

Date: 23 December, 2020

Post graduate course, Monsoon semester

**VMC 609: Techniques in
Immunology and Microbiology**

Topic: Antibiotic Sensitivity Testing

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Antibiotic classes

Penicillins

- Penicillin
- Amoxicillin
- Ampicillin
- Amp/Clavulanate
- Amp/Sulbactam
- 

Anti- Staph Penicillins

- Nafcillin
- Oxacillin
- Cloxacillin
- Dicloxacillin

Anti-Pseudomonal Penicillins

- Piperacillin/Tazobactam
- Ticarcillin/Clavulanate



Cont'd

- Carbapenems

- Imipenem
- Meropenem
- Ertapenem
- Dorapenem

- Monobactam

- Aztreonam

Cephalosporins

- First generation
 - Cefazolin
- Second generation
 - □ Cefotetan
 - Cefoxitin
 - Cefuroxime
- Third generation
 - Cefotaxime
 - Ceftriazone
 - Ceftazidime
 - Cefpodoxime
- Fourth generation
 - Cefepime



The Rules for Susceptibility Testing

1. CLSI - Clinical Laboratory Standards Institute Approved standards for the testing & reporting of susceptibility results/ updated yearly
 1. Charts with appropriate antibiotics to test
 2. How to interpret the laboratory results
 3. QC standards and proper testing procedures
2. Susceptibility Tests are tests of bacterial stasis - not killing



Methods/Bacteria in Review

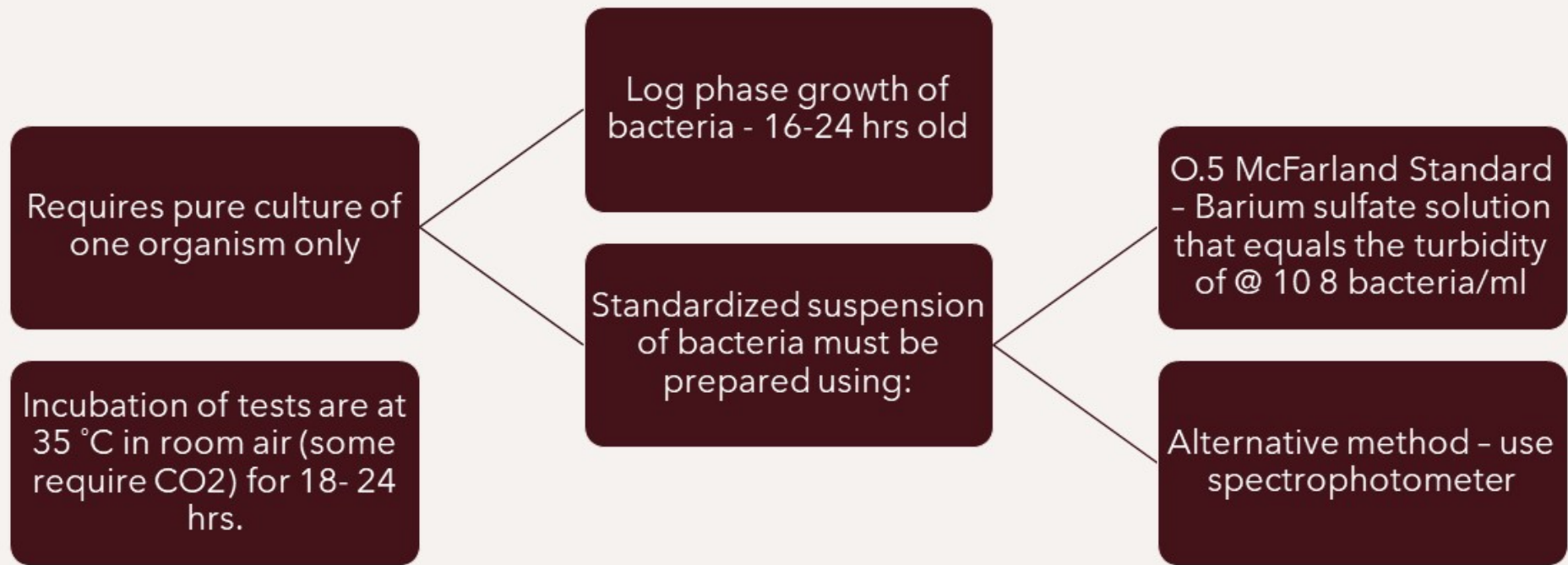
- METHODS

1. Kirby Bauer disk diffusion
2. E Test Strip Minimum inhibitory concentration (MIC)
3. Broth dilution Minimum inhibitory concentration (MIC)
4. Beta lactamase enzyme detection

