

General Microbiology  
Antimicrobial susceptibility Test  
Lab 6

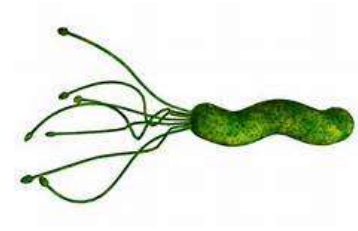
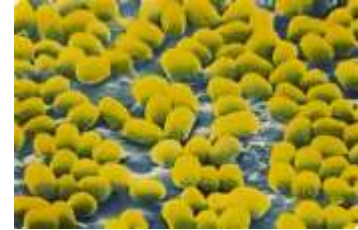
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# Medical Application



New antibiotics are continuously being developed

+



different bacteria acquire new resistant genes to the available antibiotics

determine the antibiotic susceptibility or resistance is required to determine most suitable antibiotic therapy

# Methods of Antimicrobial Susceptibility Testing

1. Standardized filter-paper disc-agar diffusion (Kirby-Bauer method)



**Qualitative Antimicrobial Susceptibility Testing**

2. Minimum Inhibitory concentration (MIC)  
& Minimum lethal concentration (MLC)

3. Epsilon meter test (E-test)

**Quantitative Antimicrobial  
Susceptibility Testing**

# Standardized filter-paper disc-agar diffusion

## Procedure



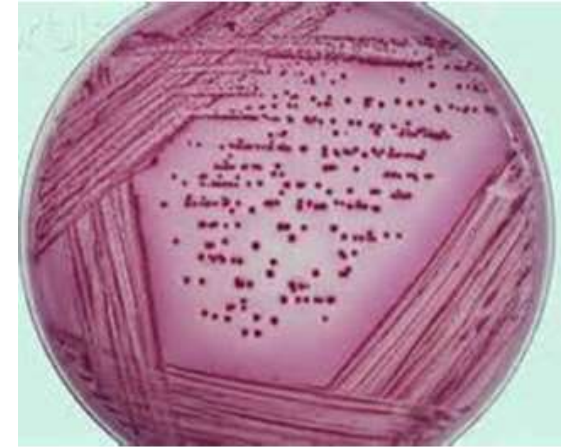
Urine sample



MacConkey agar



Gram negative bacilli  
Lactose fermenter



Biochemical reactions

**Antibiotic  
susceptibility  
test**



*E. coli*



Glucose	A, G
Lactose	A, G
Maltose	A, G
Mannitol	A, G
Sucrose	A, G

indole	+ve
MR	+ve
VP	-ve
Citrate	-ve
Urease	-ve
H <sub>2</sub> S	-ve

# Standardized filter-paper disc-agar diffusion

## Principle



Mueller Hinton agar



Confluent growth



Applying antibiotic disks



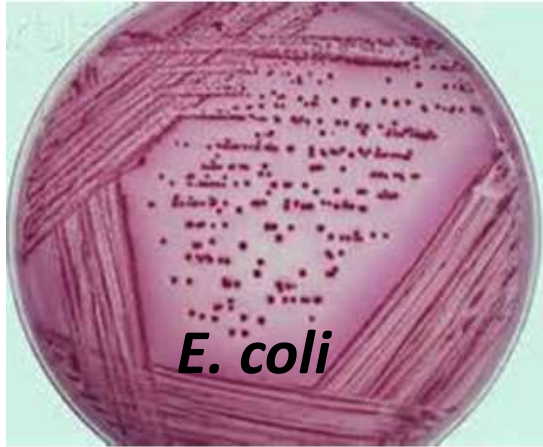
Incubation 24h at 37°C



Read the diameter of the inhibition zone

# Standardized filter-paper disc-agar diffusion

## Procedure



**1**

→ Transfer at least three to five well-isolated colonies of the same morphological type into nutrient broth tube

**2**



Incubated between 2 to 6 hrs



**3**



**4**

Compare the turbidity of the nutrient broth to the 0.5 McFarland standards by either a photometric device or visually.

