

General Microbiology
Antimicrobial susceptibility Test
Lab 6

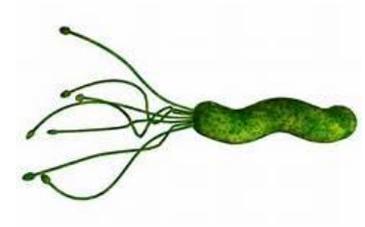
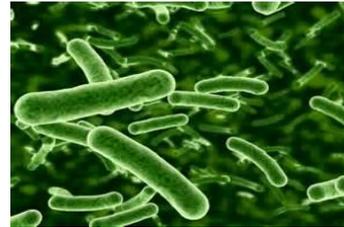
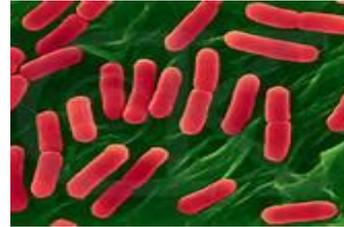
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Medical Application



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



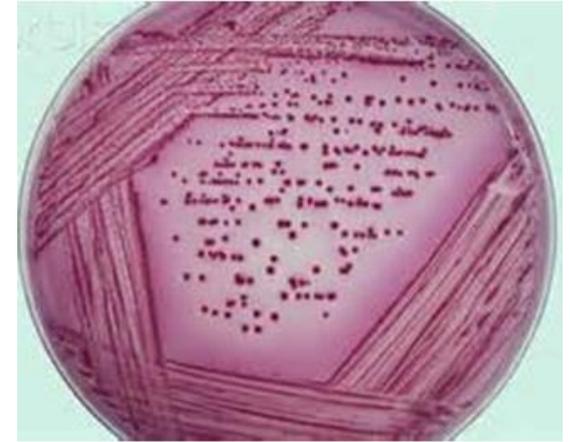
Urine sample



MacConkey agar



Gram negative bacilli
Lactose fermenter



Biochemical reactions

