

- Broth microdilution
- Broth macrodilution / Tube dilution
- Agar dilution method
- Gradient diffusion method (E-Test)

Disk Diffusion/ Kirby- Bauer

Procedure

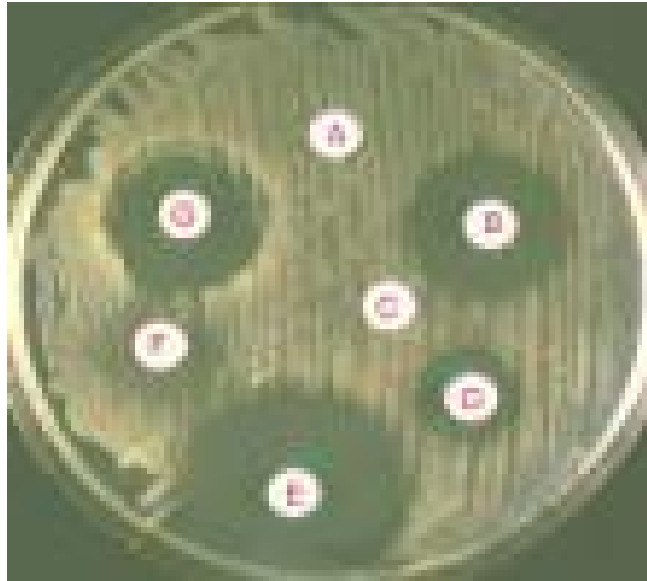
- Use a well-isolated, 18-24 hour old organism
- Transfer organism to a broth (Either tryptic soy/sterile saline)
- Ensure a turbidity of 0.5 McFarland (Standard provided by companies equivalent to 10^7 CFU of bacteria /ml of any sample)

Standardization of Antimicrobial Susceptibility Testing

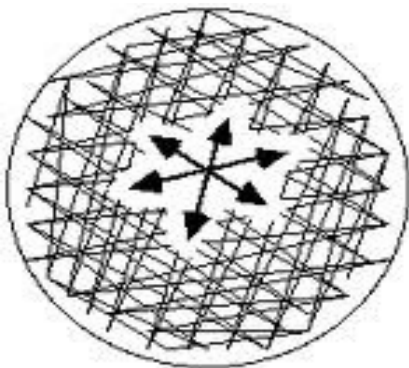
- Inoculum Preparation: Use 2-3 colonies NOT just 1 colony
- Inoculum Standardization: done by using 0.5 McFarland standard
- Turbidity of the bacterial suspension should be like that the black lines on the paper behind the test tube are just visible.