# Standardized filter-paper disc-agar diffusion

## Procedure

5

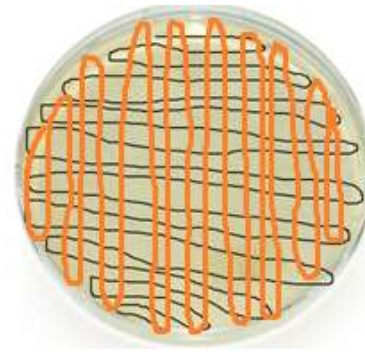


Dip a sterile cotton swab into a well-mixed saline test



6

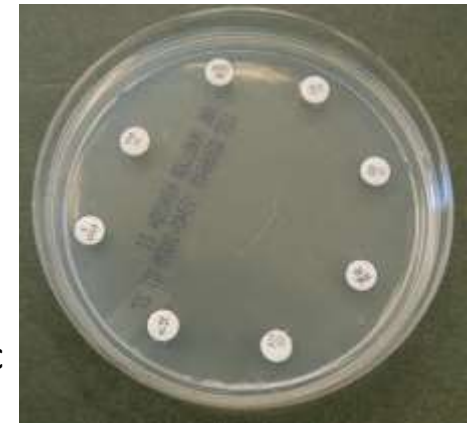
streak the entire agar surface horizontally, vertically, and around the outer edge of the plate



Confluent streaking

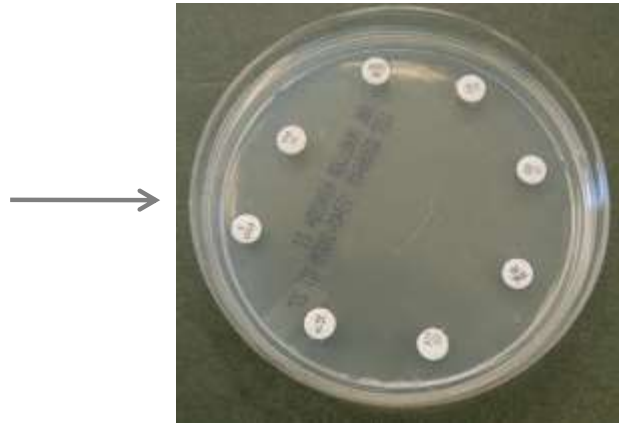
7

Carefully place the provided antibiotic discs onto the plate at equal distances using a sterile forceps and lightly touch each disc to make sure it will stay in place