**Antibiotic
susceptibility
test**



E. coli

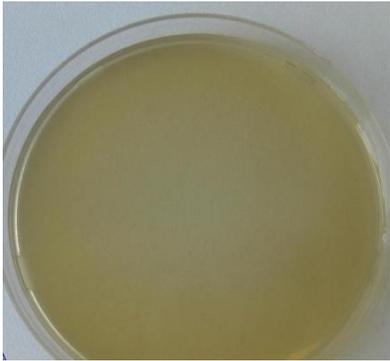


Glucose	A, G
Lactose	A, G
Maltose	A, G
Mannitol	A, G
Sucrose	A, G

indole	+ve
MR	+ve
VP	-ve
Citrate	-ve
Urease	-ve
H ₂ S	-ve

Standardized filter-paper disc-agar diffusion

Principle



Mueller Hinton agar



Confluent growth



Applying antibiotic disks



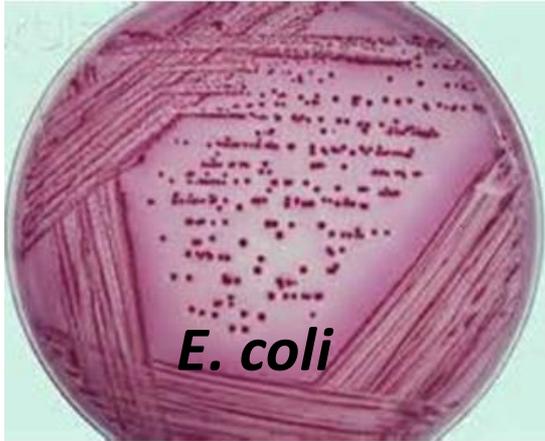
Incubation 24h at 37°C



Read the diameter of the inhibition zone

