

# Antibiotic susceptibility test Kirby-Bauer disk diffusion method

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**Antibiotic susceptibility test Kirby-Bauer disk diffusion method** (also known as the disc diffusion method): is the most widely used antibiotic susceptibility test, in determining what choice of antibiotics should be used when treating an infection.

-This method relies on the inhibition of bacterial growth measured under standard conditions.

Let's start to know the procedure of sensitivity testing

## The first step

-To begin the procedure of sensitivity testing at first we select 2 to 3 pure colonies of one of the organisms to be tested using a sterile wire loop.

- And aseptically emulsify the colonies to a sterile enrichment growth media which is called **Mueller-Hinton growth media**.

-Mix it thoroughly to ensure that there is no solid material from the colony is visible in the saline solution.

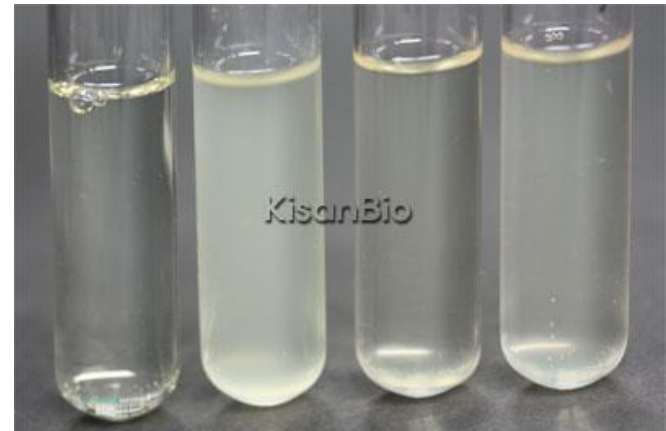
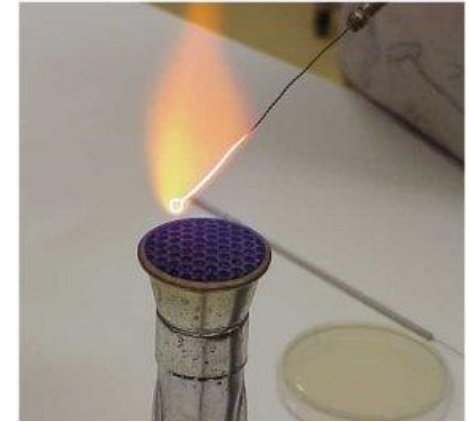
-Wait until the turbidity of the bacterial broth visually match that of 0.5 McFarland tube, when the turbidity of the bacterial growth visually matches that of 0.5 concentration McFarland tube we do the next step .

\*\* ( **the McFarland tube** is a standard turbidity tube with different concentrations used as a reference in order to adjust the turbidity of the liquid bacterial suspension)

**Isolated colonies**



**Avoid mixed colony**



MB-M1034 Mueller Hinton Broth  
Uncultivated  
/ Cultivated *E.coli* / *Staphylococcus aureus* / *Enterococcus*



## The second step

- In this step we take a sterile swab, dip it into the broth culture of the organism and spread the tested organism all over a Mueller-Hinton agar media several times for an even growth using the swab.

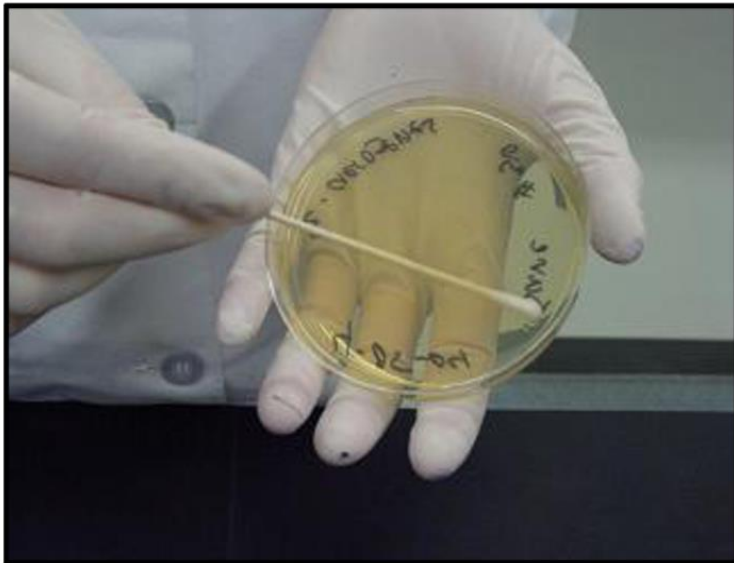
-We place antibiotic discs using a sterilised forceps on the agar plate where the bacteria have been swabbed