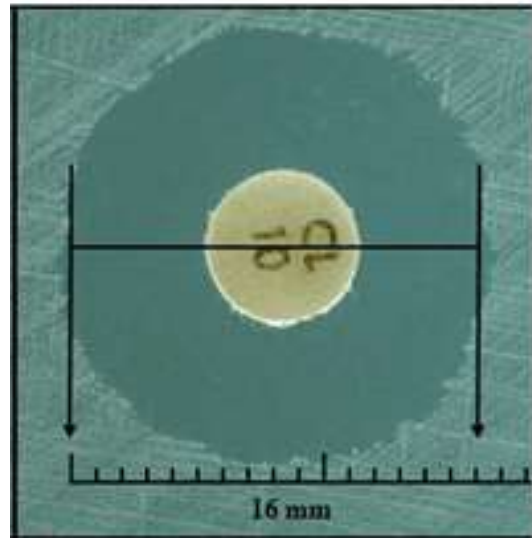


# Standardized filter-paper disc-agar diffusion

## Procedure



Incubation 24h  
At 37°C

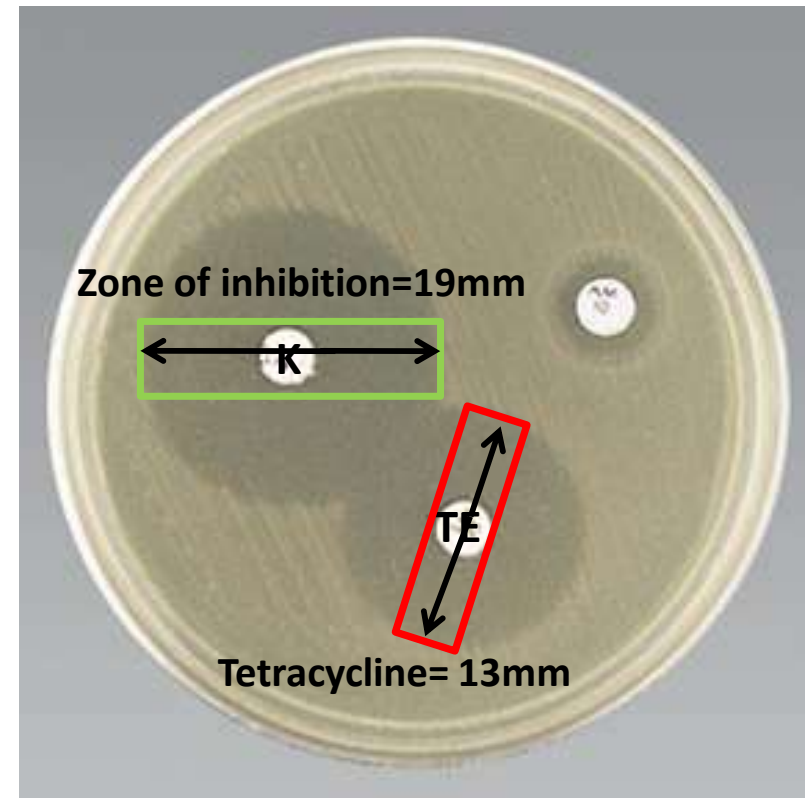




# Standardized filter-paper disc-agar diffusion

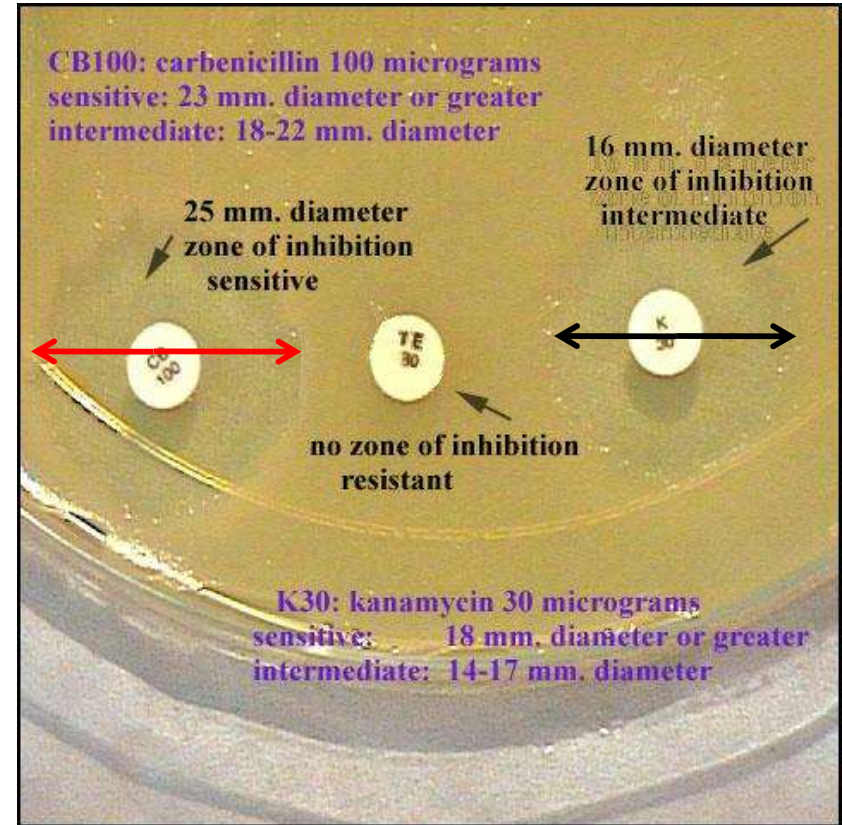
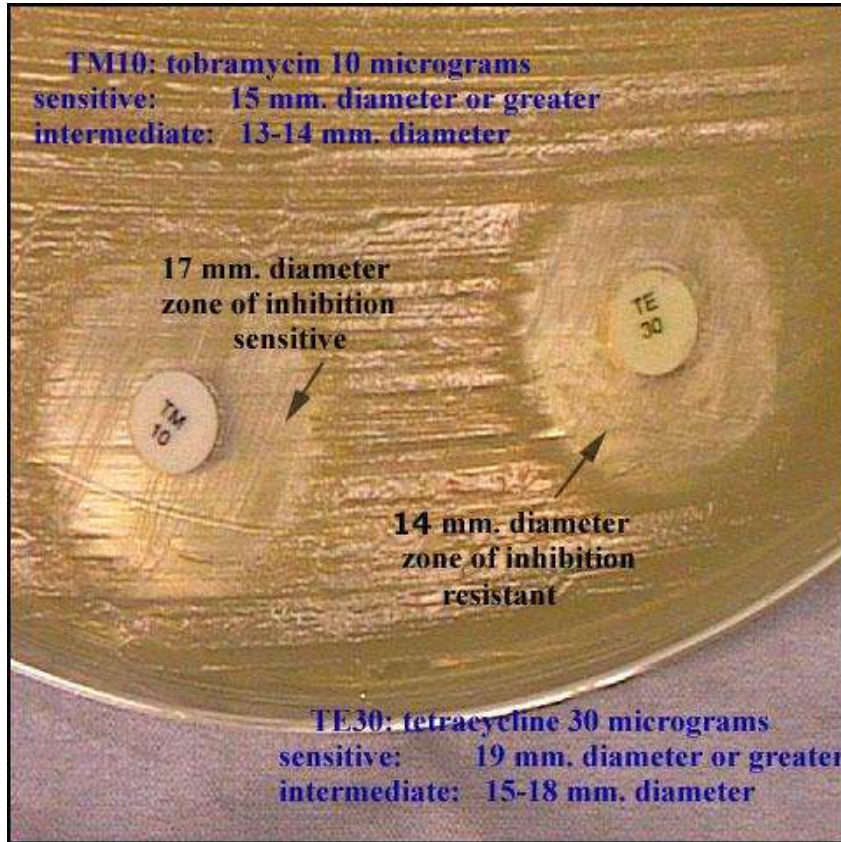
## Results

