Interpreting Antimicrobial Susceptibility Reports

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The views expressed in this presentation are the personal views of the speaker

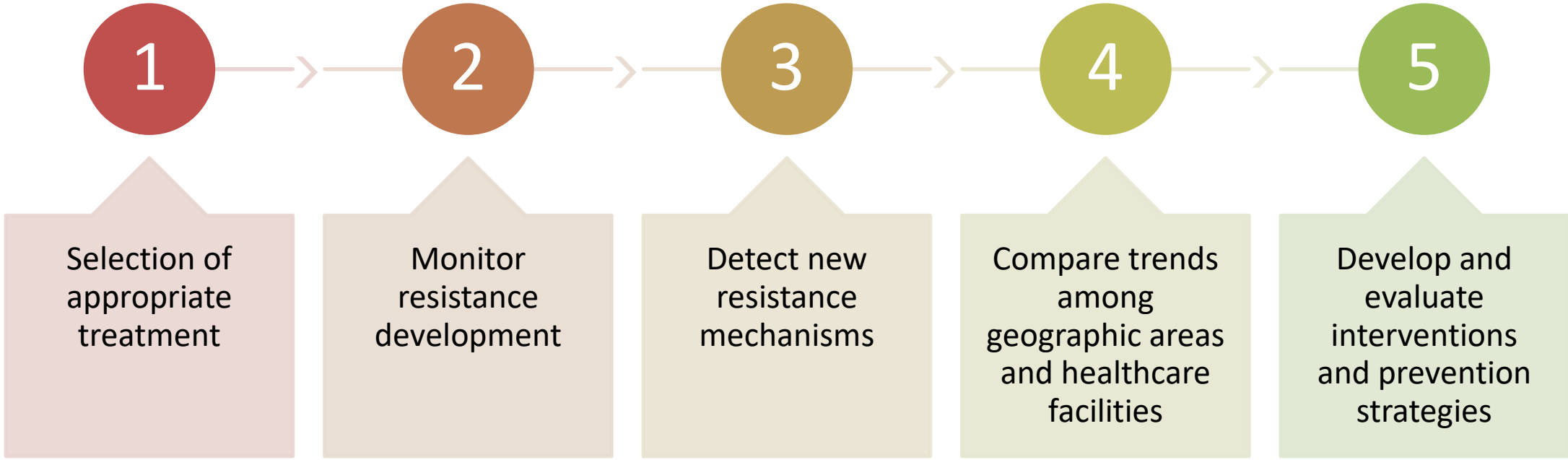
Conflicts of interest: none

Content Flow

Antimicrobial Susceptibility Testing (AST)
• Different methods of AST
• Breakpoints
• Epidemiological cut off
• Interpretation of AST reports
• Genotypic AST, POCT & Biomarkers

Antimicrobial Susceptibility Testing (AST)

Antimicrobial Susceptibility Testing (AST) - **Why is it necessary?**



Importance of appropriate sampling

- Collection technique, storage and transport varies for each sample

	Blood	CSF	Nasopharyngeal swabs
Collection and inoculation	Inoculate immediately on the blood-culture media to prevent clotting in the syringe	If N. meningitidis is suspected to be the cause of the illness and a delay of several hours in processing specimens is anticipated, incubating the CSF at 35°C in a 5% CO_2 atmosphere may improve bacterial survival	Inoculate the swab on appropriate medium for direct plating
Storage	Blood culture bottle can be kept at room temperature (20°- 25°C) for up to 8 hours. **Do not refrigerate**	**Do not refrigerate the CSF specimen** or expose it to temperature extremes	STGG can also be used for storage and transport (for a several hours at room temperature; for up to 8 weeks at -20°C; and, for at least 2 years at -70°C)
Transport	Sample should be receive by the laboratory within 12-18 hours for subculture. It should be protected from temperature extremes (<18°C or >37°C)	As soon as the CSF has been collected, it should be transported to the microbiology laboratory	Place the swab in STGG (Skim- milk tryptone glucose glycerol) transport medium for transportation to the laboratory
Recommendation	Ideally, the blood samples should be processed in a bacteriology laboratory within 2 hours of collection	Examine CSF sample within 1 hour of collection	Short-term storage of STGG is best at -70°C although a freezer at -20°C may also be used

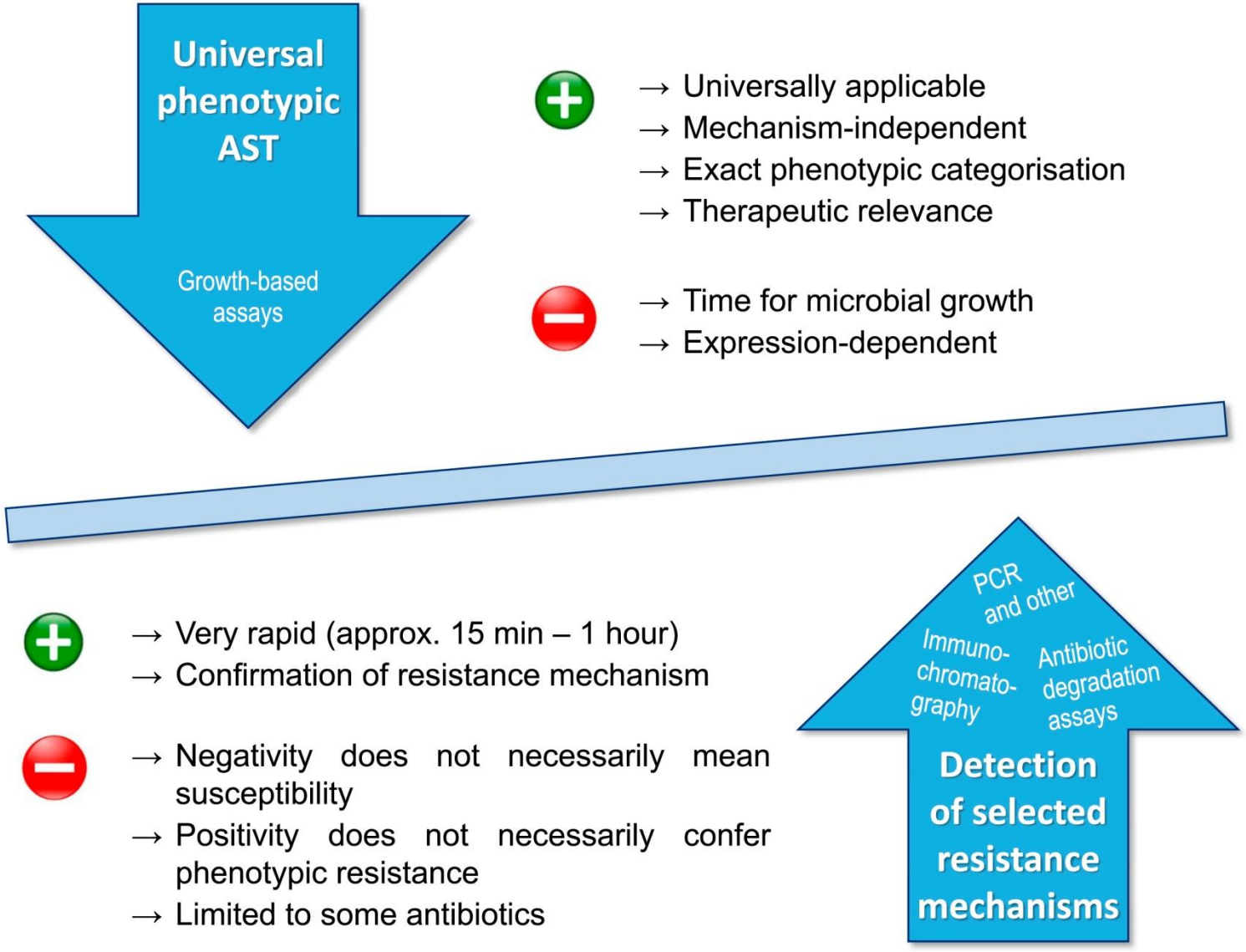
Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health concern in the developing world. WHO. Appendix 3, 5. Available at: https://www.who.int/csr/resources/publications/drugresist/en/VAMRManual.pdf?ua=1. Accessed on March 2021.

AST Testing Types

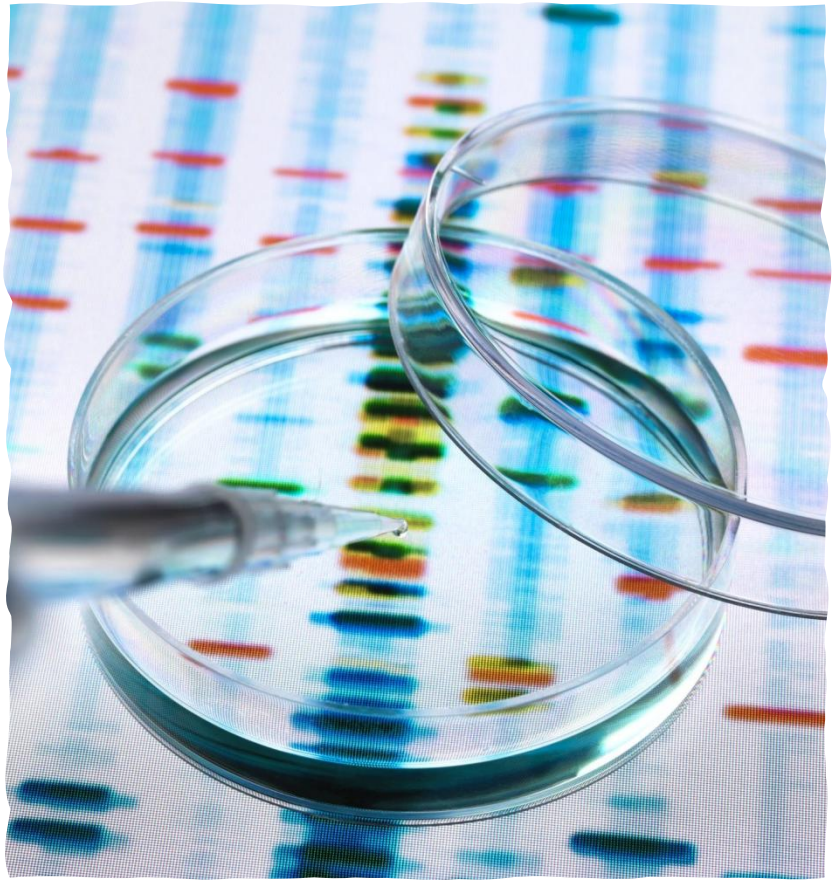
- **Phenotypic**
- **Genotypic**

An important limitation to genotypic testing is that resistance detection is not the same as susceptibility testing, i.e., a negative result does not necessarily imply susceptibility. A number of alternative resistance mechanisms can still cause microbial resistance and, hence, treatment failure. For instance, despite lack of carbapenemase detection Gram-negative bacteria may be resistant due to reduced permeability or increased efflux

In contrast, phenotypic susceptibility testing is universal, mechanism-independent and allows exact phenotypic categorization with direct therapeutic relevance.



AST Phenotypic Testing - Methodologies



Selection of an AST method may be based on numerous factors

- Ease of performance
- Flexibility
- Adaptability to automated or semi-automated systems
- Cost
- Reproducibility
- Reliability
- Accuracy
- Preference

Only few methods have been shown to be reproducible and repeatable:

- Disk diffusion
- MIC (Broth dilution)
- E – Test

What does AST provide?