\*\* (**The antibiotic discs** are a small filter paper discs saturated with a standard amount of antibiotic)

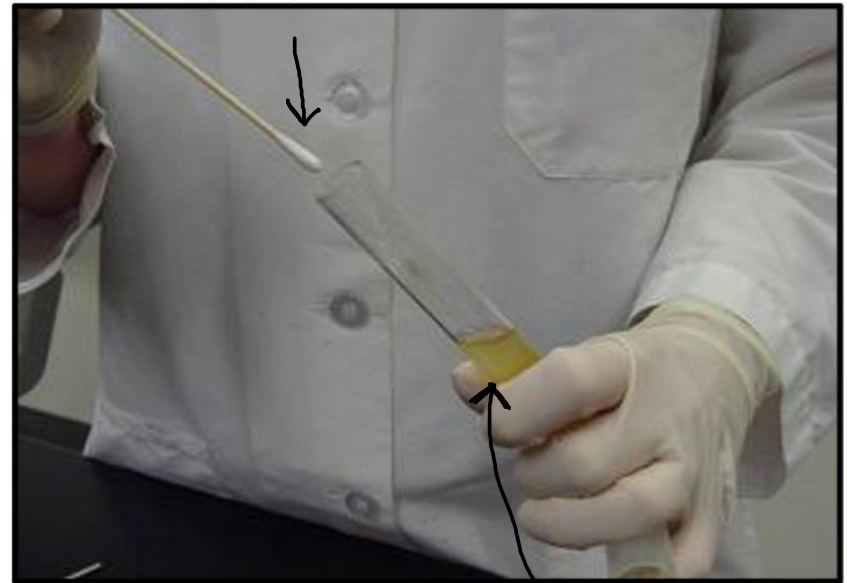
-Then we carefully invert the inoculated plates and incubate them for 24 hours at 37 centigrade ( celsius) .