Antibiotic	Disk Conc.	Diameter of zone of inhibition (ZOI)		
		Resistant	Intermediate	Susceptible
Amikacin	10 µg	≤11	12-13	≥14
Ampicillin	10 µg	≤11	12-13	≥14
Bacitracin	10 units	≤8	9-11	≥13
Cephalothin	30 µg	≤14	15-17	≥18
Chloramphenicol	30 µg	≤12	13-17	≥18
Clindamycin	2 µg	≤14	15-16	≥17
Erythromycin	15 µg	≤13	14-17	≥18
Gentamicin	10 µg	≤12	13-14	≥15
Kanamycin	30 µg	≤13	14-17	≥18
Lincomycin	2 µg	≤9	10-14	≥15
Methicillin	5 µg	≤9	10-13	≥14
Nalidixic acid	30 µg	≤13	14-18	≥19
Neomycin	30 µg	≤12	13-16	≥17
Nitrofurantoin	0.3 mg	≤14	15-16	≥17
Penicillin				
vs. staphylococci	10 units	≤20	21-28	≥29
vs. other organisms	10 units	≤11	12-21	≥22
Polymyxin	300 units	≤8	9-11	≥12
Streptomycin	10 µg	≤11	12-14	≥15
Sulfonamides	0.3 mg	≤12	13-16	≥17
Tetracycline	30 µg	≤14	15-18	≥19
Vancomycin	30 µg	≤9	10-11	≥12



# Standardized filter-paper disc-agar diffusion

## Results



# McFarland standard

<b>McFarland Standard No.</b>	<b>0.5</b>	1	2	3
<b>Approx. cell density (1X10<sup>8</sup> CFU/mL)</b>	<b>1.5</b>	3.0	6.0	9.0
<b>Absorbance at 600 nm</b>	<b>0.08 to 0.1</b>	0.257	0.451	0.582