Standardized filter-paper disc-agar diffusion

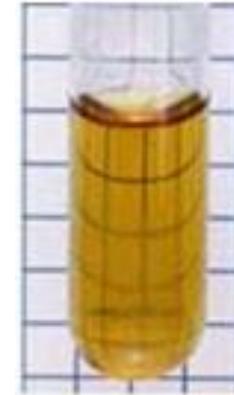
Procedure



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



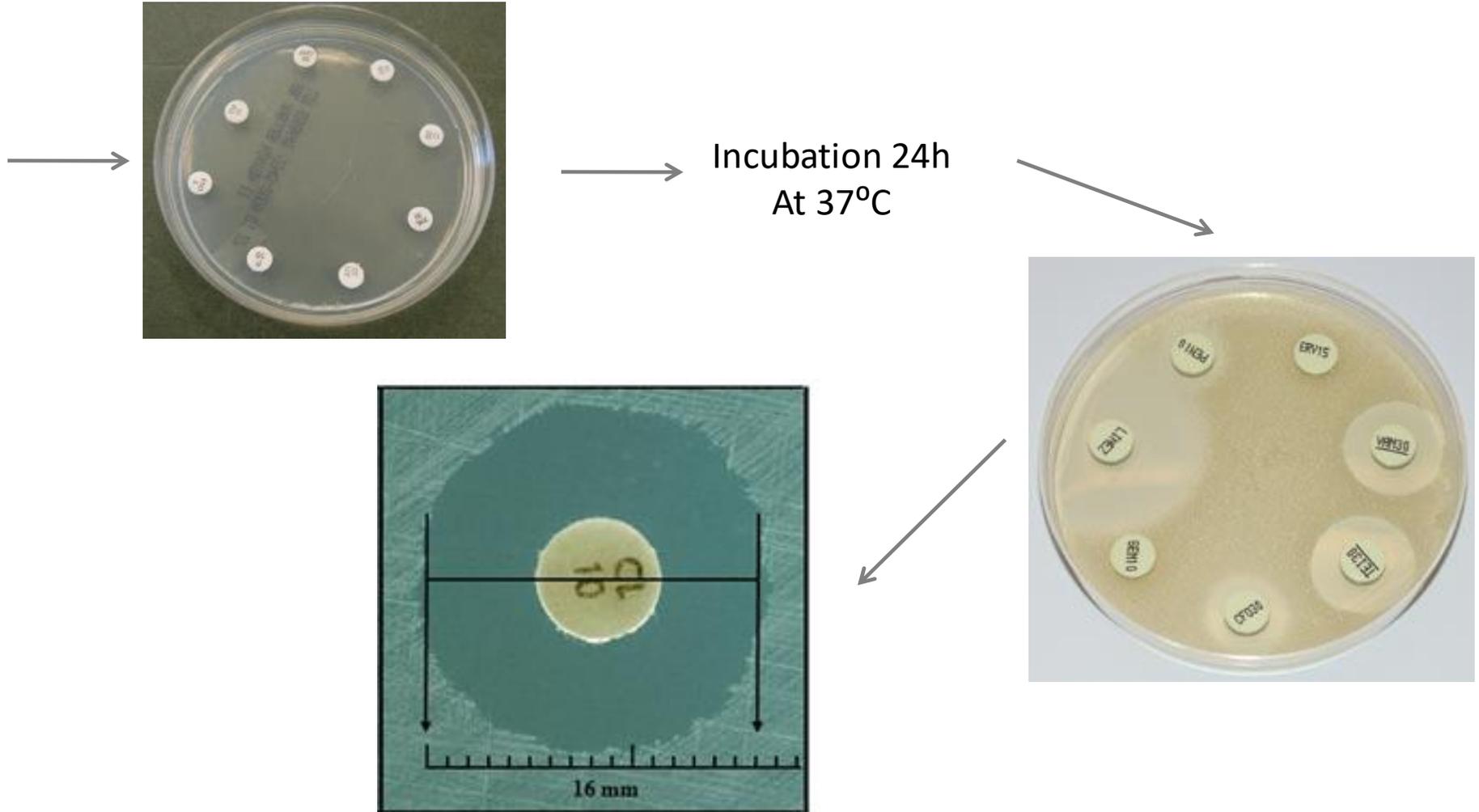
3



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

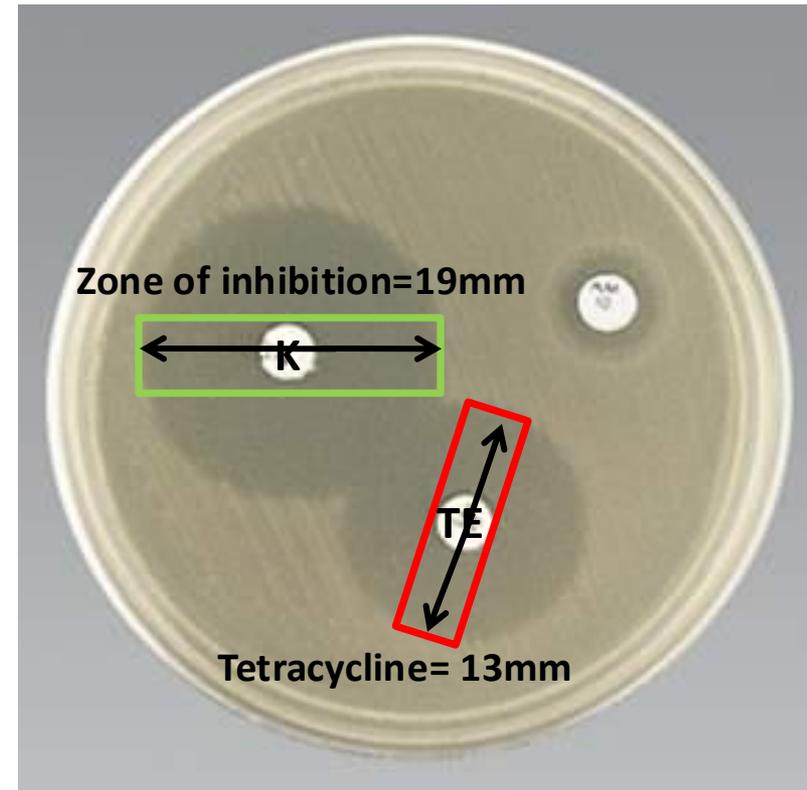
Procedure



Standardized filter-paper disc-agar diffusion

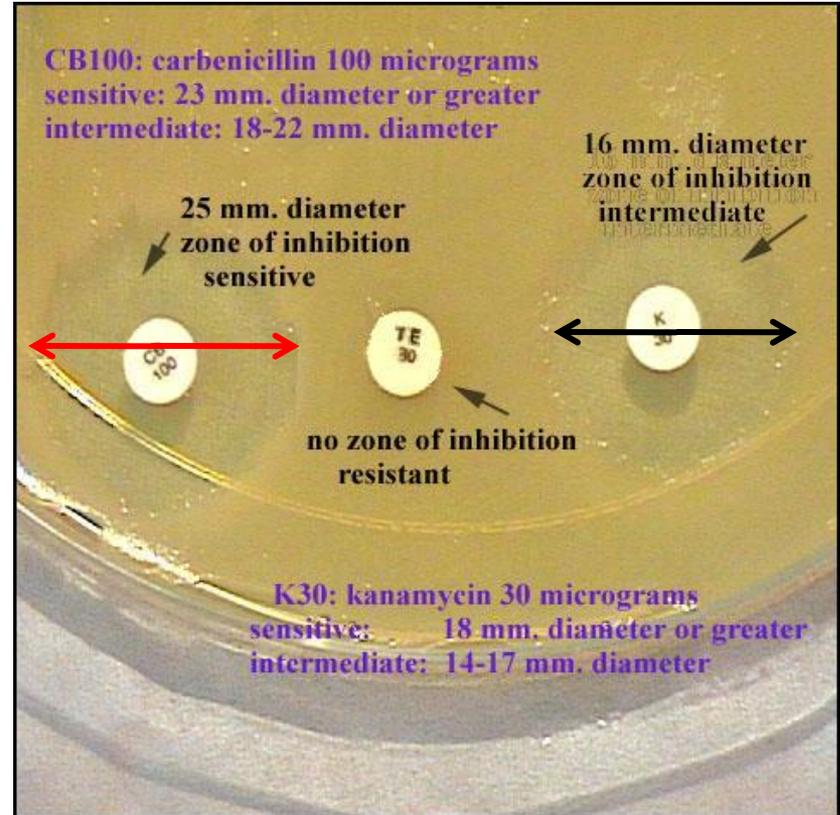
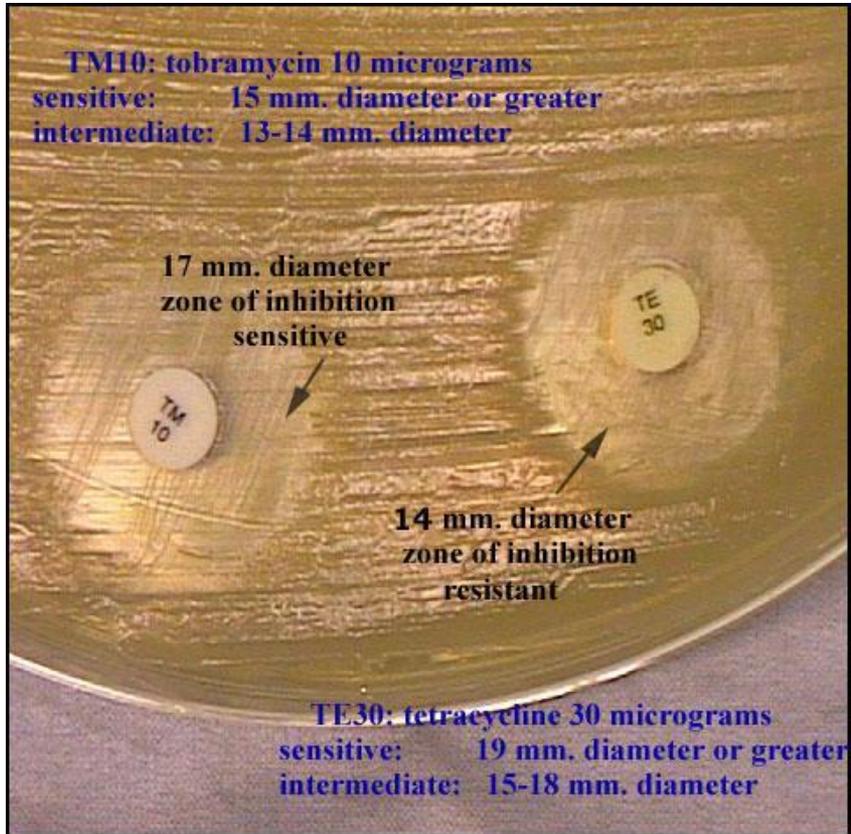
Results

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

McFarland Standard No.	0.5	1	2	3
Approx. cell density (1X10⁸ CFU/mL)	1.5	3.0	6.0	9.0
Absorbance at 600 nm	0.08 to 0.1	0.257	0.451	0.582



Different McFarland standards



0.5

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



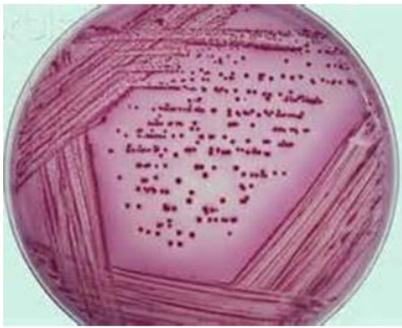
**The broth
used to
inoculate the
Hinton
Muller agar**

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.