Standardized testing may provide qualitative or quantitative results

- **Qualitative** results indicate how a drug may respond to a drug *in vivo*

Resistant: Treatment failure can be expected.

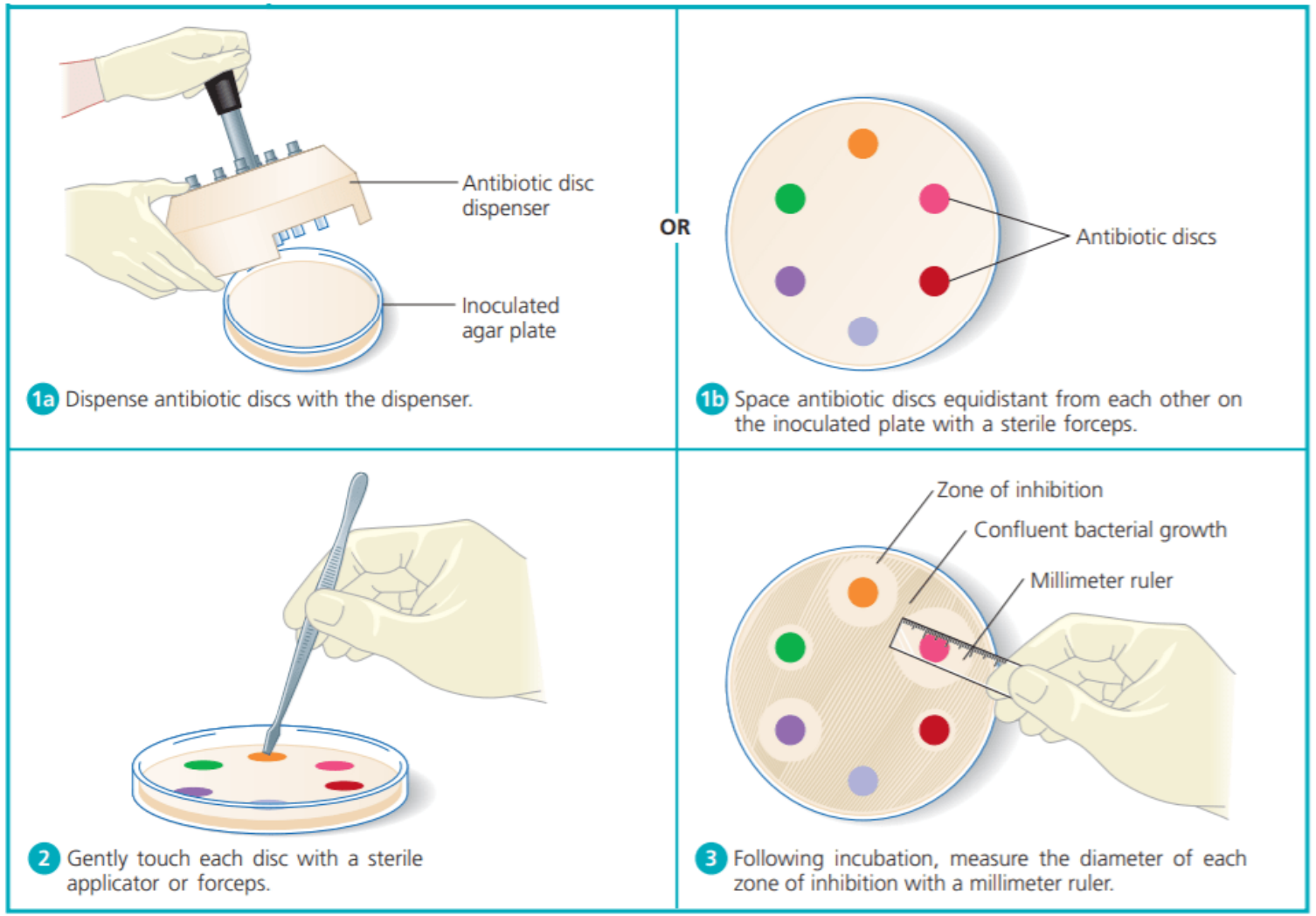
Intermediate: Treatment is possible at high dosages. It is also known as Susceptible Dose Dependent (SDD).

Sensitive: Successful treatment can be expected at the approved dosage.

- **Quantitative** results are reported as Minimum inhibitory concentration (MIC)
  - Lowest antimicrobial concentration that will inhibit the growth or kill the test organism over a define range related to the organism's growth rate. Most basic measurement of antimicrobial activity against a target organism

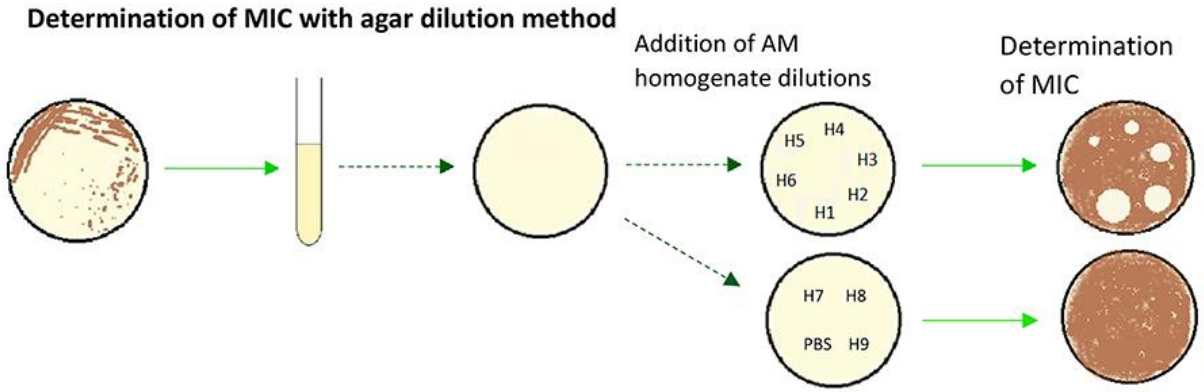
Kirby-Bauer Disc Diffusion Testing

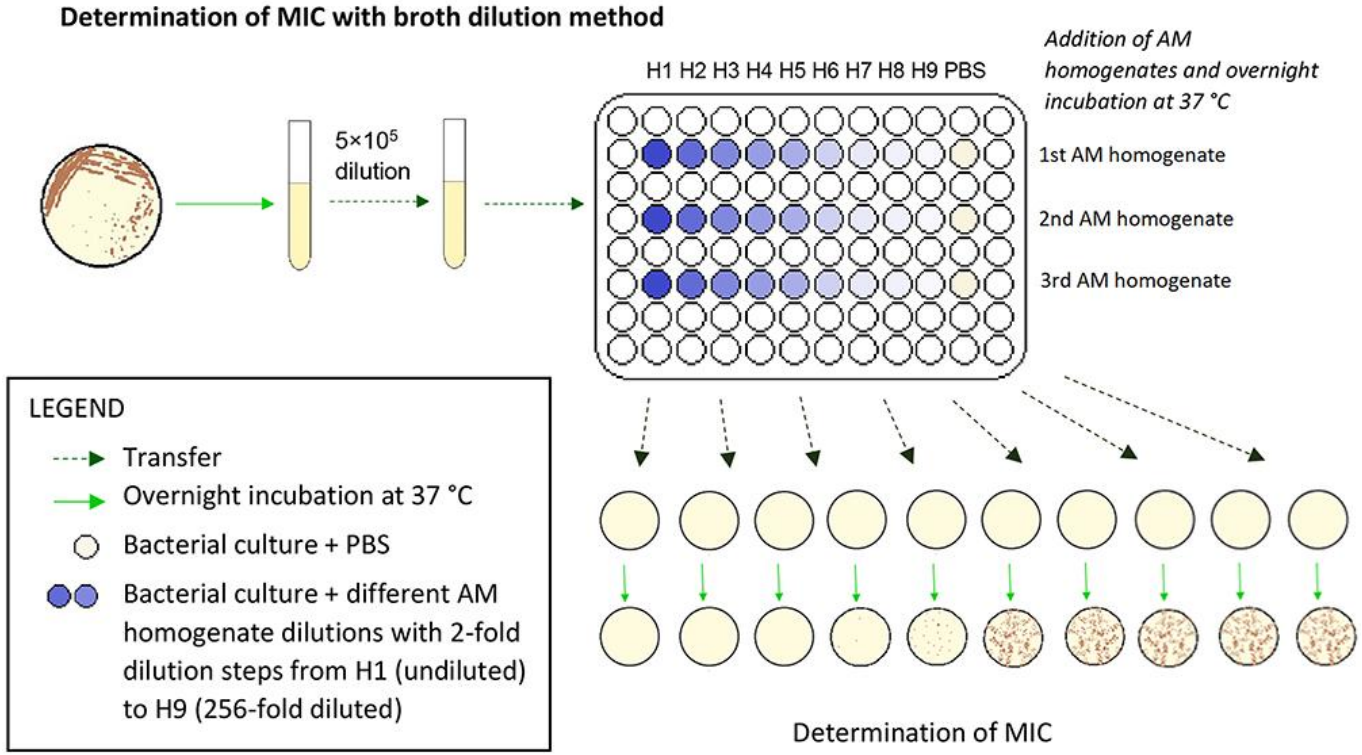
- **Principle:** A paper disk with a defined amount of antibiotic is used to generate a dynamically changing gradient of antibiotic concentrations in the agar in the vicinity of the disk
- The antibiotic contained in a reservoir is allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms.
- The disk is applied to the surface of an agar plate inoculated with the test organism.
  - The antibiotic diffuses out of the disk to form the gradient.
  - The test organisms start to divide and grow and progresses toward a critical mass of cells.
- Inhibition zone edge is formed at the critical time where a particular concentration of the antibiotic is just able to inhibit the organism before it reaches an overwhelming cell mass or critical mass.