Different McFarland standards



0.5

**Absorbance at 600 nm  
( 0.08 to 0.1)**



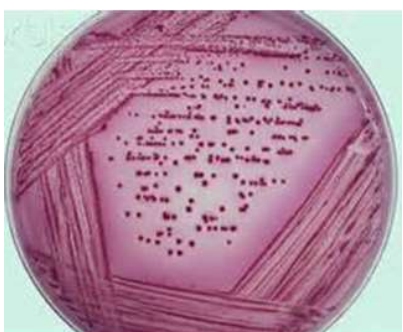
**The broth  
used to  
inoculate the  
Hinton  
Muller agar**

When equal turbidity=  
150,000,000 CFU/ml

# Minimum Inhibitory concentration (MIC) & Minimum lethal concentration (MLC)

**MIC:** is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation

**MLC (MBC):** Is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent.



**2**

Minimum Inhibitory concentration

**1**

$1 \times 10^5$  CFU

$1 \times 10^5$

$1 \times 10^5$

$1 \times 10^5$

$1 \times 10^5$

$1 \times 10^5$

$1 \times 10^5$

**4**



32 µg/ml



16



8



4



2



1



0.5

**3**



Antibiotic serial dilution



0.5



1



2



4



8



16



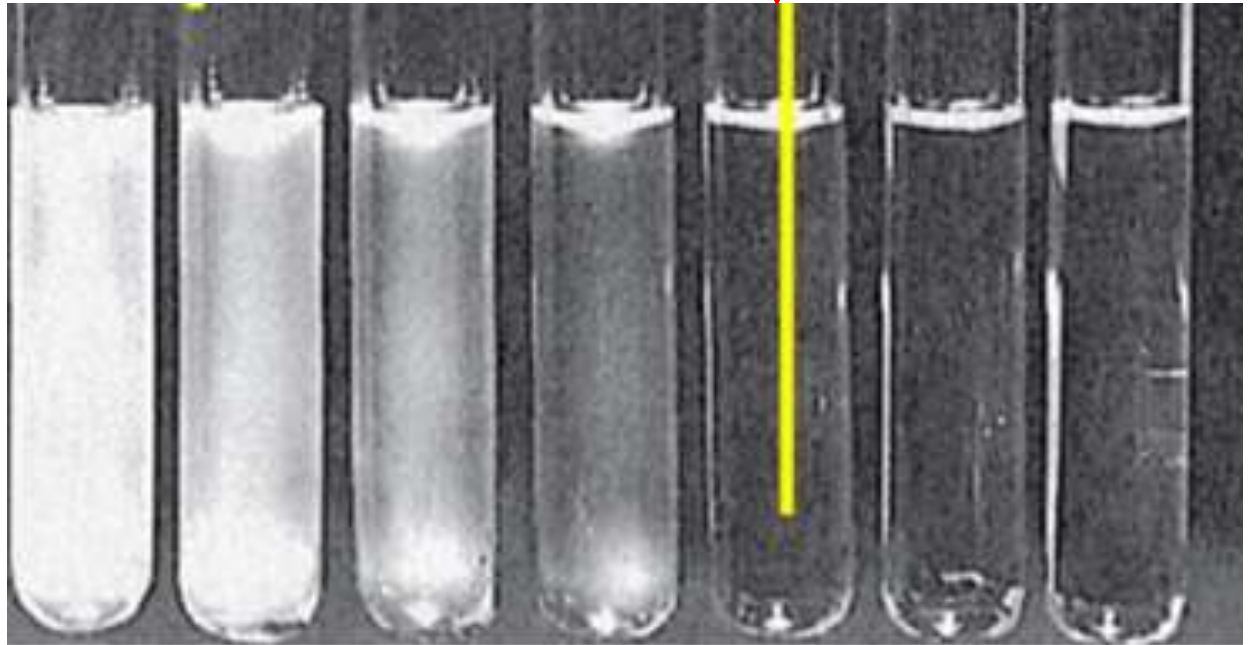
32

**MIC**

**5**

# Minimum Inhibitory concentration

Minimum Inhibitory concentration