- RESISTANT BACTERIA IN THE NEWS

- MRSA methicillin resistant *Staphylococcus aureus*
- VRE vancomycin resistant enterococcus
- ESBL Extended Spectrum Beta Lactamase Gram neg rods
- KPC *Klebsiella pneumonia* Carbapenemase or CRE (Carbapenamase Resistant Enterics)
- *Streptococcus pneumonia*
- *Neisseria gonorrhoeae*





Preparation of Bacteria for all Susceptibility Methods

0.5 McFarland Standard which is a turbidity standard made from Barium sulfate - the turbidity is equal to 10^8 CFU/ml bacteria



McFARLAND, J. (1907). THE NEPHELOMETER: AN INSTRUMENT FOR ESTIMATING THE NUMBER OF BACTERIA IN SUSPENSIONS USED FOR CALCULATING THE OPSONIC INDEX AND FOR VACCINES. JAMA: The Journal of the American Medical Association, XLIX(14), 1176.



McFarland Standard

- It consists essentially of a series of standardizing tubes containing a suspension of fine precipitate *approximating* bacterial suspensions in opacity and a holder for making their comparison easy



McFarland Standard	1% BaCl ₂ (ml)	1% H ₂ SO ₄ (ml)	Approximate Cell Count Density (x10 ⁸ cells)
0.5	0.05	9.95	1.5 x 10 ⁸
1.0	0.1	9.9	3.0 x 10 ⁸
2.0	0.2	9.8	6.0 x 10 ⁸
3.0	0.3	9.7	9.0 x 10 ⁸
4.0	0.4	9.6	12.0 x 10 ⁸

- McFarland, J. (1907). *The nephelometer:an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. JAMA: The Journal of the American Medical Association, XLIX(14), 1176.*

McFarland Standard

Agar Disk Diffusion (Kirby Bauer Method)