MIC method

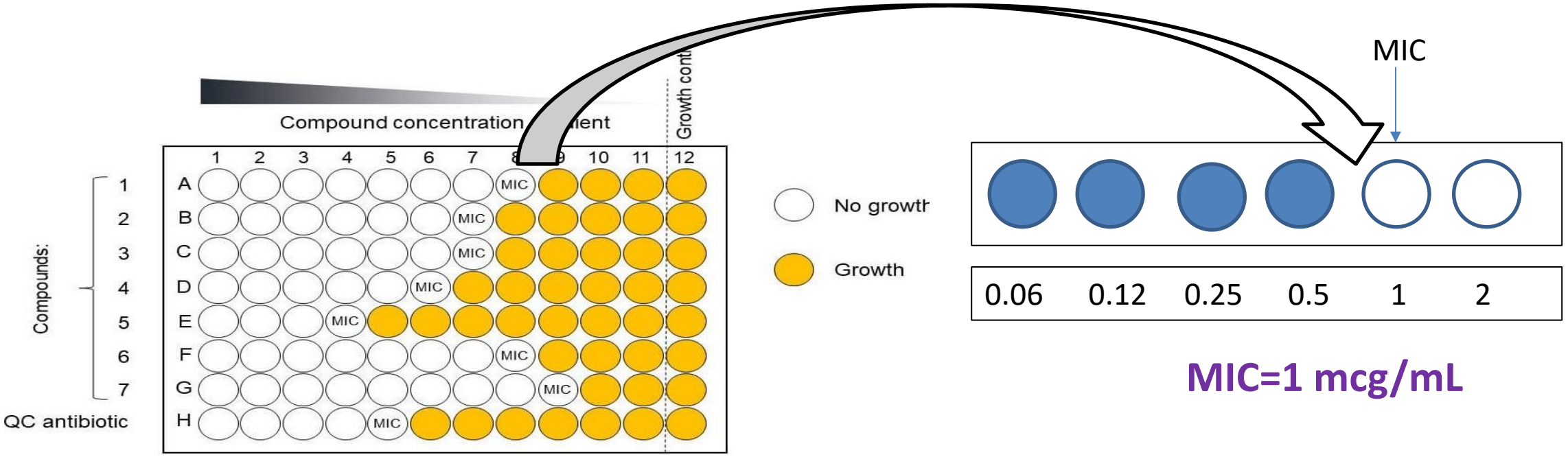
- The lowest concentration of drug that inhibits the growth of the bacteria isolated from the patient.
- The MIC is determined by inoculating the organism isolated from the patient into a series of wells/plates containing progressive dilutions of the drug
  - Patient's organism is added to wells/plates containing decreasing amounts of the antibiotic
  - Incubation at 37°C overnight
  - Lowest concentration of drug that inhibits growth is the Minimum Inhibitory Concentration (MIC)





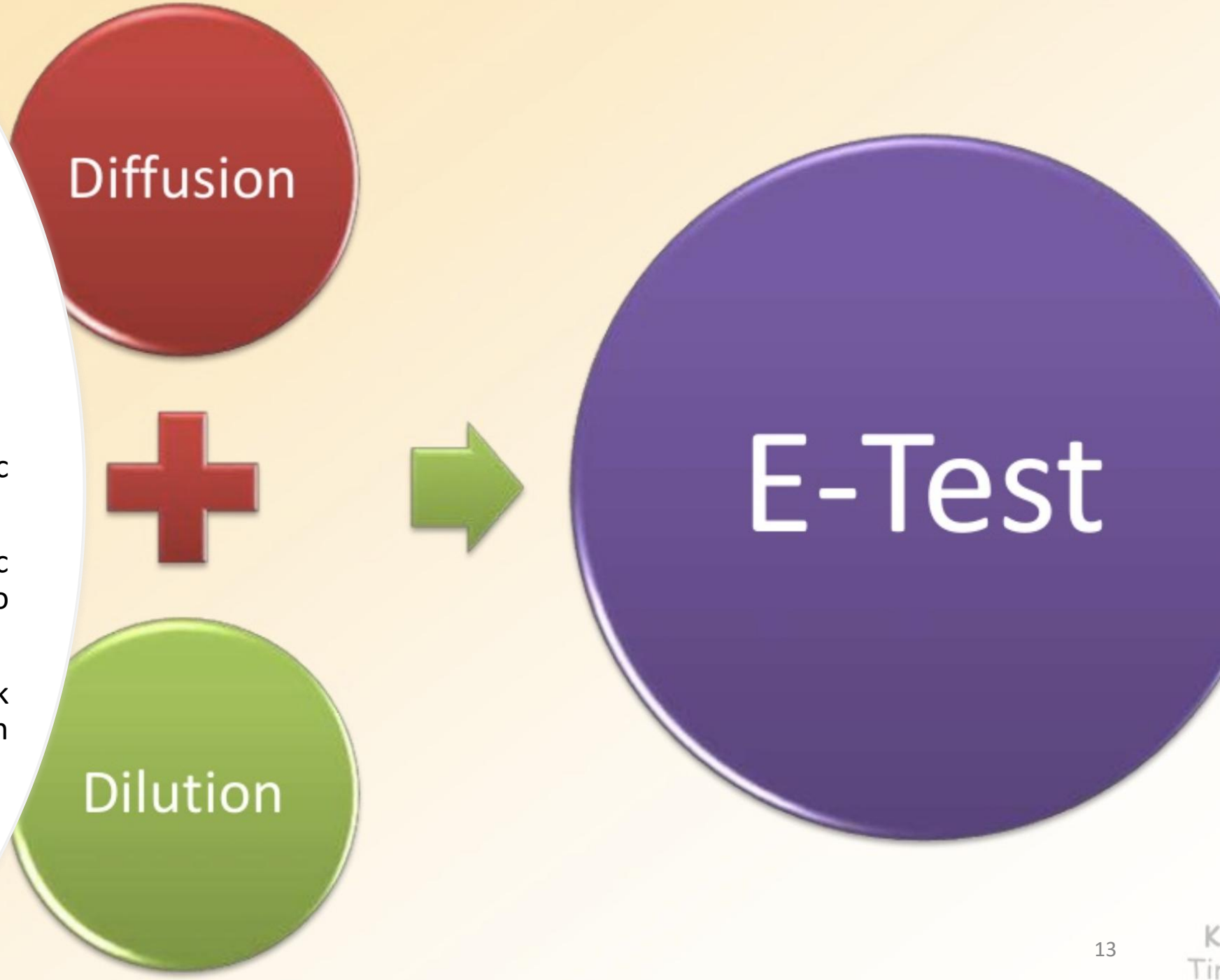
Broth MIC Interpretation

- The broth microdilution test is the widely used method for determining levels of resistance to an antibiotic.
- The lowest concentration of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC)

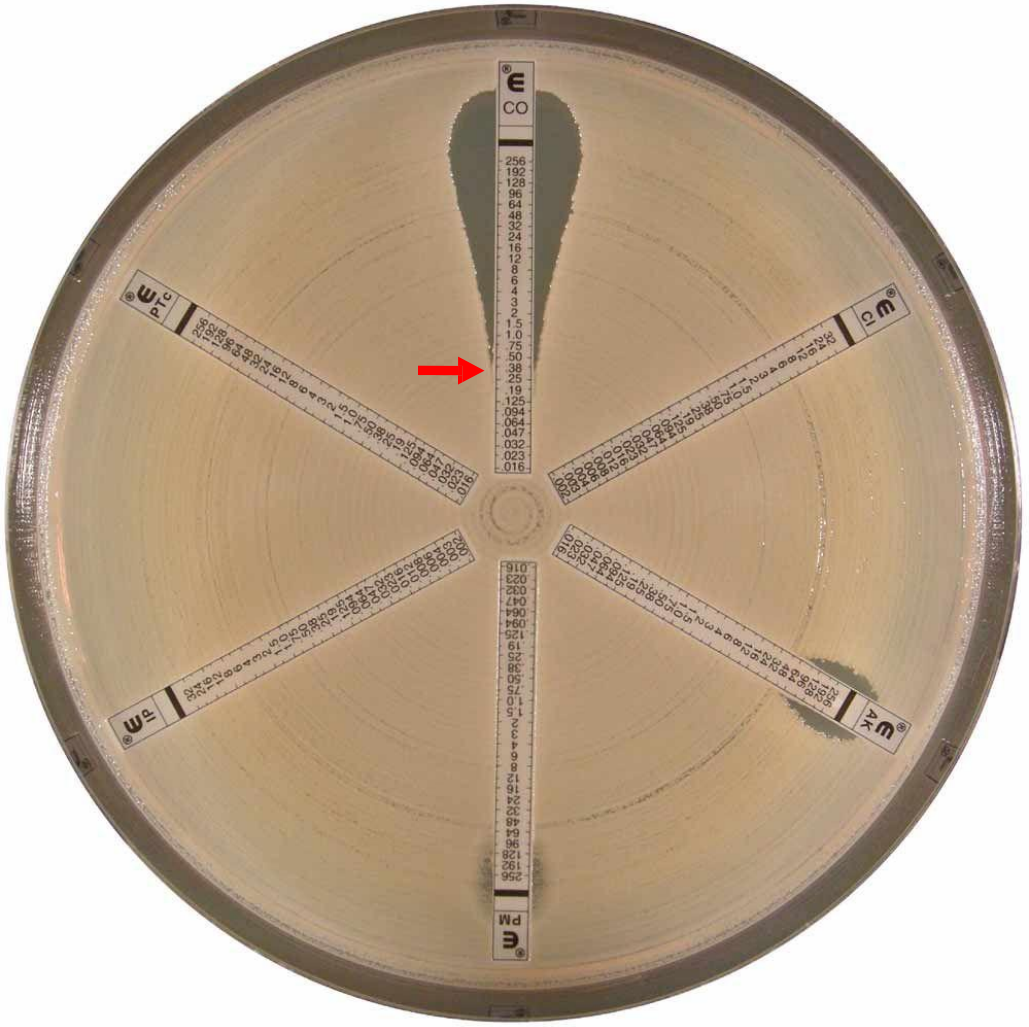


E-test

- Epsilometer Test
- Quantitative method of antibiotic sensitivity testing.
- Applies both dilution of antibiotic and diffusion of antibiotic into the medium.
- Combines the principles of disk diffusion and agar dilution methods
- Generates MIC values



Antimicrobial Gradient Testing - E-test



- Polymer strip with graded concentration of antibiotic is placed on a plate with spreaded test pathogen
- After incubation, the strip develops zone of inhibition
- The lowest part of strip from where the inhibition zone starts would be considered as MIC
- The graded concentration gives widest range and provides an accurate MIC
- As per the MIC the pathogen is categorized as Susceptible, Intermediate or resistant

Automated Microbiology instruments

- Advanced method of AST
- Completely Automated
- Broth microdilution method is used for testing
- It also identifies the bacteria and provides complete antibiogram of pathogen isolated from patients
- This method provides direct susceptibility pattern and guidance about possible antibiotic treatment

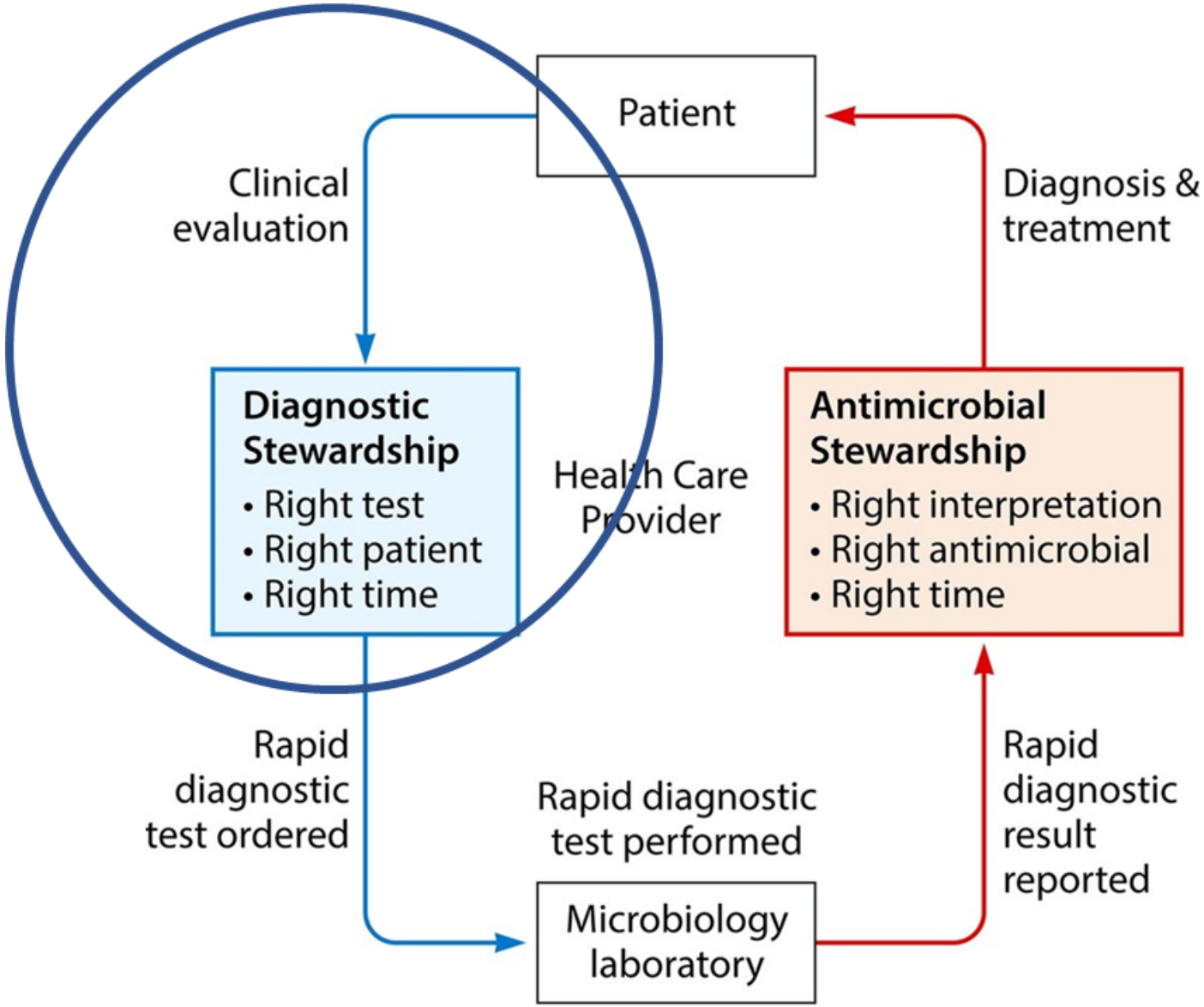
1. MicroScan WalkAway *plus* System: https://www.beckmancoulter.com/products/microbiology/microscan-walkaway-plus-system Accessed on 2nd February 2020.
2. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.
3. The Vitek 2 System: https://www.biomerieux-usa.com/vitek-2 Accessed on 2nd February 2020.

