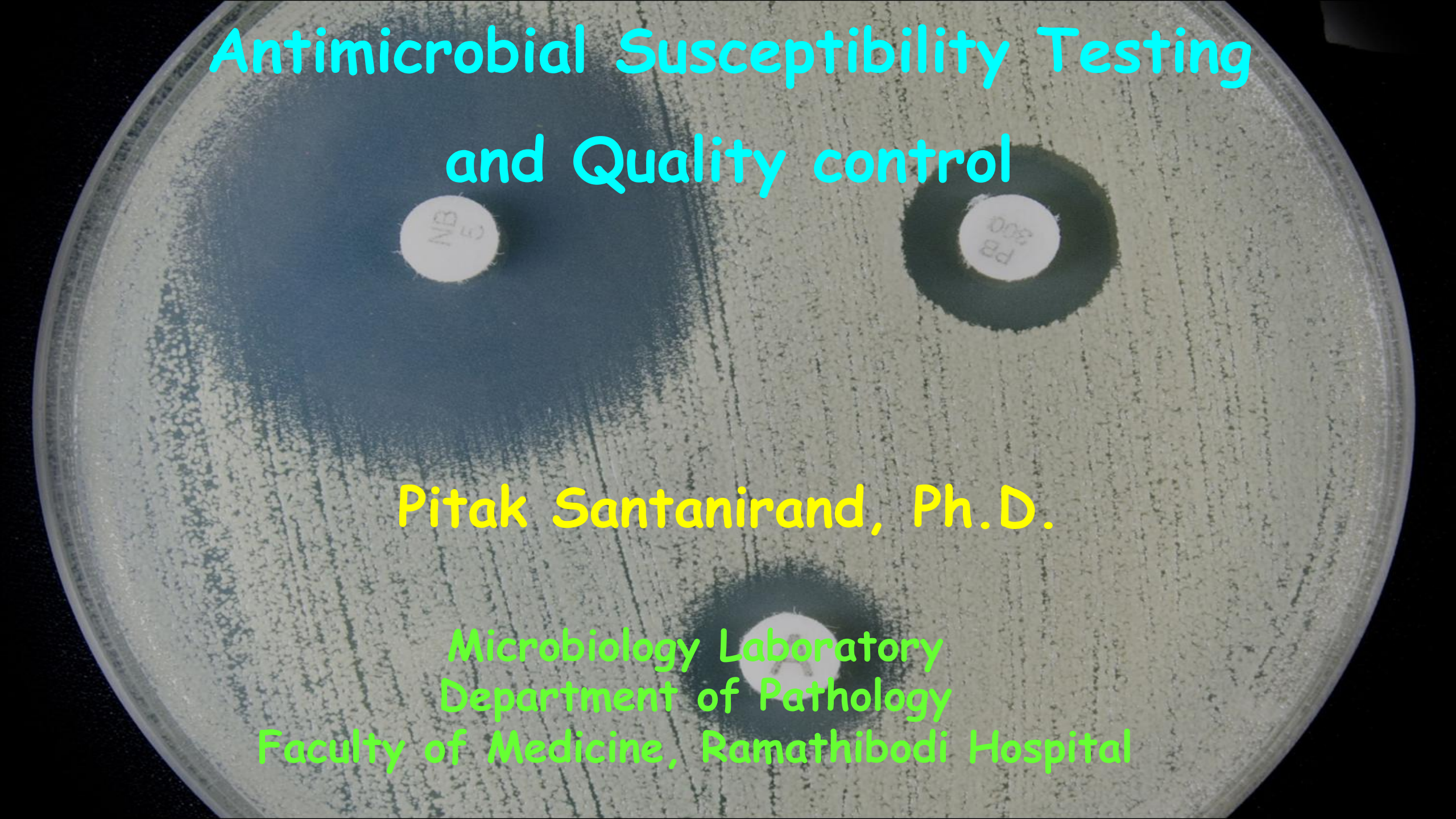
Antimicrobial Susceptibility Testing and Quality control



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Susceptibility testing techniques

Broth dilution: MIC & MBC (μg/ml)

- Broth macrodilution (test tube)
- Broth microdilution (microtitre plate)

Agar dilution: MIC (μg/ml)

Disk diffusion:

- Inhibition zone (mm) (Kirby-Bauer)
- MIC (μg/ml) (E-test)