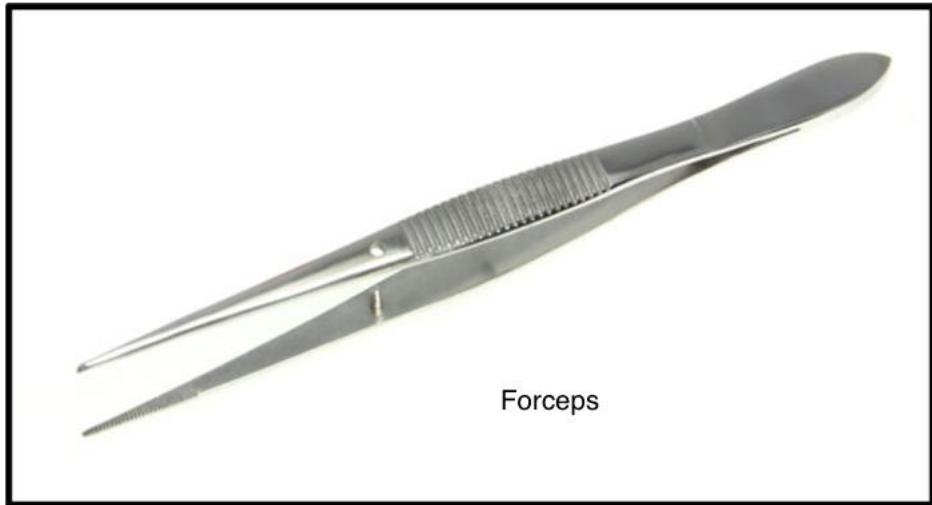


Sterile swab



Swap the plate in  
side to side motion

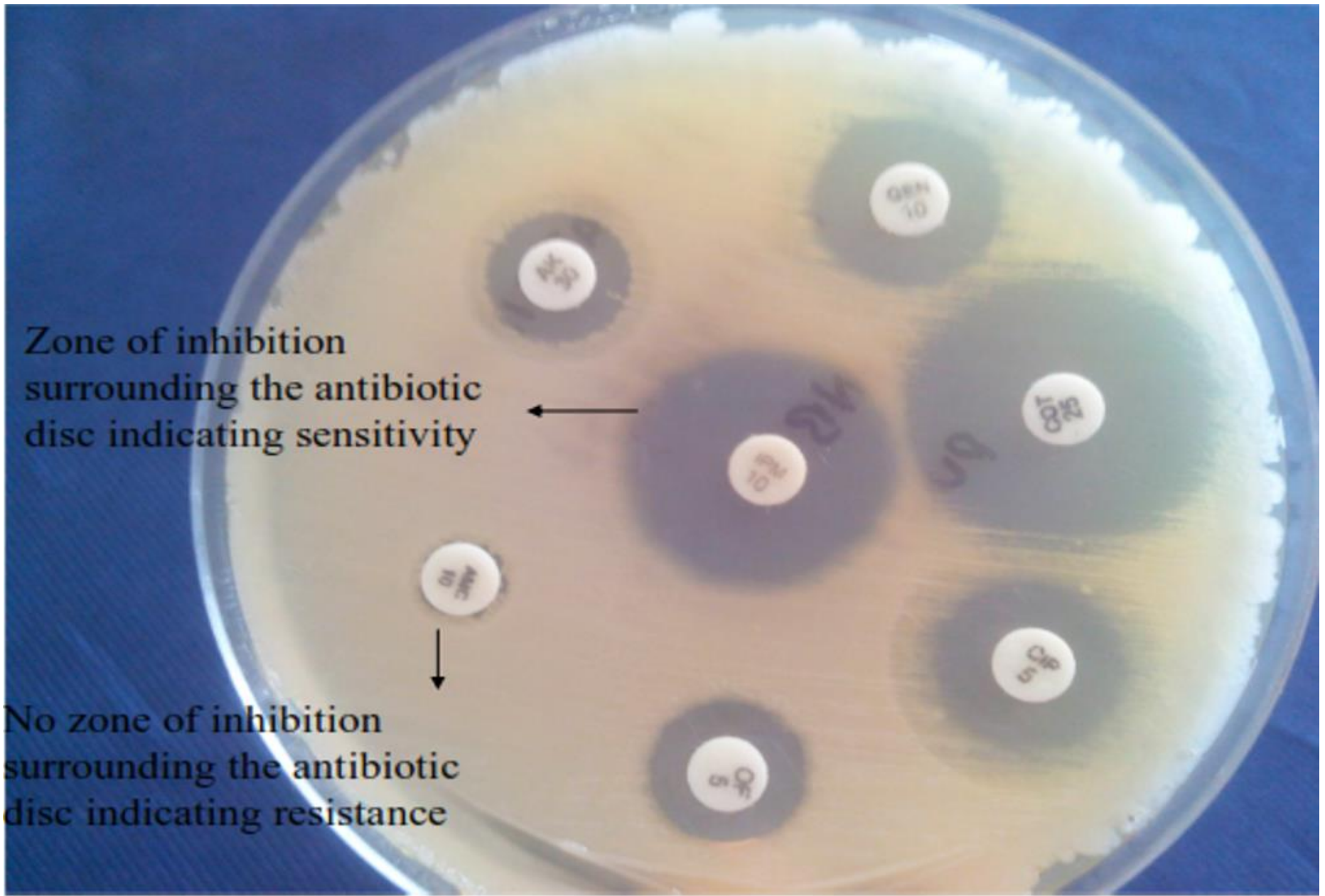




-After incubation, you can observe zones of inhibition around each antibiotic disc if present .

\*\***The zone of inhibition** :is the circular area around the antibiotic disc in which the bacterial colonies do not grow.