Disc diffusion	MIC	E-test	Automated Microbiology System
Discs with fixed conc of antibiotics	Agar/Broth dilution	Combination of Disc diffusion & MIC	Used for both Identification & AST
Qualitative	Quantitative	Easy to perform	Advanced method
Inhibition zone is interpreting criterion	Inhibition concentration is interpreting criterion	Paper Strips with graded antibiotic concentration	Broth microdilution method
Less Reproducible Data	Gold Standard of AST	Generates MIC value	Quick & provides detail antibiogram

Reports Interpretation dilemmas

- Will the physician understand the test result?
- Will the physician appropriately modify antimicrobials based on test results?
- Will the physician act promptly on the test result?
- Both diagnostic and antibiotic stewardship are required to optimize use of resources and outcomes

Roles of Clinical Microbiologist in Diagnostic Stewardship



Interpretation

The main concept is the "*clinical categorization*"

- There is a well-developed system of interpreting the results of all these methods and it is called as Breakpoints of antibiotics.
- Breakpoints help to categorize the pathogen into the either of the 3 categories, susceptible, intermediate or resistant.
- Strains are sorted according to level of Minimal Inhibitory Concentration (MIC) versus reference breakpoints

Susceptible			Intermediate			Resistant
MIC	≤	**c**	<	MIC	≤	**C**	< MIC

c and C are the breakpoints..

Interpreting the AST results

- Optimal interpretation of MICs requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites
- Common terminologies used are,
  - **Susceptible (S)**: indicates that the patient's organism should respond to therapy with that antibiotic using the dosage recommended normally for that type of infection and species
  - **Resistant (R)**: indicates that an organism should not be inhibited by the concentrations of the antibiotic achieved with the dosages normally used with that drug
  - **Intermediate (I)**: indicates that clinical response is likely to be less than with a susceptible strain.

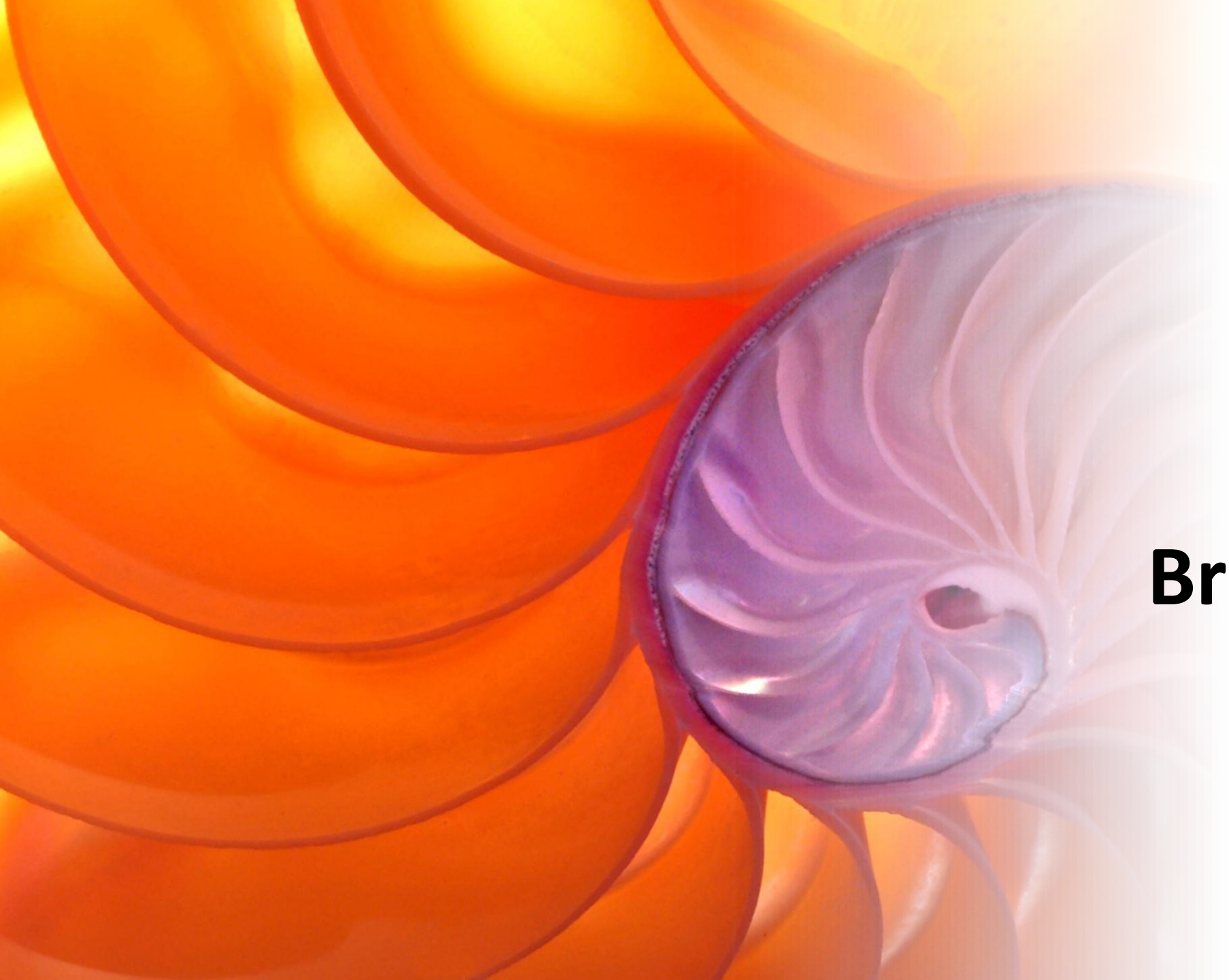
Jorgensen JH, et al. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clinical Infectious Diseases. 2009;49(11):1749-1755.

MICs vs BREAKPOINTS

MIC: Minimum concentration of an antibiotic needed to inhibit visible growth of a single isolate of an organism. Important for definitive treatment of an individual patient.

Breakpoint: Discriminatory concentrations used in the interpretation of results of susceptibility testing to define isolates as susceptible, intermediate, or resistant (determined by various organizations - FDA, CLSI, EUCAST)

Updates on antimicrobial stewardship. Available at: https://www.sahealth.sa.gov.au/wps/wcm/connect/6b86eddf-3bd4-4dfc-ada4-2ccc95244318/MICS_Breakpoints_AMS_Updates_Issue1__2018_SAAGARversion.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-6b86eddf-3bd4- 4dfc-ada4-2ccc95244318-niQTcCC. Accessed on Feb 2021.



Breakpoints

The pharmacological concept for breakpoints

- The concentration range tested for a drug and the interpretative criteria for various categories are based on extensive studies that correlate with
  - Serum achievable levels for each antimicrobial agent
  - Particular resistance mechanisms
  - Successful therapeutic outcome
- **In practice situations the entire range may not be used for decision making and therefore the breakpoint is needed..**

Agencies That Determine **Antimicrobial Breakpoints**

- In the United States, the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards, and the Food and Drug Administration (FDA) are responsible for setting breakpoint standards. In Europe, the main agencies are the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) and the European Medicines Agency (EMA).
- The 2 main, nongovernmental agencies, CLSI and EUCAST, differ in philosophy and approach toward setting antimicrobial breakpoints as well as in how they report to their respective governmental counterparts, the FDA and EMA (Table).

	CLSI	EUCAST
Members	Representatives from different fields and agencies	Representatives from different fields and agencies
Role of government	Recognized by FDA, but FDA still determines its own breakpoints. Breakpoints determined by FDA can be modified by CLSI after 2 yr	Functions as breakpoint committee for EMA
Role of industry	Part of decision process	Consultative role
Decision making	Consensus by group votes	Consensus of executive committee (no vote)
Funding	Industry, government and sales of documents	ESCMID, ECDC, government
Data and Rationale documents	For sale www.clsi.org	Free www.eucast.org
Meetings	2 per year	5 per year
Breakpoints	Clinical breakpoints Retains intermediate category, expanding concept of SDD (susceptible dose dependent)	Epidemiological cutoffs Clinical breakpoints Largely abandoned intermediate category

CLSI

EUCAST

Such kind of agencies are required in India as well

Matthew J. Labreche, Christopher J. Graber, Hien M. Nguyen, Recent Updates on the Role of Pharmacokinetics-pharmacodynamics in Antimicrobial Susceptibility Testing as Applied to Clinical Practice, *Clinical Infectious Diseases*, Volume 61, Issue 9, 1 November 2015, Pages 1446–1452

Reference Materials for Execution/Interpretation

- **CLSI** (www.clsi.org)
  - Methods dilution AST for bacteria that grow aerobically, approved standard (M7)
  - Performance standards disks susceptibility testing (M02)
  - Methods for dilution and disk susceptibility testing of infrequently isolated or fastidious organisms, approved guideline (M45)
  - Performance standards, informational supplement (M100)
  - Performance standards disk and dilution susceptibility - bacteria isolated from animals, approved standard (VET01-A4)
  - Performance standards disk and dilution susceptibility - bacteria isolated from animals, informational supplement (VET01-S2)

Reference Materials for Execution/Interpretation

- **EUCAST** (www.eucast.org)
  - – Disk diffusion method by EUCAST http://www.eucast.org/eucast_disk_diffusion_test/
    - General information
    - Implementation guidelines
    - Methodology (prep media, test)
    - Interpretative criteria
    - Diameter distributions
    - QC tables
  - – MIC testing based on CLSI method
  - – QC tables
  - – Epidemiological cut-offs
  - – Clinical breakpoints
  - – Databases (e.g. www.eucast.org):
    - MIC distributions
    - DD distributions

