ASD Govt Degree College For Women (A) Kakinada

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Drug Sensitivity

Definition and Measurement

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation.

MICs can be determined on plates of solid growth medium (called agar, shown in the "Kirby-Bauer Disk Susceptibility Test" atom) or broth dilution methods (in liquid growth media, shown in) after a pure culture is isolated.

For example, to identify the MIC via broth dilution, identical doses of bacteria are cultured in wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth. There are also several commercial methods available to experimentally measure MIC values.

Significance and Applications

An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents.

MIC scores are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

Clinicians use MIC to identify an effective dose of antibiotic.

1.Kirby-Bauer Disk Susceptibility Test

Kirby-Bauer testing measures sensitivity of bacteria to antibiotics by culturing bacteria on solid growth media .

Kirby-Bauer antibiotic testing **(also called KB testing or disk diffusion antibiotic sensitivity testing)** uses antibiotic-containing disks to test whether particular bacteria are susceptible to specific antibiotics.

First, a pure culture of bacteria is isolated from the patient. Then, a known quantity of bacteria are grown overnight on agar (solid growth media) plates in the presence of a thin disk that contains a known amount of a relevant antibiotic. If the bacteria are susceptible to the particular antibiotic present in the disk, an area of clear media observed, where bacteria are not able to grow surrounds the disk, which is known as the zone of inhibition.

A larger zone of inhibition around an antibiotic-containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk.

Kirby-Bauer test**: In Kirby–Bauer testing, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into the agar. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown**

KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic.

KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition.

If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic.

Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant.

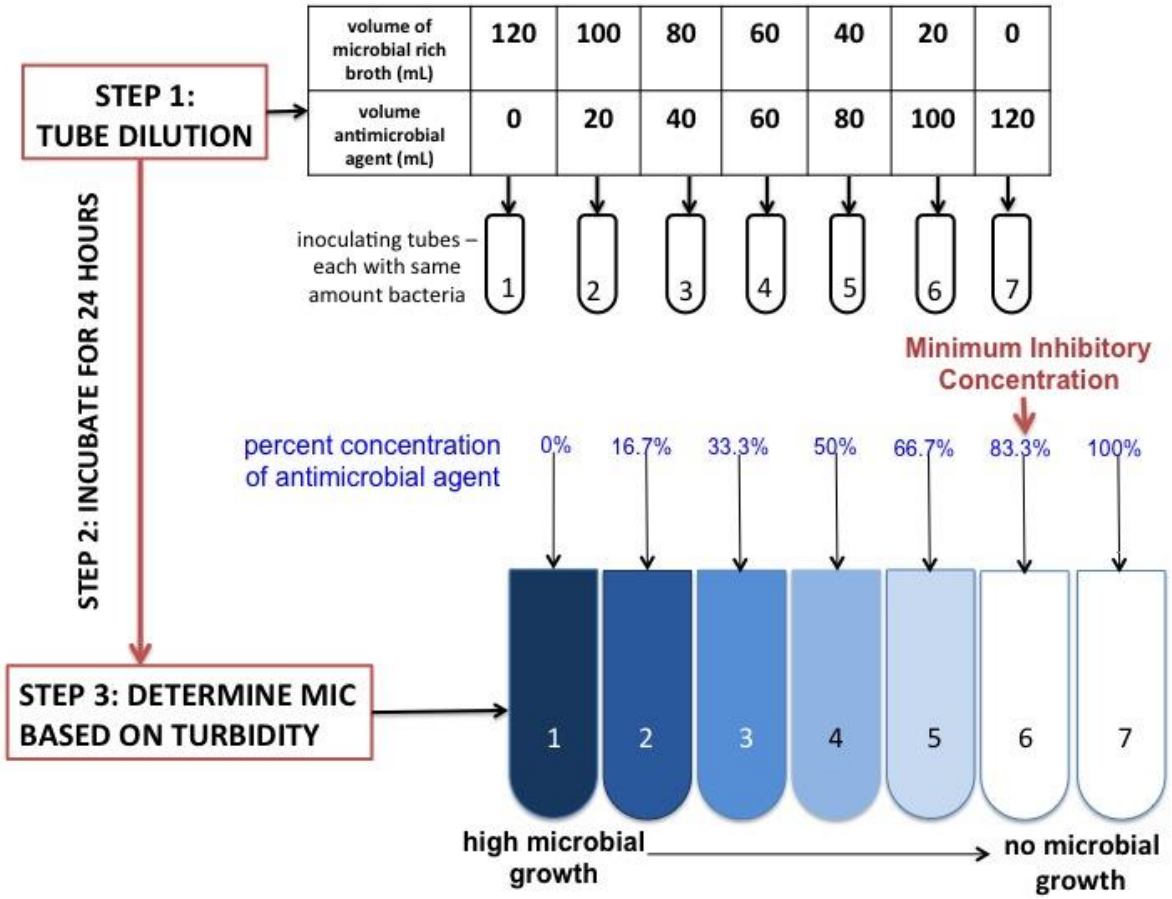
The size of a zone of inhibition in a KB test is inversely related to the minimum inhibitory concentration (MIC), which is the amount of antibiotic required to prevent bacterial growth in an overnight culture. The MIC (in μg/ml) can be calculated from known standard-curve (linear regression) graphs based on the diameter of the observed inhibition zone diameter (in millimeters).