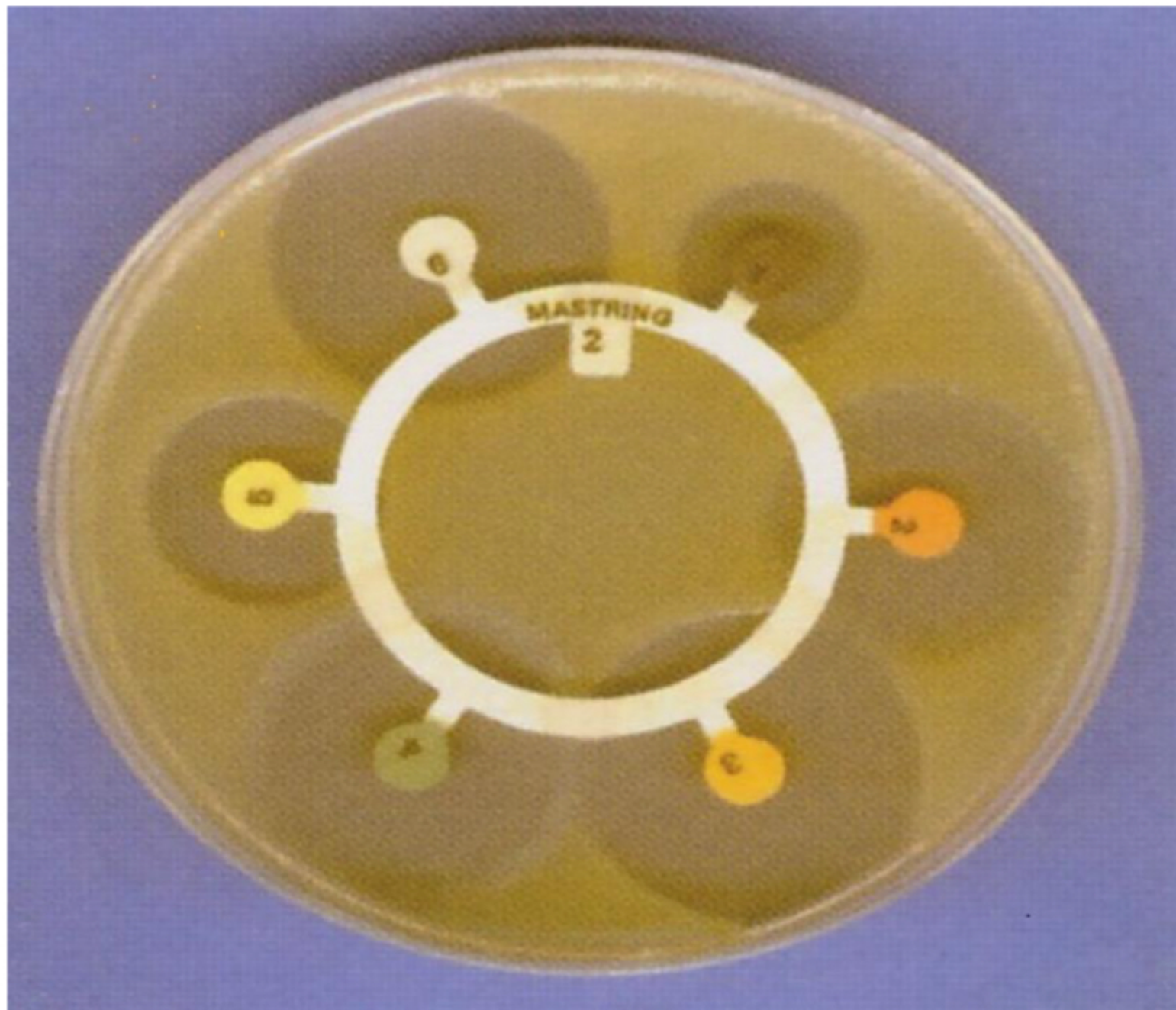
-You should know that the presence of the zone of inhibition is not automatically interpreted as susceptibility to the antibiotic, the zone diameter has to be measured and compared against a reference standard table as you see in the pictures below.