Understanding Breakpoints

- Every category (S, I, R) specifies the possibility of successful treatment of pathogen by a particular antibiotic.
- These categories are decided on the basis of breakpoints. The process of establishing a breakpoint is very crucial as it is the main driver of antibiotic treatment.
- **Breakpoints are determined using the following multidisciplinary approaches:**
    - Step 1: **Microbiological** - Epidemiological cut-off
    - Step 2: **Pharmacological** - PK-PD index
    - Step 3: **Clinical**

Interpreting “**Intermediate**” Category

- Similar terminologies for this category are – **“Susceptible-Dose Dependent” and “Susceptible, Increased Exposure”.**
- Sometime the antibiotic can still be used
  - Higher doses required to ensure efficacy
  - Antibiotic may be efficacious if concentrated in vivo in an infected body fluid (e.g., urine)
- Sometimes there is uncertainty
  - Intermediate resistance may represent a “buffer” zone that prevents strains with borderline susceptibility from being incorrectly categorized as resistant
  - Trained professionals with good quality material can 90-95% of the time attain:
    - a target MIC value +/- 1 dilution
    - a target zone diameter +/- 2 mm

Zone Diameter and Minimal Inhibitory Concentration Interpretive Standards for *Enterobacteriaceae*

Test/ report group	Antimicrobial agent	Disk content	Zone Diameter Interpretive Criteria (nearest whole mm)				MIC Interpretive Criteria (µg/mL)			
			S	SDD	I	R	S	SDD	I	R
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS (Pseudomonas aeruginosa)										
B	Piperacillin-tazobactam	100/10 µg	≥ 21		15–20	≤ 14	≤ 16/4		32/4-64/4	≥ 128/4
O	Ticarcillin-clavulanate	75/10 µg	≥ 24		16–23	≤ 15	≤ 16/2		32/2-64/2	≥ 128/2
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS (Enterobacteriaceae)										
B B B B	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanate	20/10 µg 10/10 µg 100/10 µg 75/10 µg	≥ 18 ≥ 15 ≥ 21 ≥ 20		14-17 12-14 18-20 15-19	≤ 13 ≤ 11 ≤ 17 ≤ 14	≤ 8/4 ≤ 8 /4 ≤ 16/4 ≤ 16/2		16/8 16/8 32/4-64/4 32/2-64/2	≥ 32 /16 ≥ 32 /16 ≥ 128/4 ≥ 128/2
A	Cefazolin	30 µg	≥ 23		20-22	≤ 19	≤ 2		4	≥ 8
C	Ceftaroline	30 µg	≥ 23		20-22	≤ 19	≤ 0.5		1	≥ 2

M100-S25 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards. 30th edition. 2015;35(3) page 45 & 53.

EXTRAPOLATING ANTIBIOTIC SUSCEPTIBILITY RESULTS

Antibiotic	Further extrapolation to other antibiotics
Amoxicillin	Predicts susceptibility of ampicillin.
Cephalexin	Predicts susceptibility of all first-generation cephalosporins, except cefazolin.
Clindamycin	Predicts susceptibility of lincomycin. Should not be used in horses, rabbits, and other herbivores. Not effective against aerobic gram-negative bacteria.
Erythromycin	Predicts susceptibility of azithromycin and clarithromycin. Not effective against aerobic gram-negative bacteria.
Oxacillin	Predicts susceptibility to methicillin.
Trimethoprim/sulfa	Predicts susceptibility of other potentiated sulfonamides.

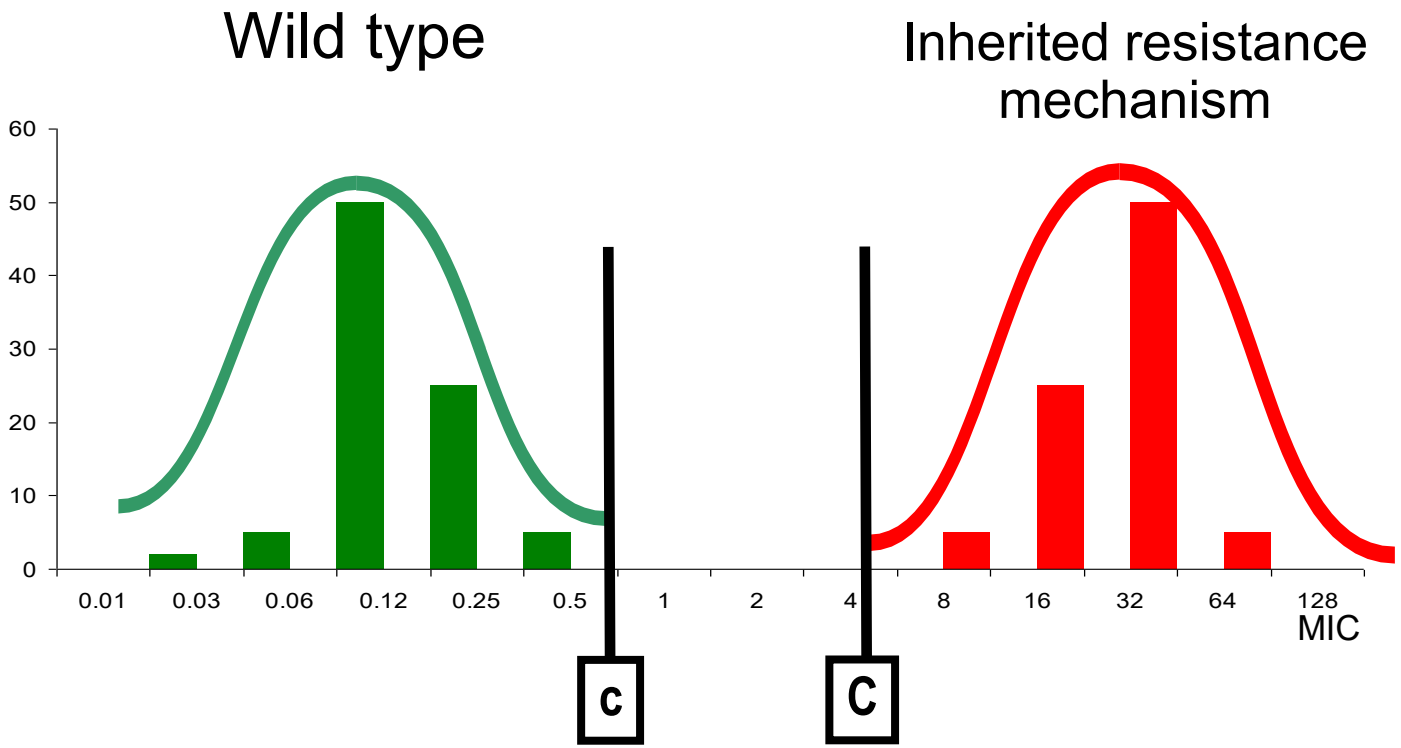
ORGANISMS INTRINSIC RESISTANCE TO ANTIBIOTICS

Organism	Intrinsic resistance
Bacteroides (anaerobes)	aminoglycosides, many β-lactams, quinolones
All gram positives	aztreonam
Enterococci	aminoglycosides, cephalosporins, lincosamides
Listeria monocytogenes	cephalosporins
All gram negatives	glycopeptides, lipopeptides
Escherichia coli	macrolides
Klebsiella spp.	ampicillin
Serratia marcescens	macrolides
Pseudomonas aeruginosa	sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, tetracycline
Stenotrophomonas maltophilia	aminoglycosides, β-lactams, carbapenems, quinolones
Acinetobacter spp.	ampicillin, glycopeptides

Epidemiological Cut off Value

Abbreviated as **ECV** (CLSI) or **ECOFF** (EUCAST), are measures of a drug MIC distribution that separate bacterial populations into those representative of a wild type population, and those with acquired or mutational resistance to the drug.

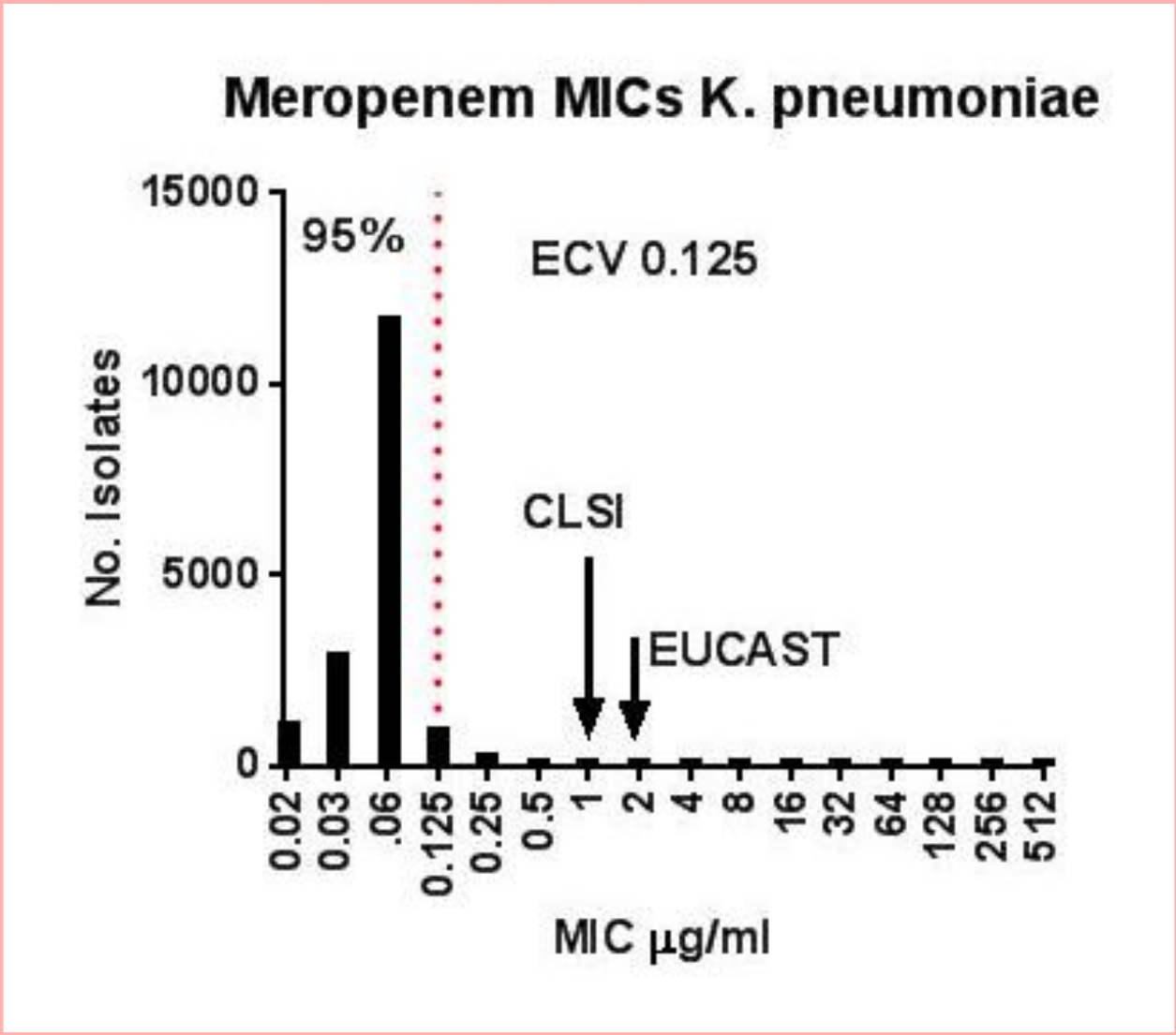
Epidemiological Cutoff Values



The minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without acquired and/or mutational resistance based on their phenotypes (wild-type or non-wild-type). The ECV defines the upper limit of susceptibility for the wild-type population of isolates.