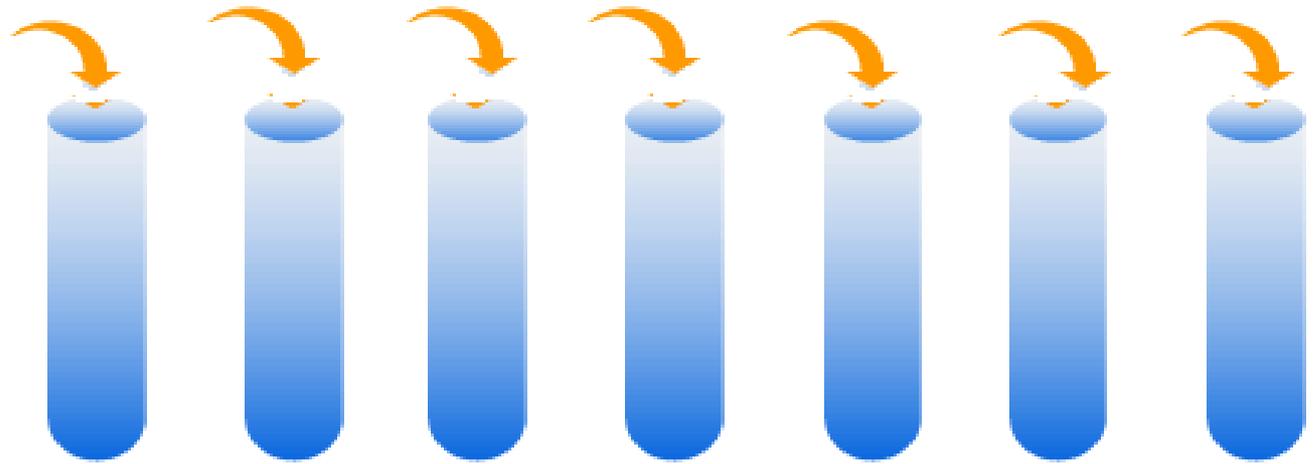


2

Minimum Inhibitory concentration

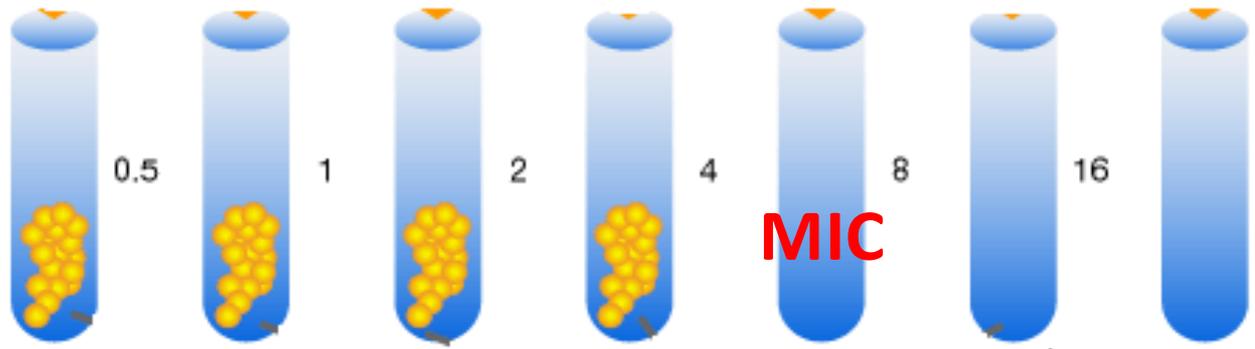
1

1 x 10⁵ CFU 1 x 10⁵ **4**



32 µg/ml 16 8 4 2 1 0.5 **3**