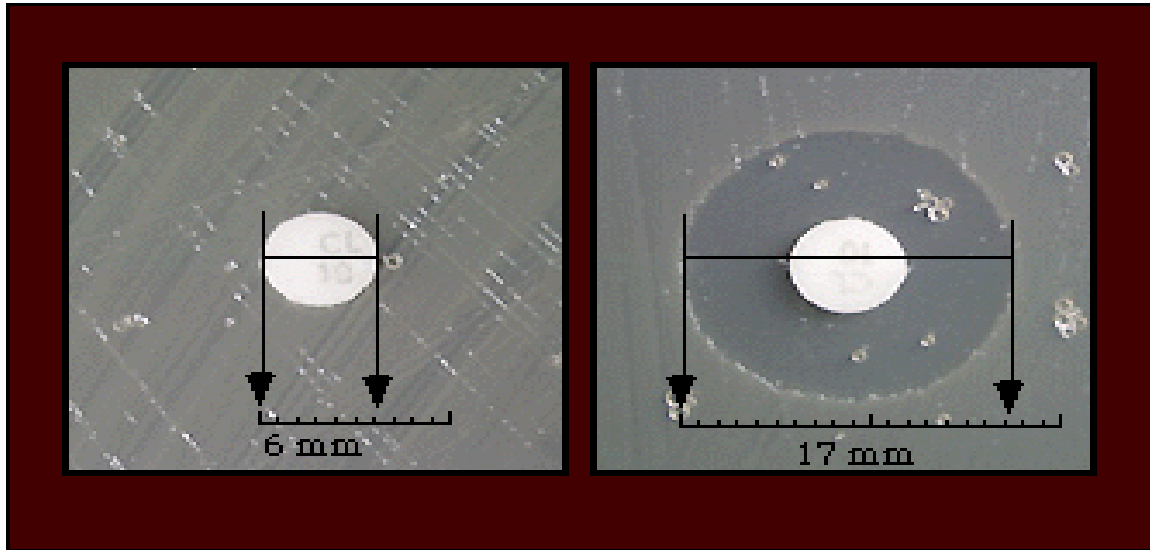


- Take a swab, dip it in 0.5 McFarland suspension of the organism and inoculate MH agar (Mueller Hinton agar) by swabbing in three different directions: “Lawn of growth”
- Place filter paper disks impregnated with antimicrobial agents on the agar (Abbreviation of the name of the drug and its quantity in the disc is mentioned on the antimicrobial disc)
- Invert and incubate the agar plate for 16-18 hours at 37 °C.



- During incubation, drug diffuses into agar
- Depending on the organism and drug, areas of no growth form a **zone of inhibition**
- Zones are measured to determine whether the organism is susceptible, intermediate, or resistant to the drug.





- Susceptible "S"
 - Interpretive category that indicates an organism is inhibited by the recommended dose, at the infection site, of an antimicrobial agent
- Intermediate "I"
 - Interpretive category that represents an organism that may require a higher dose of antibiotic for a longer period of time to be inhibited
- Resistant "R"
 - Interpretive category that indicates an organism is not inhibited by the recommended dose, at the infection site, of an antimicrobial agent.

Broth Dilution Method

Procedure

First of all we make 2 fold **dilutions of antibiotic in broth; Mueller-Hinton broth or Tryptic Soy Broth**

This is followed by inoculation of bacterial suspension (0.5 McFarland standard) and this is further incubated for 16-18 hours at 37 °C.

A tube of controls with no bacterial inoculum and no antibiotic is also put along with the test.

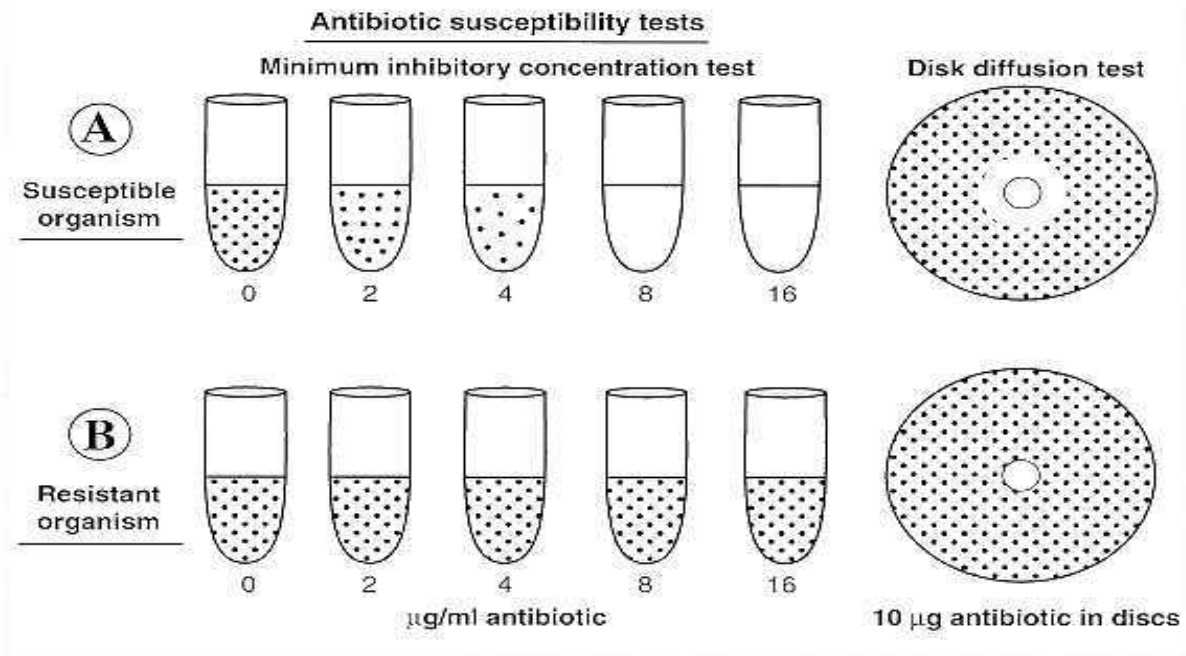
Next day we look for the turbidity produced by the growing bacteria.