Knowledge of the MIC will provide a physician valuable information for making a prescription

2. Broth Dilution Assay

Broth Dilution Assay. The MIC is determined by evaluation of turbidity of tubes with constantly increasing concentration of antimicrobial agent.
There are three main reagents necessary to run this assay: the media, an antimicrobial agent, and the microbe being tested.
The most commonly used media is Mueller Hinton Broth, due to its ability to support the growth of most pathogens and its lack of inhibitors towards common antibiotics. Depending on the pathogen and antibiotics being tested, the media can be changed and/or adjusted.

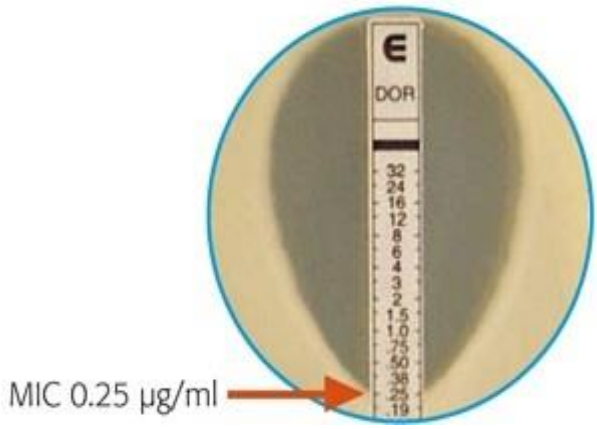
The antimicrobial concentration is adjusted into the correct concentration by mixing stock antimicrobial with media. The adjusted antimicrobial is serially diluted into multiple tubes (or wells) to obtain a gradient. The dilution rate can be adjusted depending on the breakpoint and the practitioner's needs.
The microbe, or the inoculating agent, must come from the same colony-forming unit, and must be at the correct concentration. This may be adjusted by incubation time and dilution. For verification, the positive control plate is maintained.
The microbes inoculate the tubes (or plate) and are incubated for 16–20 hours. The MIC is generally determined by turbidity.