Antibiotic serial dilution

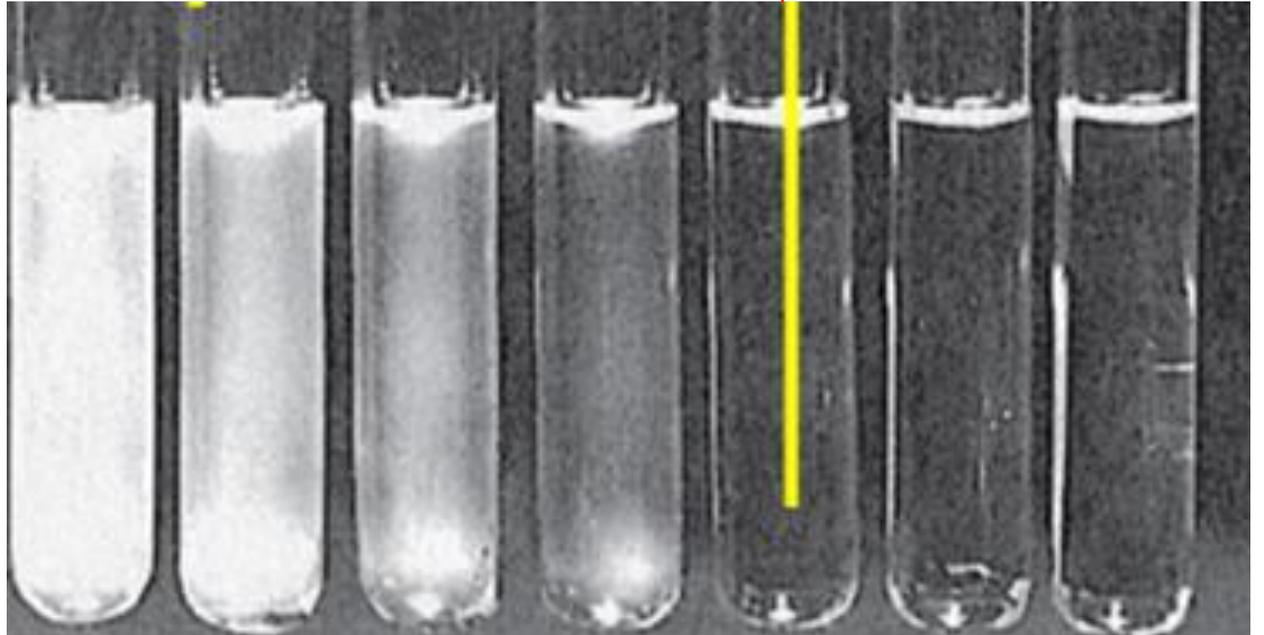


MIC

5

Minimum Inhibitory concentration

Minimum Inhibitory concentration



0.5

1

2

4

8

16

32 µg/ml

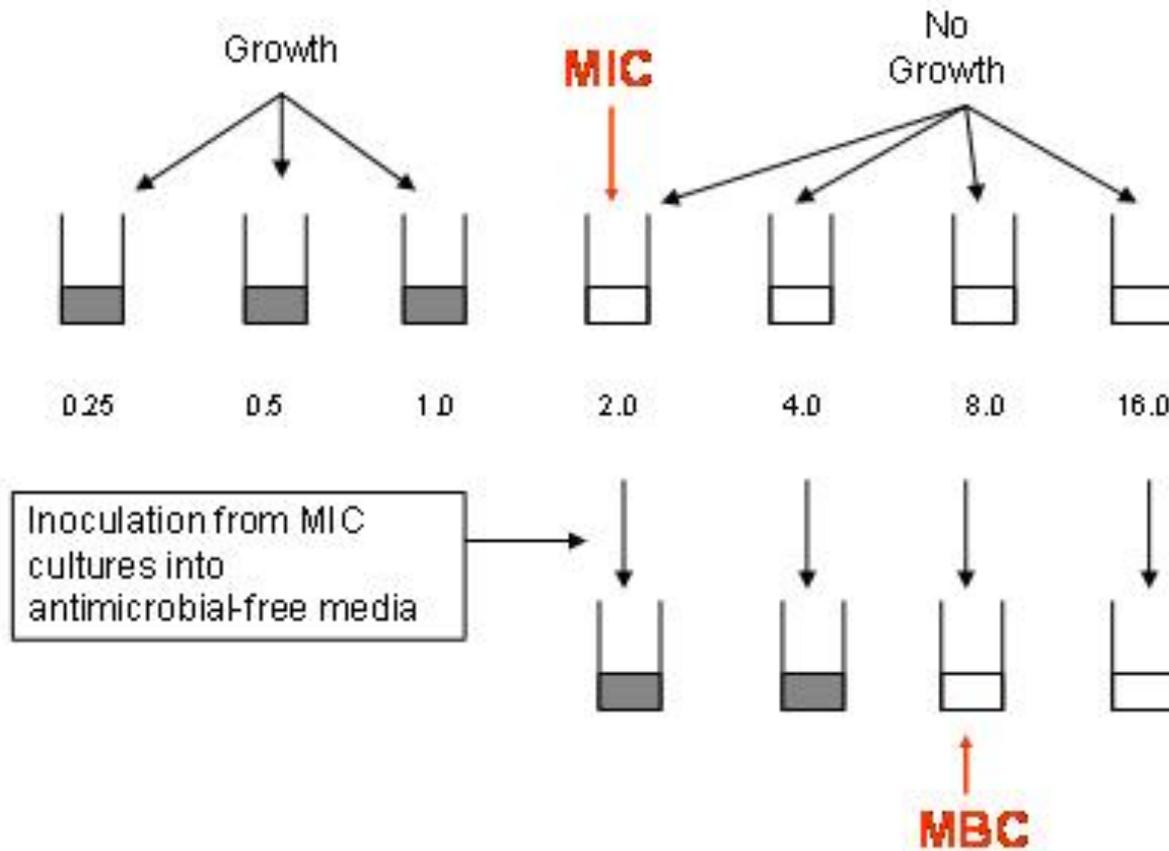