- **Wild-type** – an ECV interpretive category defined by an ECV that describes isolates with no mechanisms of acquired resistance or reduced susceptibility for the antimicrobial agent being evaluated.

- **Non-wild-type** – an ECV interpretive category defined by an ECV that describes isolates with presumed or known mechanisms of acquired resistance and reduced susceptibility for the antimicrobial agent being evaluated.



Here you can see that the population is almost all wild type, with 95% of the population being wild type. The ECV, **≦0.125 mg/L**, is well below the indicated CLSI and EUCAST breakpoints.

Source: P Edelstein, 3/20/2017, updated 1/13/2021; http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/ECV.htm

Step 1. Population Data

K pneumoniae | meropenem

MIC	Log_2MIC	Raw Count	Cum. Count	Fitted
0.001	-10	0	0	0.0
0.002	-9	0	0	0.0
0.004	-8	0	0	0.0
0.008	-7	273	273	0.0
0.016	-6	1206	1479	60.5
0.03	-5	3021	4500	4530.6
0.06	-4	11781	16281	11584.9
0.125	-3	1022	17303	1341.3
0.25	-2	355	17658	4.2
0.5	-1	188	17846	0.0
1	0	128	17974	0.0
2	1	78	18052	0.0
4	2	49	18101	0.0
8	3	32	18133	0.0
16	4	33	18166	0.0
32	5	4	18170	0.0
64	6	1	18171	0.0
128	7	0	18171	
256	8	0	18171	
512	9	0	18171	
1024	10	0	18171	

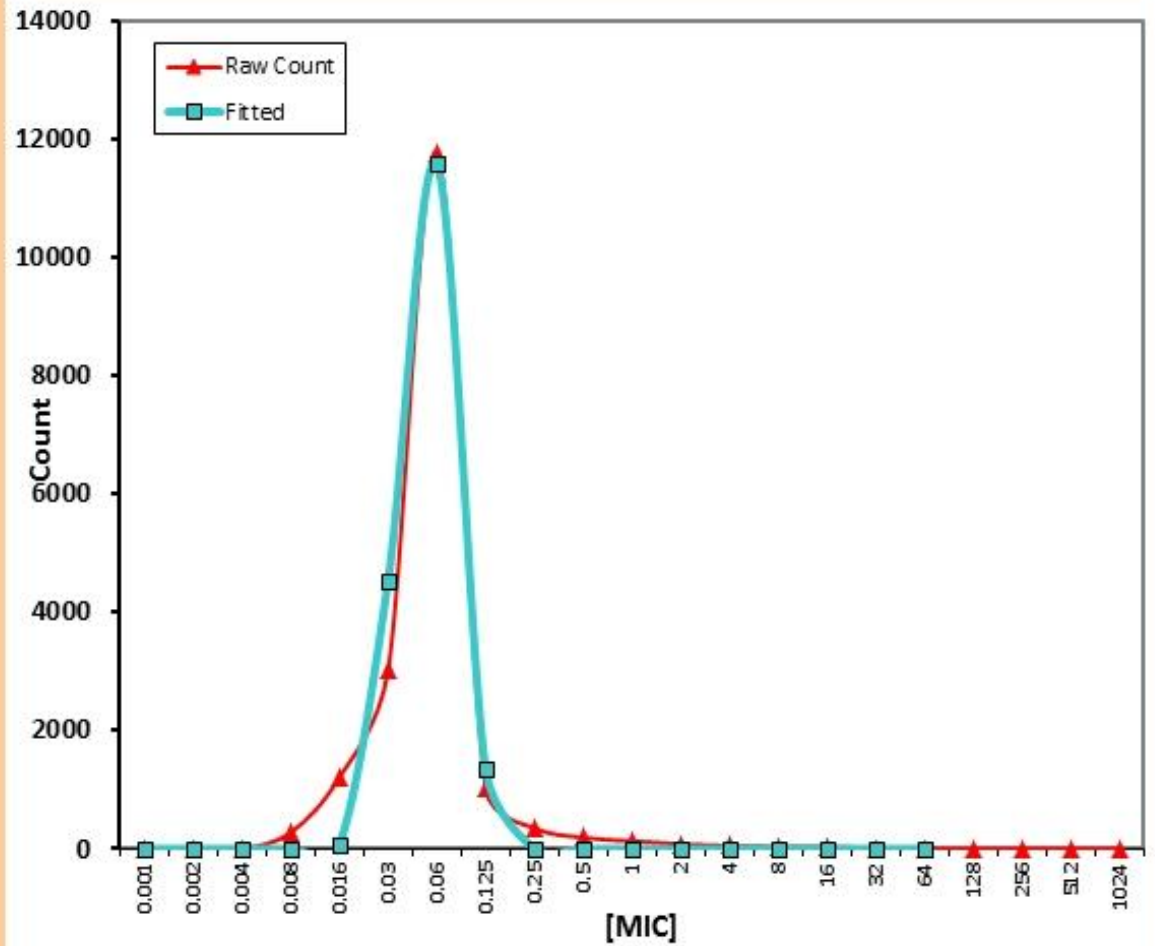
Enter/paste data in this column

Clear All

Modal MIC	0.063
Log_2MIC Mode	-4
Max Log_2MIC	6
Selected Log2 Mean	-4.69 =0.04
Selected Log2 SD	0.484

Selected CO_{WT} Values		%>
ECOFF 95.0%	0.125	4.8%
ECOFF 97.5%	0.125	4.8%
ECOFF 99.0%	0.125	4.8%
ECOFF 99.9%	0.125	4.8%

REVIEW AREA



Source: P Edelstein, 3/20/2017, updated 1/13/2021; http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/ECV.htm

Step 2. Estimate N, Mean and Standard Deviation

K pneumoniae | meropenem

	Mode MIC	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤	Subset ≤
Log_2 value	-4	-3	-2	-1	0	1	2	3	4	5	6
Value	0.063	0.125	0.250	0.500	1.000	2.000	4.000	8.000	16.000	32.000	64.000
Starting Values N_{est}→		16491.7	17521.6	17631	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
Starting Values Log2 mean→		-4.7957	-4.6913	-4.684	-4.6782	-4.6738	-4.6704	-4.6677	-4.6654	-4.6635	-4.6621
Starting Values Log2 SD→		0	0.48447	0.49336	0.50022	0.50546	0.50954	0.51277	0.51548	0.51762	0.51933

Selected Log_2 Subset <=
-2
Selected Subset Log_2 Mean
-4.691
Selected Subset Log_2 SD
0.484

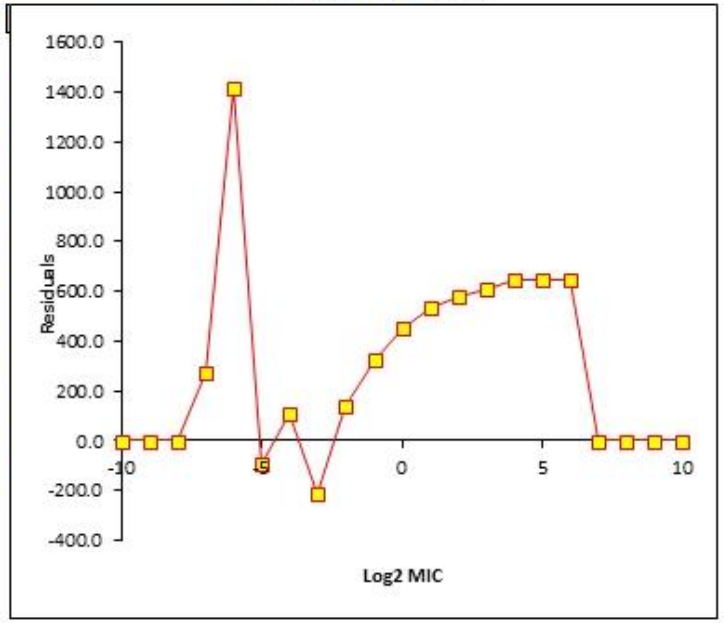
Max Log_2 MIC
6

Solve

Please review results also on the Data Entry sheet after solving

Log2MIC	Raw Count	Cum N Actual	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred	N Pred
-10	0	0	#NUM!	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0	0	#NUM!	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0	0	#NUM!	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	273	273	#NUM!	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
-6	1206	1479	#NUM!	60.5	67.4	72.9	77.4	80.9	83.8	86.2	88.2	89.7
-5	3021	4500	#NUM!	4591.1	4600.0	4607.2	4613.0	4617.6	4621.3	4624.5	4627.1	4629.2
-4	11781	16281	#NUM!	16176.1	16170.8	16166.3	16162.3	16159.0	16156.2	16153.8	16151.7	16150.0
-3	1022	17303	#NUM!	17517.3	17625.4	17710.7	17777.0	17829.0	17870.5	17905.6	17933.3	17955.5
-2	355	17658	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
-1	188	17846	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
0	128	17974	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
1	78	18052	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
2	49	18101	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
3	32	18133	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
4	33	18166	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
5	4	18170	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
6	1	18171	#NUM!	17521.6	17631.0	17717.7	17785.2	17838.3	17880.8	17916.7	17945.1	17967.8
7	0	18171										
8	0	18171										
9	0	18171										
10	0	18171										

Residuals Plot for Best Fit



Stop now		18	19	20	21	22	23	24	25	26	27
Sum-of-squares	#NUM!	2170463	2240212	2328115	2417297	2500241	2574560	2645659	2702630	2748536
N	17303	17658	17846	17974	18052	18101	18133	18166	18170	18171
ABS(N − $N_{...}$)	811.3	136.4	215.0	256.3	266.8	262.7	252.2	249.3	224.9	203.2
Relative Diff	0.04689	0.00773	0.01205	0.01426	0.01478	0.01451	0.01391	0.01372	0.01238	0.01118

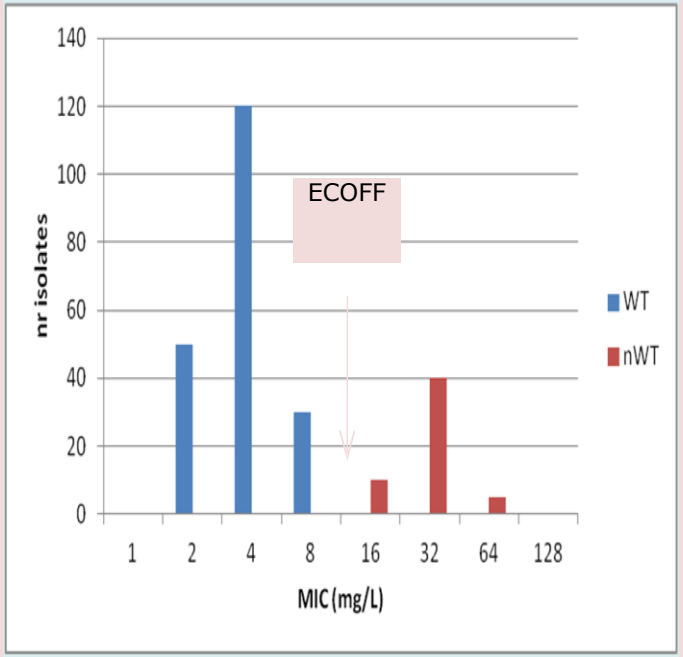
95% Subset ECOFF=	#NUM!	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
97.5% Subset ECOFF=	#NUM!	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
99% Subset ECOFF=	#NUM!	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
99.9% Subset ECOFF=	#NUM!	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
%Obs > 95%	#NUM!	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%
%Obs > 97.5%	#NUM!	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%
%Obs > 99%	#NUM!	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%
%Obs > 99.9%	#NUM!	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%	4.8%