0.5

1

2

4

8

16

32 µg/ml

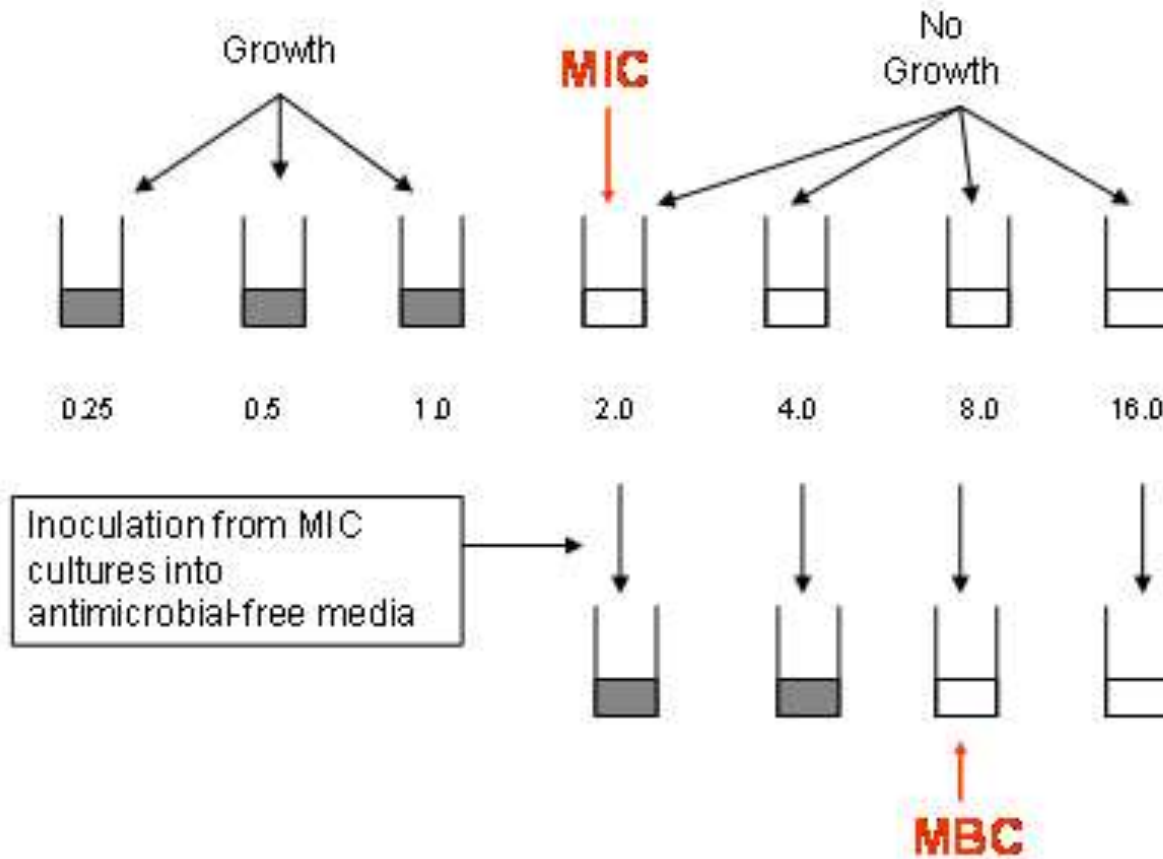
Sub-culture to agar medium

growth

No growth  
(16µg/ml  
is the  
MLC)

No growth

# Serial Dilution Susceptibility Testing



# Clinical applications for the Qualitative Antimicrobial Susceptibility Testing

MICs can also be used to reduce drug dosage and cost of antimicrobial therapy for very susceptible organisms; therefore, drugs with lower MIC scores are more effective antimicrobial agents.

This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains

MIC is used for determining treatment for patients suffering from infections such as sepsis, pneumonia, meningitis, endocarditis or osteomyelitis or managing the treatment of high-risk patients such as those suffering from cystic fibrosis or immunocompromised individuals.