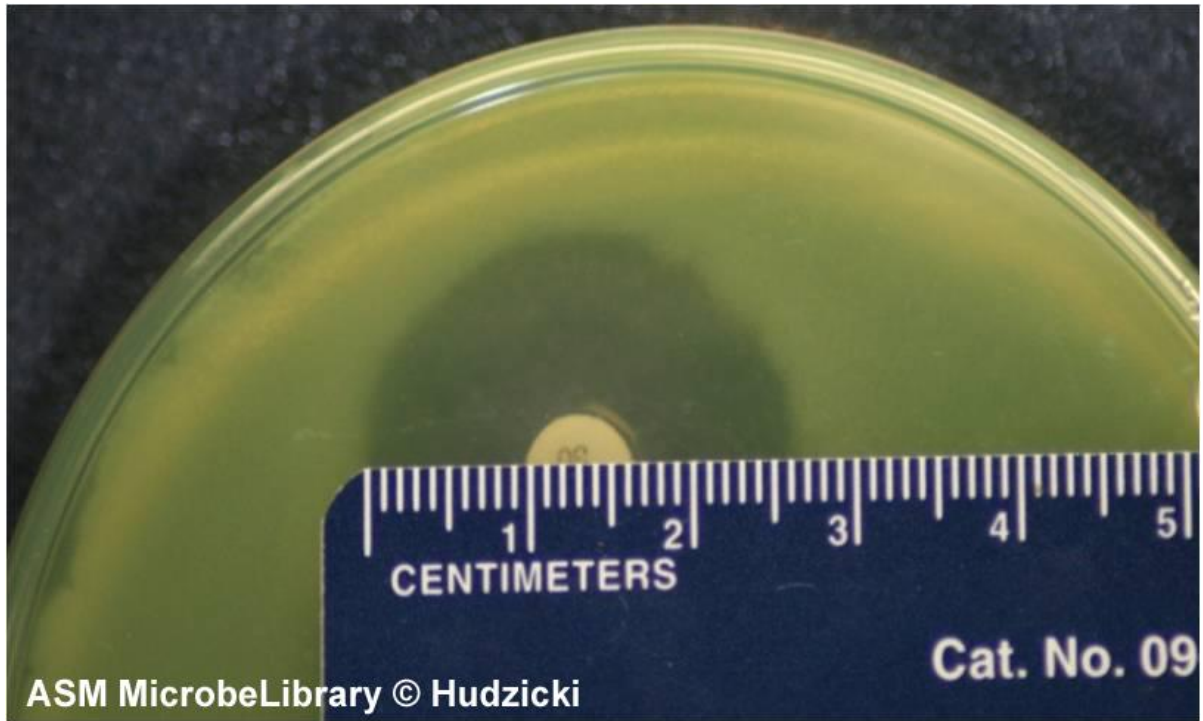
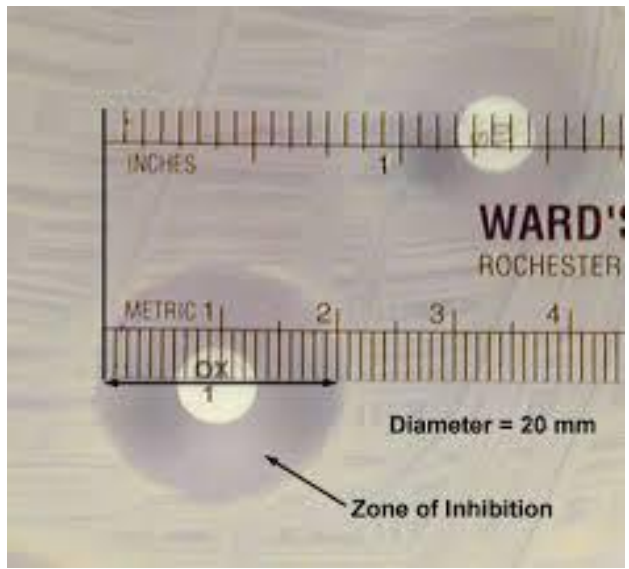