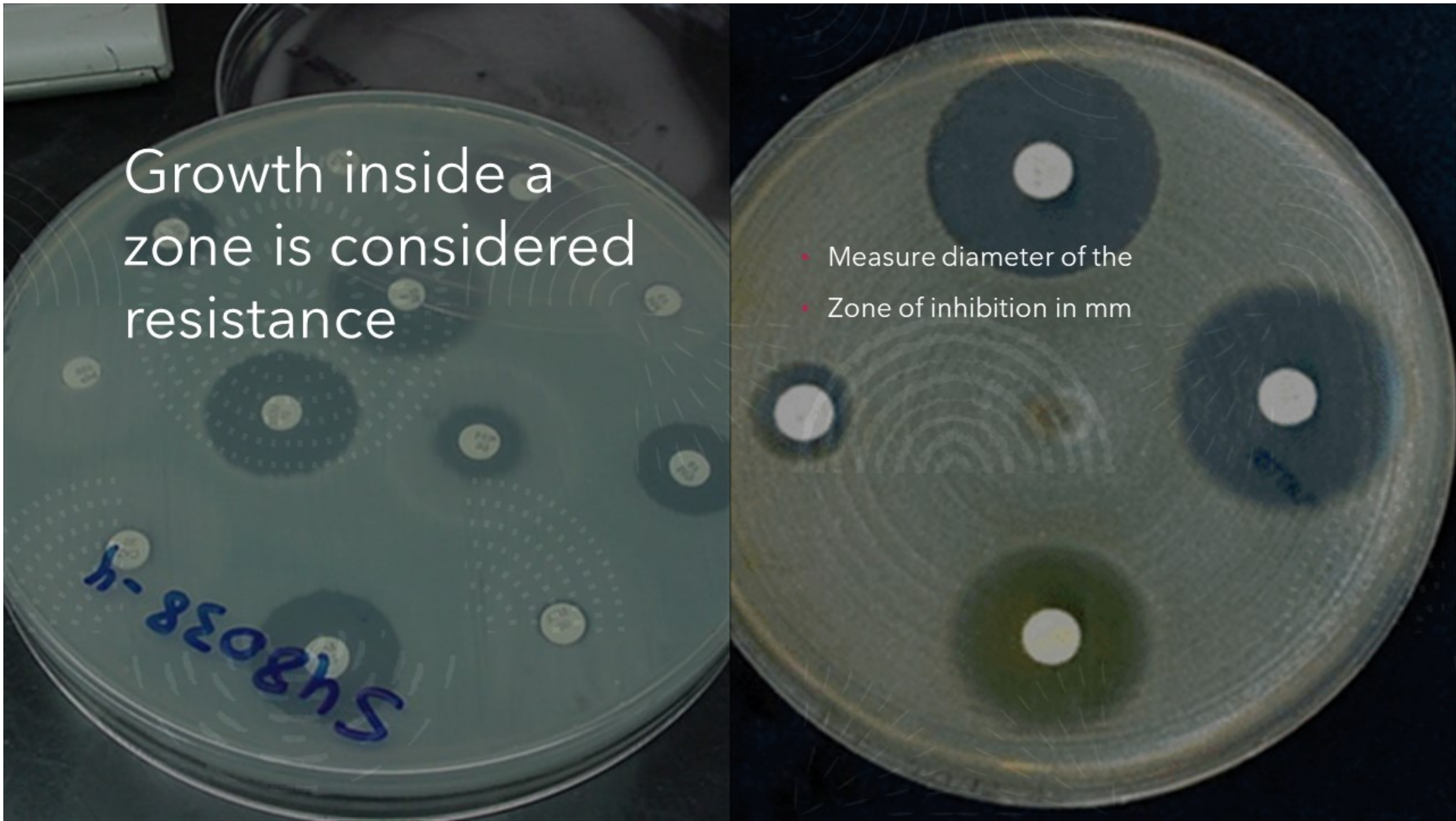
Zone of inhibition
surrounding the antibiotic
disc indicating sensitivity

No zone of inhibition
surrounding the antibiotic
disc indicating resistance

- Procedure: Qualitative Susceptibility method
 - Mueller Hinton agar -with or without blood
 - 150 mm plate diameter
 - 4mm in depth
 - Agar specifically balanced in Ca^{+} and Mg^{+} ,
 - if the ions are too high % amino-glycosides test falsely resistant,
 - if the ions too low % falsely susceptible amino-glycoside results
- Streak bacteria on plate with cotton tipped swab
- Apply 6mm paper disks that contains single antibiotic
- Incubate for 16-24 hrs at 35°C
- Measure zone of diameter of inhibition of growth (mm)

Growth inside a
zone is considered
resistance

- Measure diameter of the
- Zone of inhibition in mm



Kirby Bauer (KB)

- Concentration gradient created with the diffusing antibiotic and the increasing number of bacteria growing on the agar, this determines the zone of inhibition around disk.
- CLSI charts used to interpret the measured zone sizes as Sensitive, Intermediate or Resistant
- Cannot directly compare zone sizes between antibiotics–
 - ex: ZID of 21mm zone size is as sensitive as a GM of 14mm - zone sizes differ for organism/antibiotic combinations
 - Regression analysis can be used to calculate MIC value related to KB zone size