Selected ECOFF values
0.125
0.125
0.125
0.125
4.8%
4.8%
4.8%
4.8%

Source: P Edelstein, 3/20/2017, updated 1/13/2021; http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/ECV.htm

Guidelines For Interpretation- Epidemiological Cut-off

Most adequate for monitoring purposes and detection of resistances



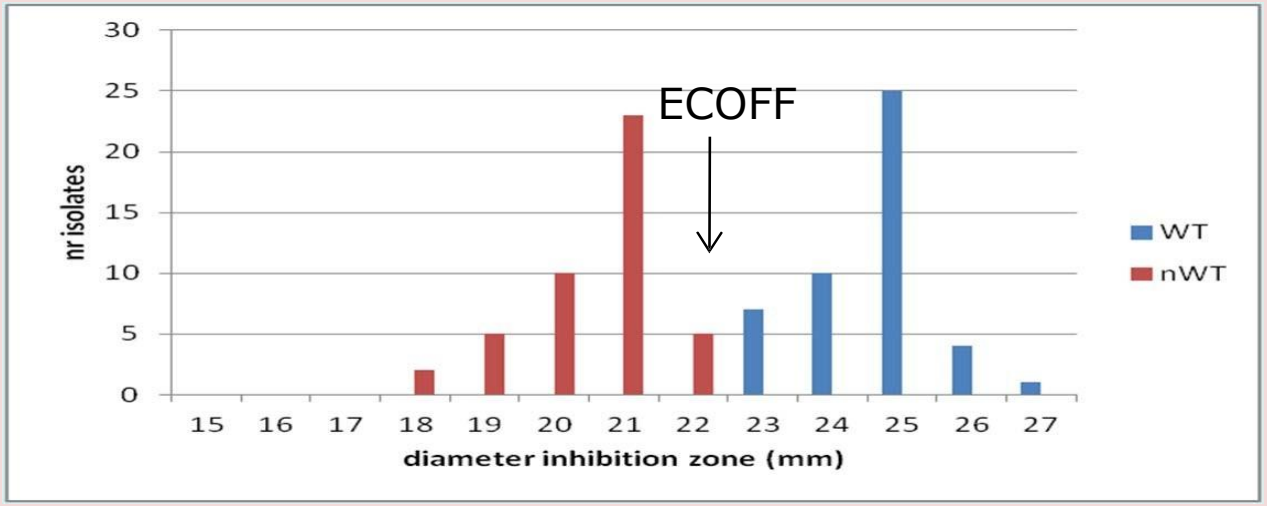
Epidemiological cut offs are normally used (www.eucast.org):

- **Wild Type (WT)**: Strain belongs to normal distribution for particular species vs antimicrobial drug.
- **Non Wild Type (nWT)**: Likely to harbour a resistance determinant

GUIDELINES

For Interpretation- Epidemiological Cut-off

ECOFFs are also available for some disk diffusion inhibition zone diameter distributions



- **Wild Type (WT)**: Strain belongs to normal distribution for particular species vs antimicrobial drug
- **Non Wild type (nWT)**: Likely to harbour a resistance determinant

M100 S28 Tables G1-G3 ECVs

Table G1. Epidemiological Cutoff Values for *Enterobacteriaceae*

Antimicrobial Agent	Disk Content	Zone Diameter ECV (mm)		MIC ECV (µg/mL)		Comments
		WT	NWT	WT	NWT	
Azithromycin[1-5]	15 µg	≥ 16	≤ 15	≤ 8	≥ 16	For use with *S. flexneri*. See Table 2A for azithromycin and *Salmonella* spp.
	–	–	–	≤ 16	≥ 32	For use with *S. sonnei*.
Colistin	–	–	–	≤ 2	≥ 4	For use with *K. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae* and *R. ornitholytica*. **The only approved method for testing colistin is MIC by broth microdilution. Disk diffusion and gradient diffusion methods should not be used.**

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non wild type; WT, wild type

Table G2. Epidemiological Cutoff Values for *Neisseria gonorrhoeae*

Antimicrobial Agent	MIC ECV (µg/mL)		Comments
	WT	NWT	
Azithromycin[1-3]	≤ 1	≥ 2	For use with *N. gonorrhoeae*.

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type

Table G3. Epidemiological Cutoff Values for Specific Anaerobic Species

Antimicrobial Agent	MIC ECV (µg/mL)		Comments
	WT	NWT	
Vancomycin	≤ 2	≥ 4	For use with *Cutibacterium (Propionibacterium) acnes*[1-4] and *Clostridioides (Clostridium) difficile*[5-7].

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type

M57 Guidance on Reporting ECVs

Table 2. Example Report Explaining the Significance of ECVs for Yeasts

Isolate	Antifungal Agent	MIC	ECV	Suggested Comment for Discussion and for the Report
Candida lusitaniae	Anidulafungin	2 µg/mL	1 µg/mL	There are currently no breakpoints or interpretive criteria for *C. lusitaniae* and anidulafungin. The anidulafungin MIC is above the WT MIC, which suggests that this isolate may have an acquired mechanism of resistance and could be considered NWT. The clinical implication of finding a NWT MIC is currently unknown. If the agent in question is being used for treatment, the patient should undergo clinical review, and in consultation with an infectious diseases physician or pharmacist, the decision should be made to continue the agent at the current dose, increase the dose of the agent, or switch to an alternative agent.

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

M57 Guidance on Applying ECVs

WHEN NOT TO USE EPIDEMIOLOGICAL CUT OFF VALUE:

- When Breakpoints have been published
- As a strong predictor of clinical response to therapy but rather as an indicator of possible acquired resistance mechanisms that could affect response to treatment

Problems with ECVs Being "The Breakpoint"

- **Does Not Account For PK/PD Or Clinical Response Correlations**
  - Just Because An Isolate Is "Wild Type", Therefore Has No Known Resistance Mechanism To The Antimicrobial, Does NOT Mean That Concentrations Are Adequate Or That PD Targets Are Met
    - May Not Be Able To Administer Adequate Doses Due To Toxicity (Colistin)
  - Conversely, Just Because An Isolate Is "Non-Wild Type", And Has A Known Resistance Mechanism, Does NOT Mean That The PD Targets Are Not Met
    - For Some Cephalosporins Animal Studies Demonstrate That PD Targets Are Met For Some Organisms With Relevant Resistance Mechanisms

Therefore

Need To Make Certain That Labs Do NOT Report Or Imply That ECVs Predict Clinical Response

Otherwise

Patients May Be Harmed

Interpreting a C/S Report

WHAT IT TELLS YOU

Identifies bacteria/fungus present (most of the time)

Sensitivity results based on lab data

- Human vs lab

WHAT IT DOES NOT TELL YOU

Does not identify infection vs colonization vs contamination

Don't treat colonization or contamination

Does not tell you which antibiotic to use

Susceptibility testing is an *in vitro* phenomenon and does not necessarily reflect or predict *in vivo* efficacy.

Susceptibility testing is subject to great variability depending on pathogen tested, media used, conditions of incubation, and method of accessing bacterial growth

Case study-
What does AST for MDR organisms look like?

- **Patient name:** Mr. XYZ

- **Age:** 49 years
- **Medical history:** The patient was admitted into a XYZ nursing home with complaints of fever, chills and persistent coughing.
- Patient had been put on following medications: Amoxycillin (500mg) + Clavulanic Acid (125mg), Azithromycin 250mg and Paracetamol 650mg.
- The patient started complaining of blood in mucus, severe chest pain and even shortness of breath. He has been referred to ABC multispecialty hospital for advanced care. Headmitted to ICU ward and put on ventilator.

- **Diagnostics test:**

- Trans-Tracheal aspiration was performed to collect sample from the
- lungs and lower airways for antimicrobial susceptibility testing using automated method.
- Collected specimen was containing *Klebsiella pneumoniae* identified by their morphology and biochemical characteristics. In Gram staining, Gram-negative, short, blunt rods were seen.
- The antimicrobial sensitivity of the test strains of Gram Negative antibacterial drugs was done using the automated method.

Susceptibility testing report

Date: 16-01-2023

Name: Mr. ABC Age: 49 years
Gender: Male

Antibiotic	MIC	
	µg/ml	Interpretation
Meropenem	8	R
Imipenem	8	R
Piperacillin-tazobactam	128	R
Ceftriaxone	8	R
Levofloxacin	16	R
Colistin	**1**	**S**
Ampicillin	64	R
Gentamycin	16	R
Ceftazidime/avibactam	**2**	**S**
Ciprofloxacin	4	R
Cefotaxime	4	R

Report assessment

- Lab reports indicate that patient is infected with *Klebsiella pneumoniae* (*K. pneumoniae*) which is resistant to several antibiotics.
- Reports indicate that the bacteria is sensitive to colistin and it is also susceptible to Ceftazidime/avibactam
- Taking in consideration of issues related to colistin in ICU setting and positive data of avibactam/ceftazidime, this patient was started on Ceftazidime/avibactam therapy

Case study: Which antibiotic should you use?

Antibiotic	MIC	Interpretation
Aztreonam	8	S
Ceftriaxone	> 32	R
Ceftazidime	4	S
Ciprofloxacin	≤ 1	S
Gentamicin	2	S
Meropenem	≤ 1	S
Piperacillin/tazobactam	≤ 16	S

Rule #1

- **ALWAYS START WITH A BETA-LACTAM IF POSSIBLE,**
- **ESPECIALLY IN SEVERE INFECTIONS**

- They have the best data supporting their use and are in general excellent drugs

  Exception: atypical infections

Rule #2

DO NOT COMPARE MICS BETWEEN DRUGS

Each antibiotic has

- Different serum concentrations
- Different tissue concentrations
- Different pharmacokinetics

- Each antibiotic has different pharmacodynamic parameters
  - Time dependent antibiotics vs Concentration dependent antibiotics vs

    AUC/MIC dependent antibiotics

Rule #3

- **IF "≤" YOU CAN USE THE DRUG**
  - **(NOTE EXCEPTIONS BELOW)**

- **Exceptions**
  - Drug doesn't get to the site of action
  - Drug doesn't achieve its goal pharmacodynamics parameters
  - Drug doesn't have inducible resistance
  - Patient-specific factors
  - Drug cost

Rule #4

MICROBIOLOGY ALWAYS HAS MORE INFORMATION THAN WHAT IS REPORTED

- They may have results before they are reported in the computer
- Antibiotics may be suppressed
- They can perform additional testing

Assessment question #1

Which antibiotic should you use?