Zone of inhibition surrounding the antibiotic disc indicating sensitivity

No zone of inhibition surrounding the antibiotic disc indicating resistance





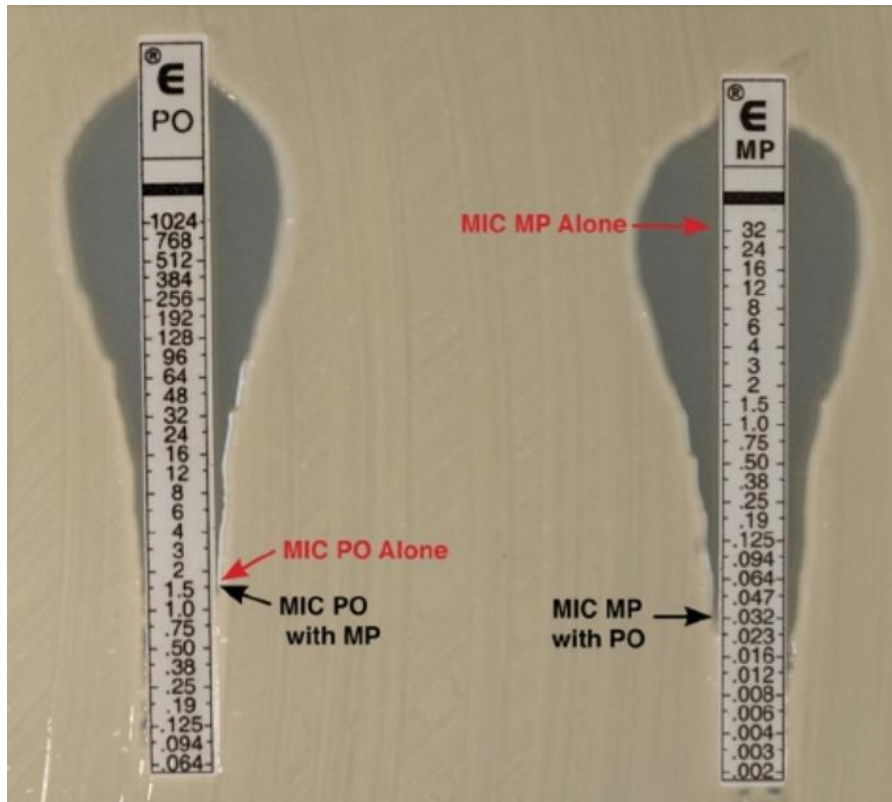


Antibiotics used	Zone diameter measured (mm)		
	Sensitive	Intermediate	Resistant
Ampicillin, 10 $\mu$ g	$\geq 10$	N/A	$\leq 9$
Chloramphenicol, 30 $\mu$ g	$\geq 23$	12 - 22	$\leq 11$
Clindamycin, 2 $\mu$ g	$\geq 18$	16 - 17	$\leq 15$
Ciprofloxacin, 5 $\mu$ g	$\geq 24$	19 - 23	$\leq 18$
Erythromycin, 15 $\mu$ g	$\geq 19$	16 - 18	$\leq 15$
Gentamicin, 10 $\mu$ g	$\geq 23$	N/A	$\leq 22$
Kanamycin, 30 $\mu$ g	$\geq 18$	14 - 17	$\leq 13$
Nalidixic acid, 30 $\mu$ g	$\geq 15$	N/A	$\leq 14$
Streptomycin, 10 $\mu$ g	$\geq 15$	12 - 14	$\leq 11$
Tetracycline, 30 $\mu$ g	$\geq 33$	16 - 32	$\leq 15$
Vancomycin, 30 $\mu$ g	-	-	-