The tube which is clear without any turbidity is referred as determinant of MIC (minimum inhibitory concentration).

All the tubes showing no turbidity are subcultured on solid agar culture plate (without any antibiotic).

The tube corresponding to the plate showing no growth of bacteria is considered as tube representing the MBC (minimum bactericidal concentration) of the drug.

Creating Dilutions



E- test/ Gradient Diffusion Method

- “MIC on a stick”
- Plastic strips impregnated with antimicrobial on one side
- MIC scale on the other side
- Read MIC where zone of inhibition intersects E strip scale



Automated Antimicrobial Susceptibility Test Methods (Modern approach)

- Detect growth in microvolumes of broth with various dilutions of antimicrobials
- Detection via photometric, turbidimetric, or fluorometric methods
- Types:

- BD Phoenix
- Microscan Walkaway
- TREK Sensititre
- Vitek 2 compact



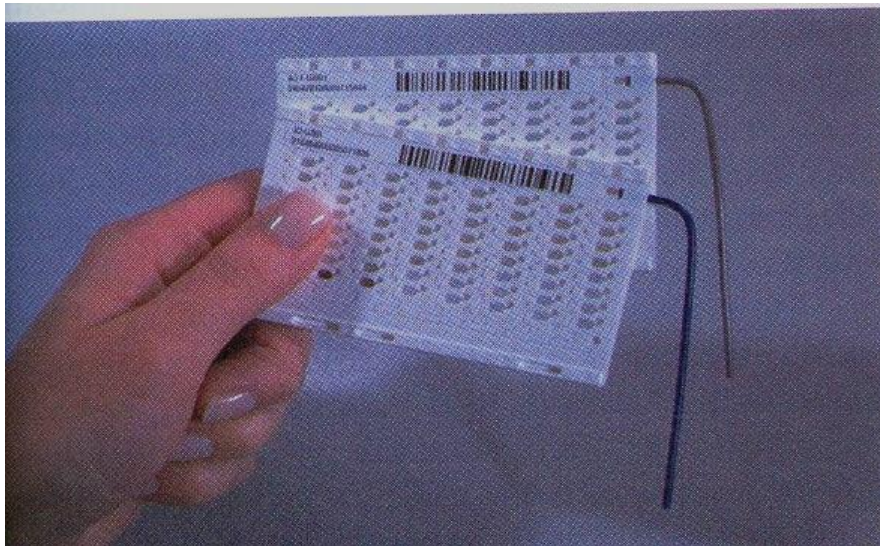
Automated Antimicrobial Susceptibility Test Methods

- Advantages
 - Increased reproducibility
 - Decreased labor costs
 - Rapid results
 - Software
 - Detects multi-drug resistances
 - ESBLs
 - Correlates bacterial ID with sensitivity
- Disadvantages

– Cost

Vitek 2 compact automated identification system uses two different plastic cards/cassettes, one for microbial identification and another for determination of antibiotic susceptibility.

VITEK 2 identification card consists of 64 wells, containing 41 fluorescent biochemical tests.



Reference:

1. Mackie and Mc Cartney; Practical Medical Microbiology 14th Edition; Chapter 8, page 151-178
2. Bailey and Scott's; Diagnostic Microbiology 12th Edition; Chapter 12; page 187-214
3. Ananthanarayan and Paniker's; Textbook of Microbiology 8th Edition; Chapter 66; page 618-620
4. Patrick R Murray; Manual of Clinical Microbiology 9th Edition; Chapter 17, page 245-257