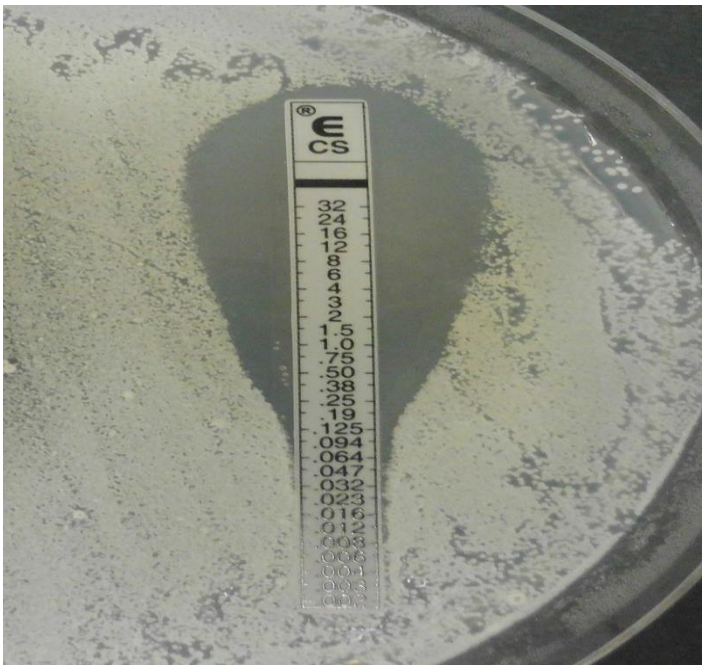
3. E- Test

Epsilometer test (E- test) is an 'exponential gradient' method of determination of antimicrobial resistance. The E-test has been developed to provide a direct quantification of antimicrobial susceptibility of microorganisms. This is a quantitative method that applies both the dilution of antibiotic and diffusion of antibiotic into the medium.

E-test is a laboratory test used to determine minimum inhibitory concentration (MIC) .The principle of E test was first described in 1988 and was introduced commercially in 1991 by AB BIODISK.



E-Test showing MIC



E test strip is a non-porous plastic strip immobilized with predefined continuous and stable gradient of 15 antibiotic concentrations on one side and printed with an MIC scale on the other side of nylon strips.

Principle of E-Test

E test is a quantitative technique that is based on combination of concept of both dilution and diffusion principle for susceptibility testing. E test strip is placed on to an inoculated agar plate; there is an immediate release of antibiotics from the plastic carrier surface into the agar surface. After incubation, bacterial growth becomes visible, symmetrical inhibition ellipse along the strip is seen. The MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip.

Purpose of E-Test

1. Determine the MIC of fastidious, slow-growing or nutritionally deficient micro-organisms, or for a specific type of patient or infection.
2. Detect low levels of resistance.

Procedure of E-Test

1. Remove the E-test package from the freezer (-20°C) and kept at room temperature at least 30 minutes before the test performed.
2. Emulsify several well-isolated test strain colonies from an overnight agar plate in saline.
3. Vortex for 15 second.
4. Adjust the suspension turbidity to 0.5 McFarland standards saline
5. Soak a sterile cotton swab into the inoculum suspension and remove the excess fluid by pressing it against the inside wall of the test tube.
6. Streak the swab over the entire agar surface in 3 directions by rotating the plate 60^{o} on Mueller Hinton Agar (MHA). (*NOTE: For Haemophilus species use Haemophilus Test Medium Agar (HTM) and for S. pneumonia and viridians streptococci use Mueller Hinton Blood Agar (MHBA*)).
7. Allow the plate to dry for 5-15 minutes so that the surface is completely dry before applying E test gradient strip.
8. Place the E test strip on agar plate with MIC scale facing upward and the concentration maximum nearest the rim of the plate.
9. Incubate the plate as follows:
    10.
    - *Haemophilus* species- CO2, 35ºC for 18 hours
    - *Streptococcus pneumoniae*– CO2, 35ºC for 20-24 hours
    - *Staphylococcus aureus* and *Enterococcus* species for Methicilllin and Vancomycin- O2, 35ºC for 24 hours
    - Others- O2, 35ºC for 24 hours.
11. After incubation observe zone of inhibition. (*NOTE: Four to six (maximum) E test strips can be placed on a 150 mm agar plate. For single MICs, one or two strips can be used on a 90 mm agar plate*).

Result interpretation of E-Test

1. Read the MIC value at the point where ellipse intersects the scale/E-test strip.

2. If the MIC value between the standard two-fold dilution is seen, always round up to the highest value.
3. Read the MIC value at complete inhibition of all growth including isolated colonies.

4. If the intersect differs on either side of the strip, read the MIC as the greater value. Ignore any growth at the edge of the strip.
5. Interpret E-test MIC results as Susceptible, Intermediate or Resistant.

Precaution of E-Test

1. Aseptic procedures and precautions against microbiological hazards should be used when handling bacterial specimens.
2. E-test should be used strictly according to the procedures described.

Limitation of E-Test

1. E-test is not suitable for *Cryptococcus neoformans*.