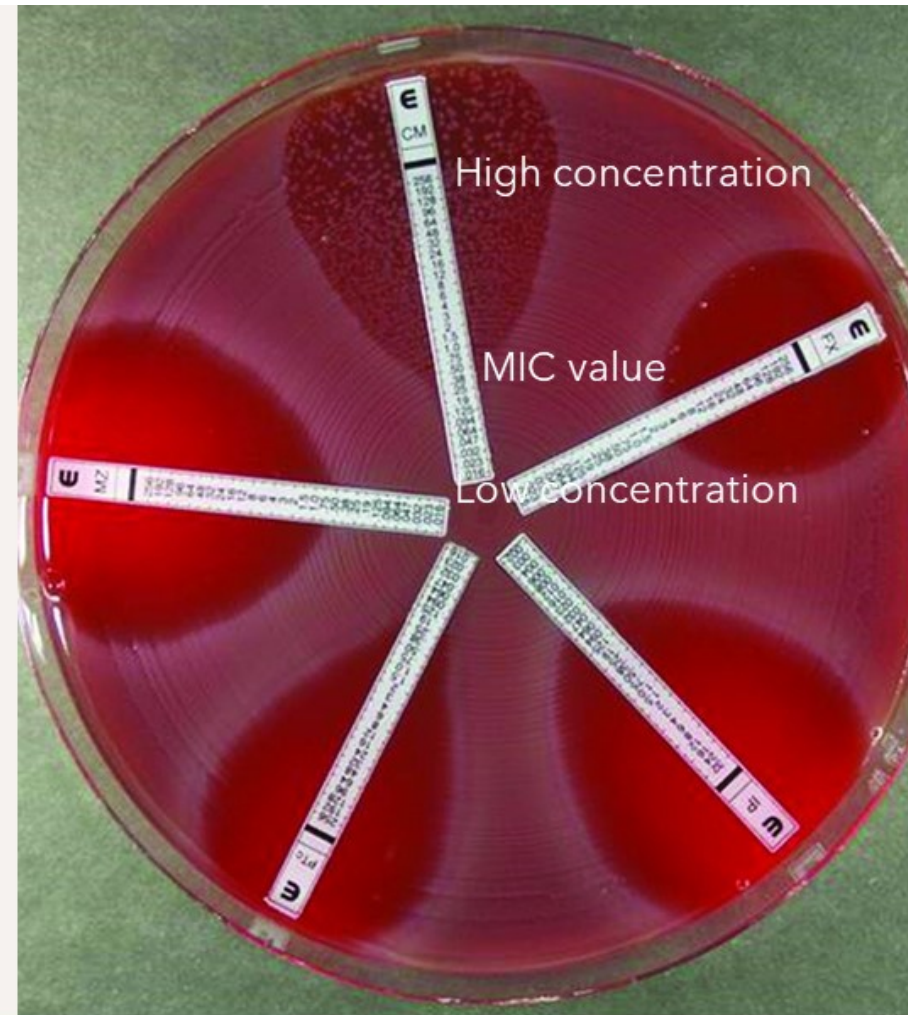
E Test

Quantitative MIC Susceptibility

- Calibrated plastic strips impregnated with one antibiotic/concentration gradient (mcg/ml) embedded in plastic / carefully placed on the agar surface
- Gradient created as antibiotic diffuses into agar in an elliptical shape
- MIC (minimum inhibitory concentration) is where the ellipse ends on the plastic strip
- Useful for any organism but a method of choice for slow growing fastidious organisms



E test



Broth Dilution

Quantitative Susceptibility Method

- Bacteria inoculum: 0.5 McFarland standard – further diluted to 5×10^5 organisms /ml in broth
- Suspension is inoculated into tubes or micro titer trays containing growth medium and known 2 fold dilutions (mcg/ml) of antibiotics