Sub-culture to agar medium

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

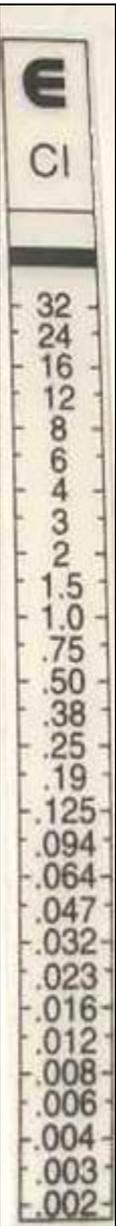
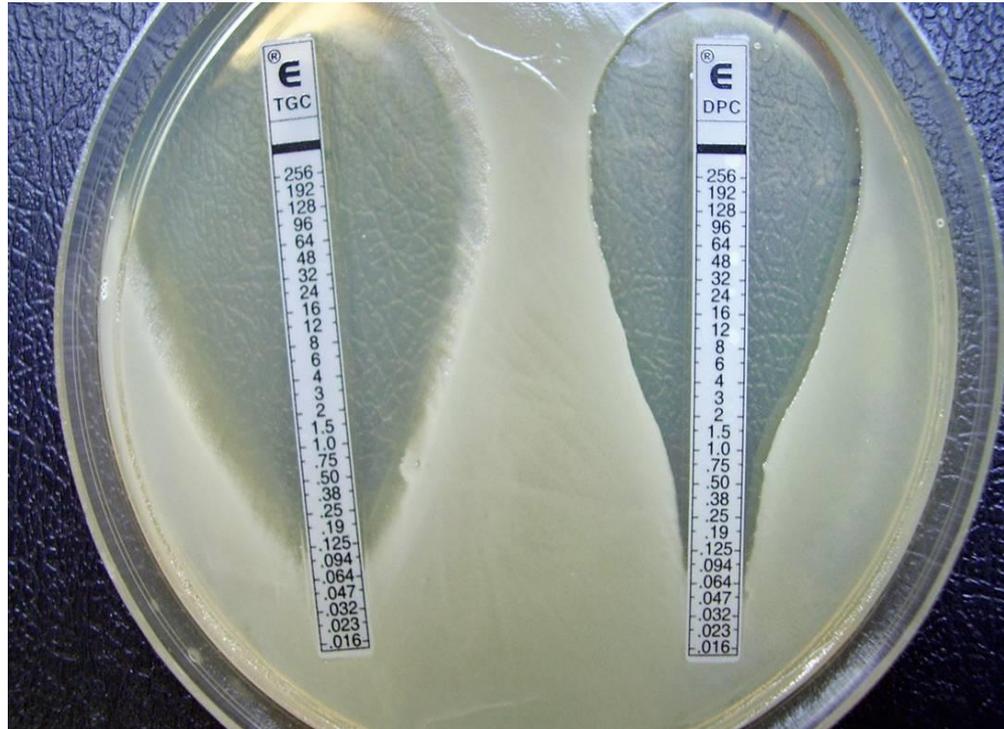
One antifungal