Blood culture: Enterococcus faecalis

Antibiotic	MIC	Interpretation
Ampicillin	<= 2	S
Daptomycin	1	S
Penicillin	2	S
Vancomycin	2	S

Assessment question #1

- **Rule #1:**
- Always start with a beta- lactam if possible
- **Rule #2:** Do not compare MICs between drugs
- **Rule #3**:
- If <= you can use the drug with some exceptions
- **Rule #4:** Micro always has more information

Blood culture: Enterococcus faecalis		
Antibiotic	**MIC**	**Interpretation**
Ampicillin	<= 2	S
Daptomycin	1	S
Penicillin	2	S
Vancomycin	2	S

Assessment question #1

Rule #1: Always start with a beta-lactam if possible

- The drug of choice for ampicillin-sensitive enterococcus is Ampicillin

Rule #2: Do not compare MICs between drugs

- Daptomycin is not “better” than ampicillin because the MIC is lower
- Amicillin is still the drug of choice if sensitive

Rule #3: If <= you can use the drug with some exceptions

- True; cost effectiveness: ampicillin > vancomycin > daptomycin

Rule #4: Microbiology always has more information that what is reported

- Microbiology also tests linezolid, which is the only oral option for treatment of this bacteremia (ampicillin is still preferred)

Answer: AMPICILLIN 2 gm IV q4h

Assessment question #2

URINE CULTURE(NEW)

Status: Edited Result - FINAL Visible to patient: Not Released Next appt: None

Culture & Susceptibility

KLEBSIELLA OXYTOCA ESBL

Antibiotic	Sensitivity	Microscan	Unit	Status
Ampicillin	Resistant	>16	mcg/mL	Final
Cefepime	Resistant	>16	mcg/mL	Final
Cefotaxime	Extended Spectrum Beta Lactamase	>32	mcg/mL	Final
Cefotetan	Sensitive	<=16	mcg/mL	Final
Ceftazidime	Extended Spectrum Beta Lactamase	>16	mcg/mL	Final
Ceftriaxone	Extended Spectrum Beta Lactamase	>32	mcg/mL	Final
Cefuroxime	Resistant	>16	mcg/mL	Final
Cephalothin	Resistant	>16	mcg/mL	Final
Ciprofloxacin	Resistant	>2	mcg/mL	Final
Gentamicin	Resistant	>8	mcg/mL	Final
Levofloxacin	Sensitive	<=2	mcg/mL	Final
Meropenem	Sensitive	<=1	mcg/mL	Final
Nitrofurantoin	Sensitive	<=32	mcg/mL	Final
Pip/Tazobactam	Intermediate	64	mcg/mL	Final
Piperacillin	Resistant	>64	mcg/mL	Final
Tigecycline	Sensitive	<=2	mcg/mL	Final
Tobramycin	Resistant	>8	mcg/mL	Final
Trimeth/Sulfa	Resistant	>2/38	mcg/mL	Final

Which antibiotic should you use?

Assessment question #2

You need more information

- Cystitis or pyelonephritis?
- If cystitis, is it a male or female?
- If female, how old?

Let's assume this is CYSTITIS in a YOUNG ADULT FEMALE with no co-morbid conditions

- Do you want IV or PO?

Assessment question #2

- **Rule #1:** Always start with a beta-lactam if possible
  - Cefotetan and meropenem are the only “sensitive” beta-lactams
- **Rule #2:** Do not compare MICs between drugs
- **Rule #3:** If <= you can use the drug with some exceptions
  - Exceptions
    - Cefotetan should not be used for ESBL-producing organisms and is IV
    - Meropenem is appropriate but is IV
    - Ciprofloxacin is resistant so levofloxacin should not be used
    - Tigecycline has poor urine penetration and is IV

      Nitrofurantoin
- **Rule #4:** Microbiology always has more information that what is reported
  - Fosfomycin can also be tested but is expensive

Answer: Macrobid 100 mg PO BID x 5 days

Guidelines For Therapeutic Decision Based On AST Results

- **In any case, results of AST need to be interpreted with some background knowledge on the biology of the microorganism and patient history before treatment is prescribed!!**

  - Do not use antimicrobials if these are not necessary!
  - Always take in account intrinsic resistances and possible resistances which are not always expressed *in vitro* testing
  - Check any unusual results!
  - The patient history is important; underlying disease and previous treatments attempted are of outmost importance
  - Drug choice needs to take in account the drug properties and distribution for predicting efficacy at target organs
  - Dosage adjustment should be precise for avoiding sublethal concentrations and or toxicity issues
  - Whenever possible, use narrow spectrum drugs and reserve critically important drugs for humans for use in exceptional cases

Rapid Diagnostic Tests

- Biomarkers of infection/inflammation

WBC

ESR

CRP

Lactate

Procalcitonin

- Gram stain

- **Molecular**

Procalcitonin (PCT) in Antimicrobial Stewardship

PCT < 0.1 ng/ml	Bacterial Infection VERY UNLIKELY	NO ANTIMICROBIALS	Consider repeat 6-24hrs based on clinical status
PCT 0.1-0.25 ng/ml	Bacterial infection UNLIKELY	NO ANTIMICROBIALS	Use of ABX based on clinical status ('unstable') & judgment
PCT > 0.25-0.5 ng/ml	Bacterial infection LIKELY	YES ANTIMICROBIALS	Repeat PCT day 3, 5, 7 (for Duration)
PCT > 0.5 ng/ml	Bacterial infection VERY LIKELY	YES ANTIMICROBIALS	CONSIDER STOP ABX when 80=90% decrease; if PCT remains high consider treatment failure

Importance of Genotyping

- Genotypic AST are effective direct methods that eliminate tedious bacterial cultures, long incubation, chances of contamination, and the spreading of deadly infections.
- PCR, DNA microarray and DNA chips, and loop-mediated isothermal amplification (LAMP) are some of the genotypic techniques for the detection of antibiotic resistance.
- Rapid detection of resistance gene within few hours that helps in making decision to use more specific antimicrobial agent rather than broad spectrum antimicrobial agent.

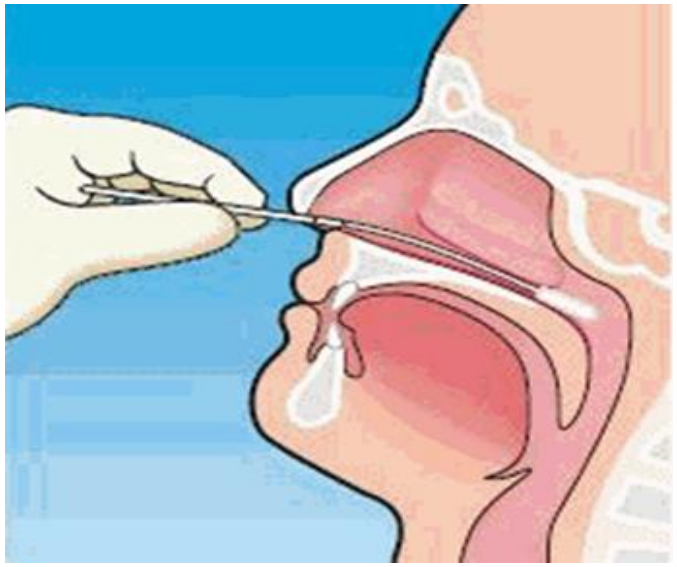
Khan ZA, Siddiqui MF, Park S. Current and emerging methods of antibiotic susceptibility testing. Diagnostics. 2019;9(2):49.
Bard JD, et al. Why Can't We Just Use PCR? The Role of Genotypic versus Phenotypic Testing for Antimicrobial Resistance Testing. Clin Microbiol Newsl . 2018;40(11):87-95.

Molecular RDTs: Culture Dependent

- Rapid biochemical identification[a]
- Proteomic identification (MALDI-TOF MS)[a]
- Rapid identification of pathogens in blood cultures[a]
  - BCID microarrays
  - PNA-FISH
- Rapid phenotypic AST[b]
- NAAT detection of selected resistance genes[a]
  - *mecA*
  - *vanA/vanB*
  - KCP

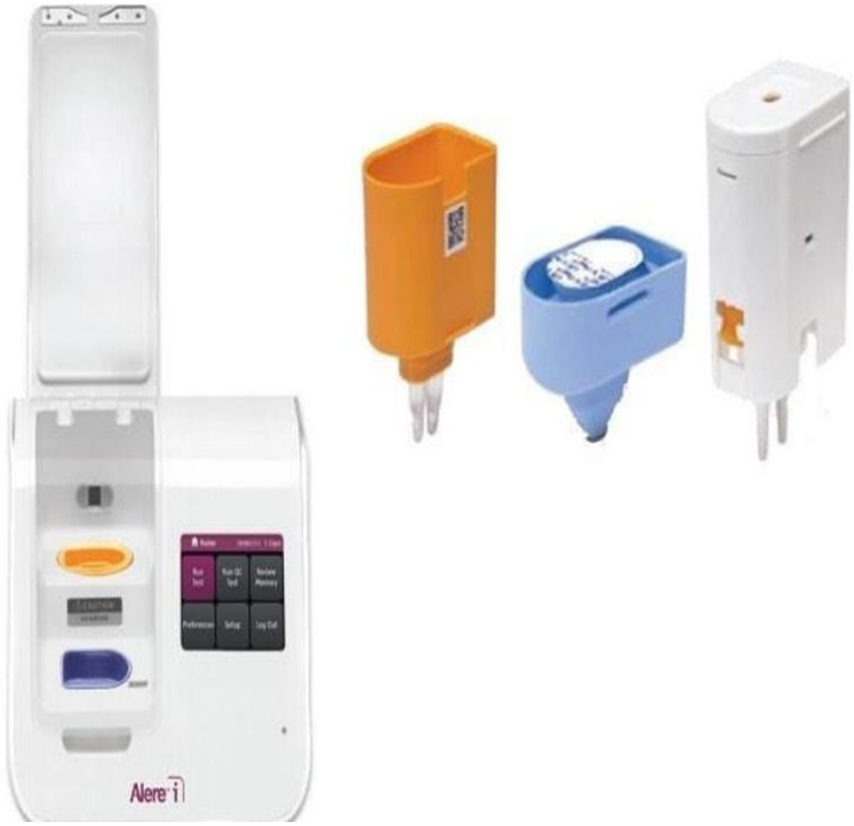
MALDI-TOF

a. Bauer K, et al. *Clin Infect Dis*. 2014;59:S134-S145.
b. Avesar J, et al. *Proc Natl Acad Sci U S A*. 2017;114:E5787-E5795.



- Respiratory Panel (FDA approved 2008)
- GI panel (FDA approved 2012)
- Blood culture panel (FDA approved 2014)
- Meningitis panel (FDA approved 2015)
- Lower Respiratory panel (FDA approved 2018)

PCR Panels in Current Use



- Swab used to collect specimen → placed in liquid medium
- Liquid pipetted into reaction container
- Barcode scanned
- Reaction container placed into instrument

<20 minute POC NAAT

Influenza A,B/ RSV/ Group A strep/COVID 19

Rapid Diagnostics

Appropriate indication and specimen collection is critical for both basic microbiology and newer diagnostics

No one rapid diagnostic platform meets all needs: select test(s) based on work flow and patient population

Rapid diagnostics can decrease diagnostic uncertainty

To be effective, rapid diagnostics have to actionable and tied to local stewardship program

Monitor for unintended consequences

Testing must be correlated with overall clinical condition of the patient

Conclusions

Antimicrobial Stewardship:

“ Use the right drug at the right time at the right dose for the right duration.”

Diagnostic Stewardship:

“ Obtain the right test in the right patient in order to use the right drug at the right time at the right dose for the right duration.”

THANK YOU

ANTIBIOTICS
USE - RESPONSIBLY