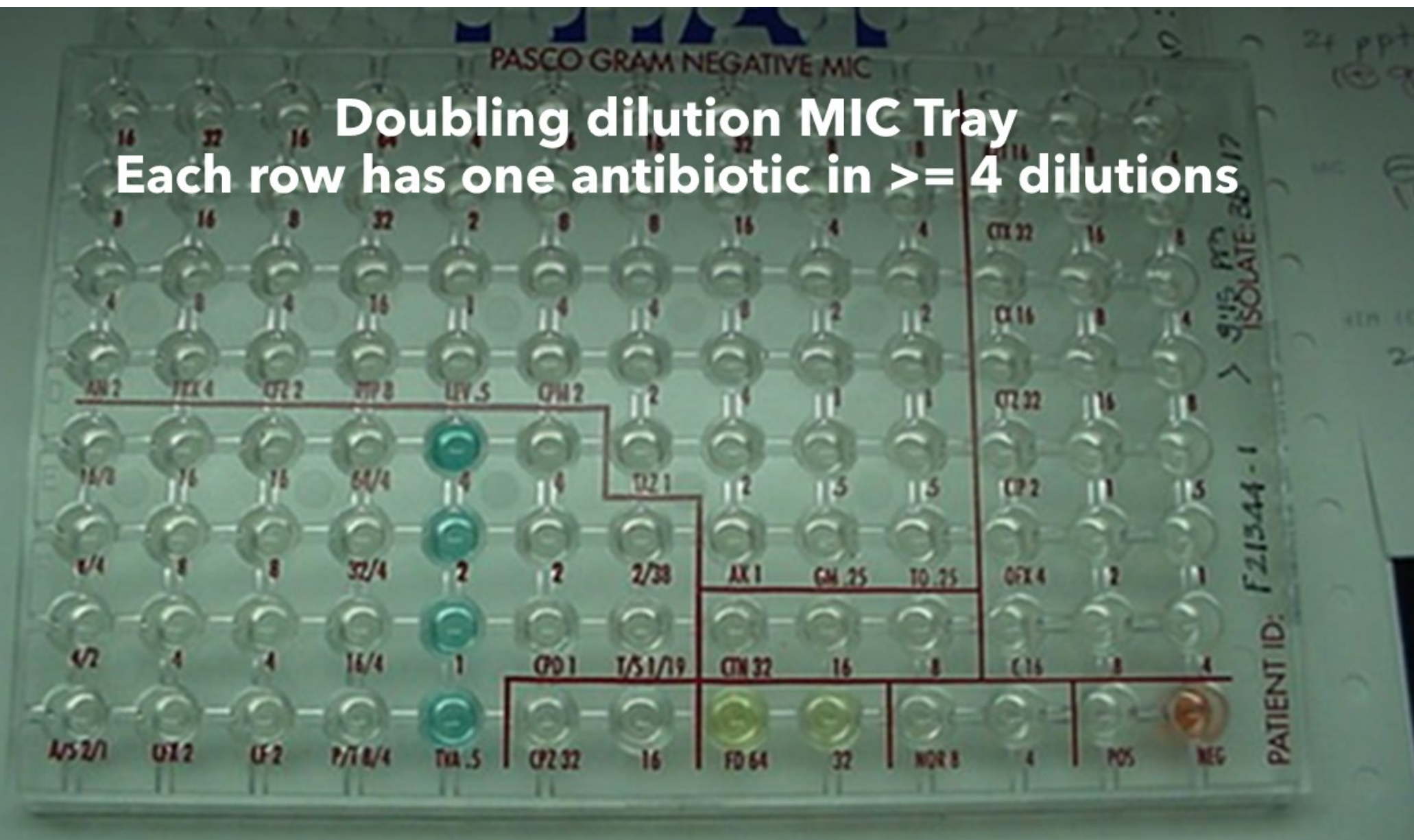


- Calibrated plastic strips impregnated with one antibiotic/concentration gradient (mcg/ml) embedded antibiotic/concentration gradient (mcg/ml) embedded in plastic / carefully placed on the agar surface in plastic / carefully placed on the agar surface
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- Useful for any organism but a method of choice for slow growing fastidious organisms Useful for any organism but a method of choice for slow growing fastidious organ



Doubling dilution MIC Tray

Each row has one antibiotic in ≥ 4 dilutions



Broth Dilution Definitions

- **MBC** - Minimum **bactericidal** concentration determined by the subculture of the contents of the wells that show no growth to solid agar - the lowest concentration of antibiotic that kills 99.9% of original inoculum is the **MBC**.

	Growth	No Growth
High	0.10	0.05
Low	0.05	0.10

- MBC = 128 MIC = 2 $128/2 = 64$ Tolerance

Antibiotic Susceptibility System





THANK YOU

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