One strip per antibiotic

Easy to use

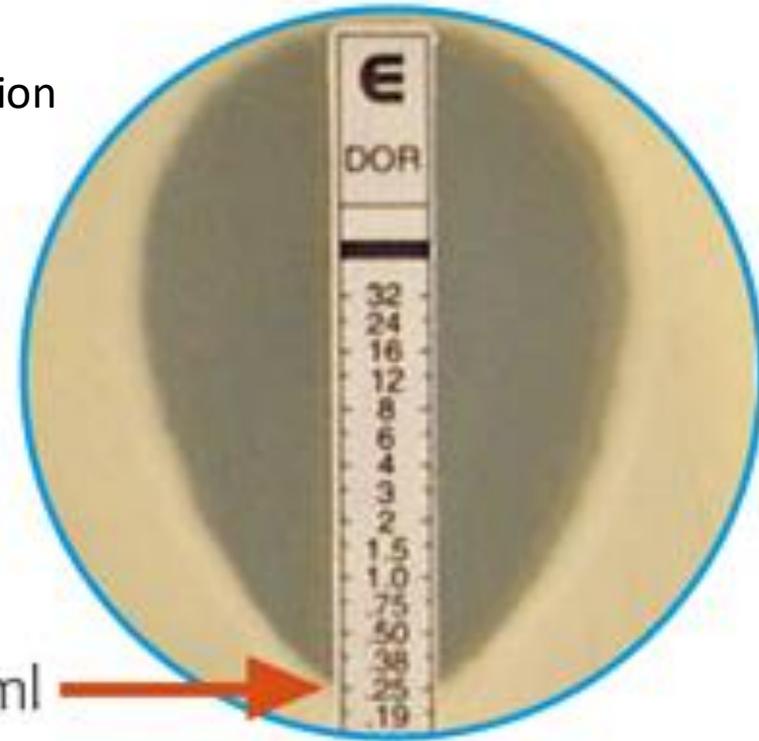
Storage at -20°C

Short shelf life, expensive



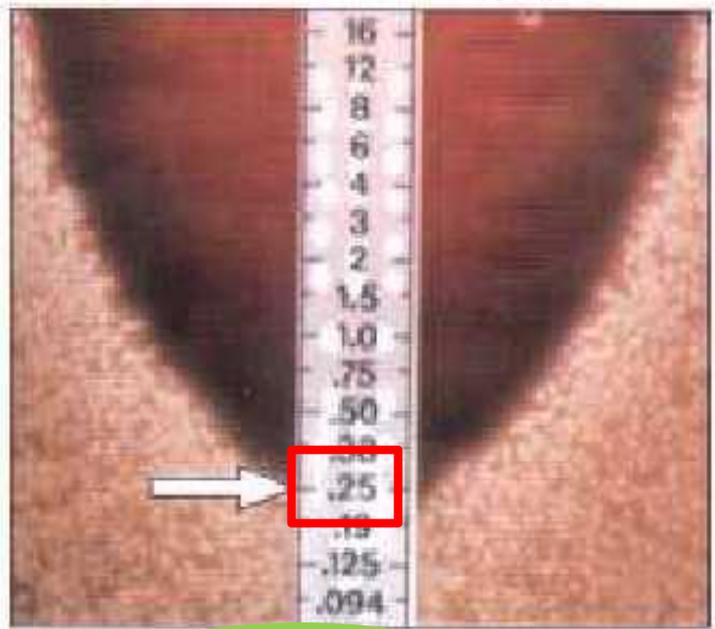
Epsilometer test (E-test)

Elliptical zone of inhibition



MIC 0.25 $\mu\text{g/ml}$

Reading E-tests



Ciprofloxacin