# Epsilometer test (E-test)

Used as a substitution for the MIC test

Plastic strips with a predefined gradient of

**One antibiotic**

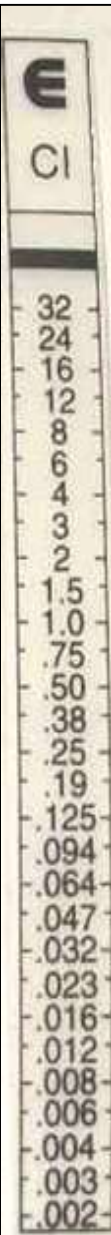
**One antifungal**

One strip per antibiotic

Easy to use

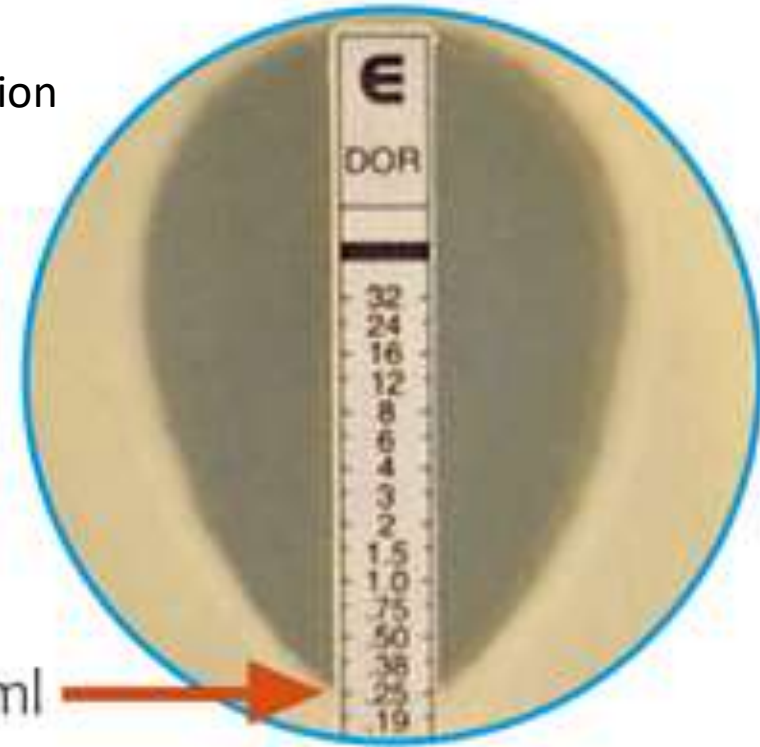
Storage at -20°C

Short shelf life, expensive



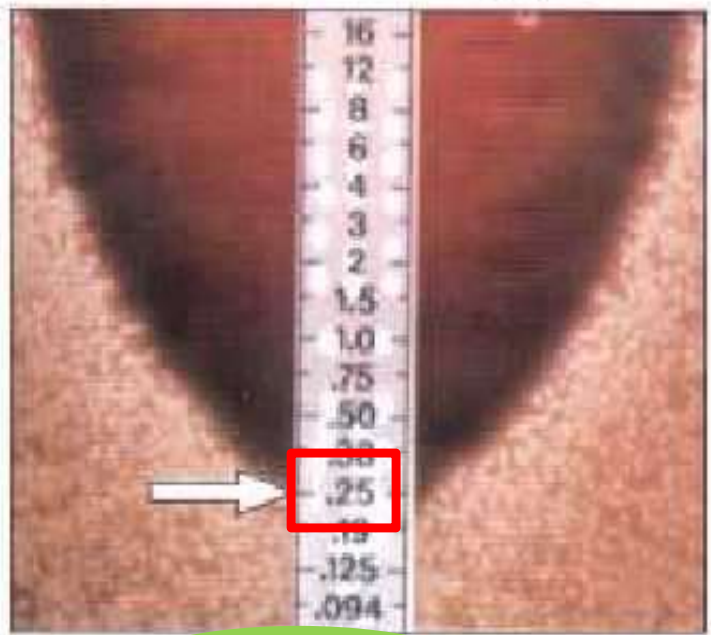
# Epsilometer test (E-test)

Elliptical zone of inhibition



MIC 0.25 µg/ml

# Reading E-tests



**Ciprofloxacin**