# E test ( Quantitative )

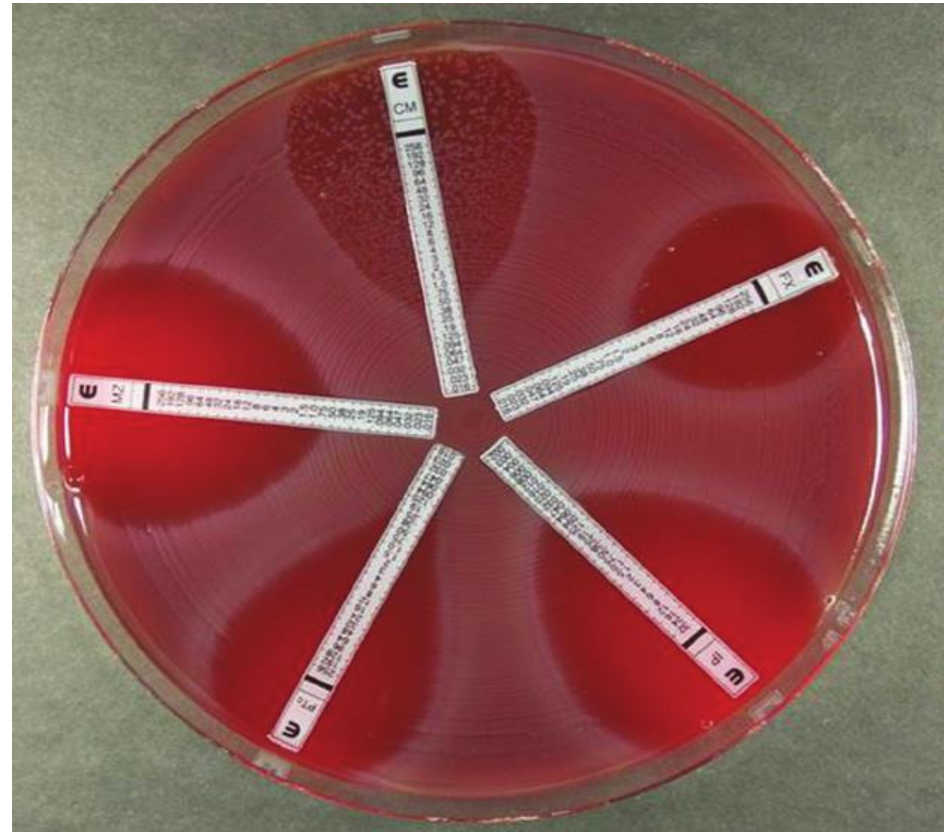
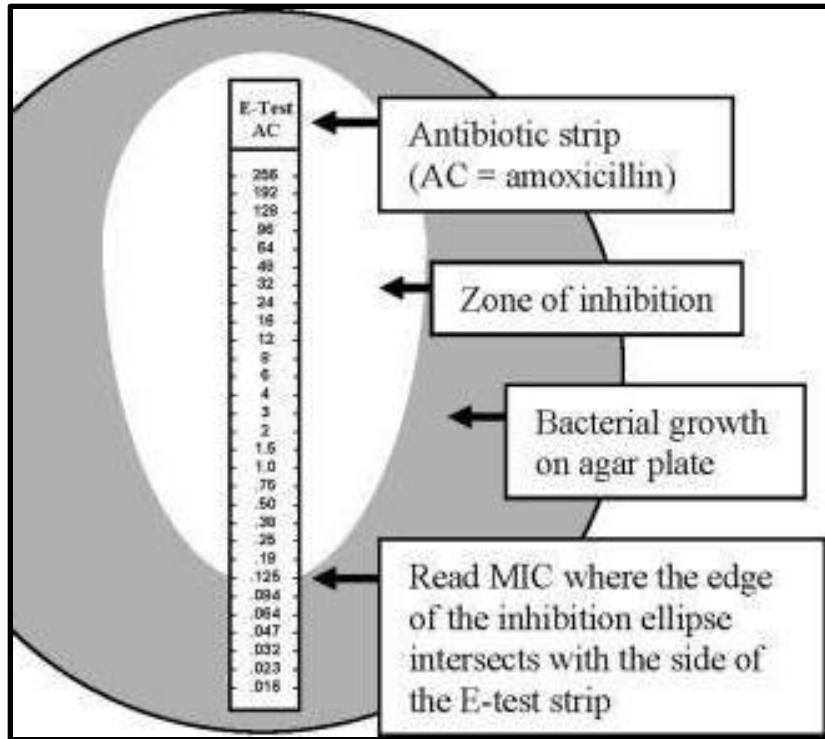
- E test consists of a predefined gradient of antibiotic concentrations on a plastic strip, it's used to determine the minimum inhibitory concentration (MIC) of antibiotics .
- This a quantitative method that applies both the dilution of the antibiotic and the diffusion of it into the media .
- The antibiotic strip consists of a predefined, continuous and exponential gradient of antibiotic concentrations immobilised along a rectangular plastics test strip.
- After spreading the bacterial colonies to be tested on a nutrient agar plate, apply the antibiotic strip using a sterile forceps, then incubate the plate for 24 to 48 hours .
- After incubation, a drop shaped inhibition zone intersects the graded test strip at the inhibitory concentration I see of the antibiotic (like we see in pictures below) .
- Read the MIC at the point where ellipse intersect the scale.

# E test ( Quantitative )



In this slide for example , the test strip on the right shows a result of 0.032 as a minimal inhibition concentration of the antibiotic , so at this point of concentration , the tested bacteria begins to be sensitive for this particular antibiotic .

# E test ( Quantitative )



®  
**E**  
TGC

- 256
- 192
- 128
- 96
- 64
- 48
- 32
- 24
- 16
- 12
- 8
- 6
- 4
- 3
- 2
- 1.5
- 1.0
- .75
- .50
- .38
- .25
- .19
- .125
- .094
- .064
- .047
- .032
- .023
- .016

®  
**E**  
DPC

- 256
- 192
- 128
- 96
- 64
- 48
- 32
- 24
- 16
- 12
- 8
- 6
- 4
- 3
- 2
- 1.5
- 1.0
- .75
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