Minimal Inhibitory Concentration (MIC)

in microbiology, is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

Minimal Bactericidal Concentration (MBC)

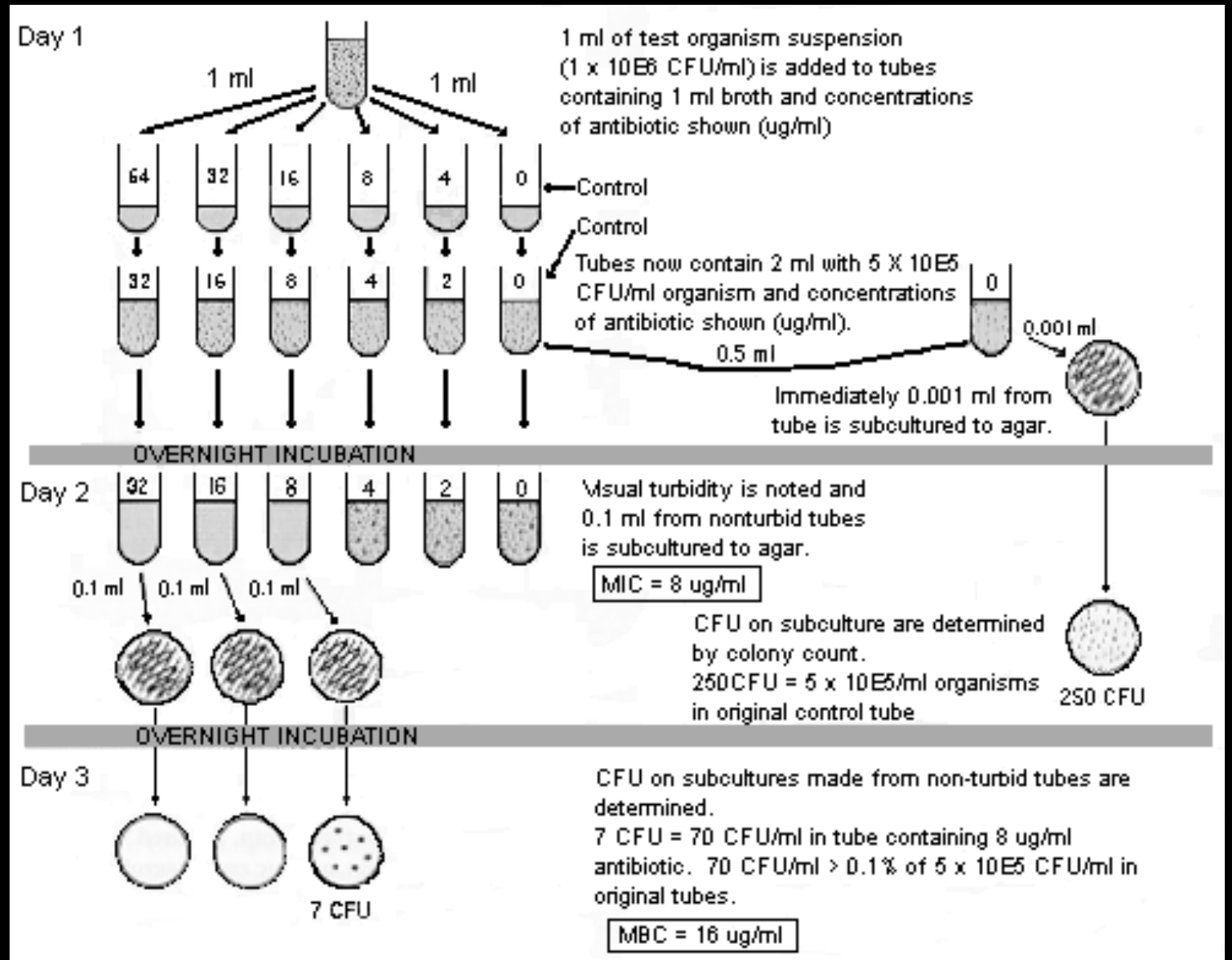


Figure 14.2 (from Bailey & Scott's Diagnostic Microbiology; 9th Edition, 1994)
Determining MIC and MBC for one organism and one antibiotic

CFU = colony forming unit

Antimicrobial Susceptibility Test
Broth Microdilution Dilution

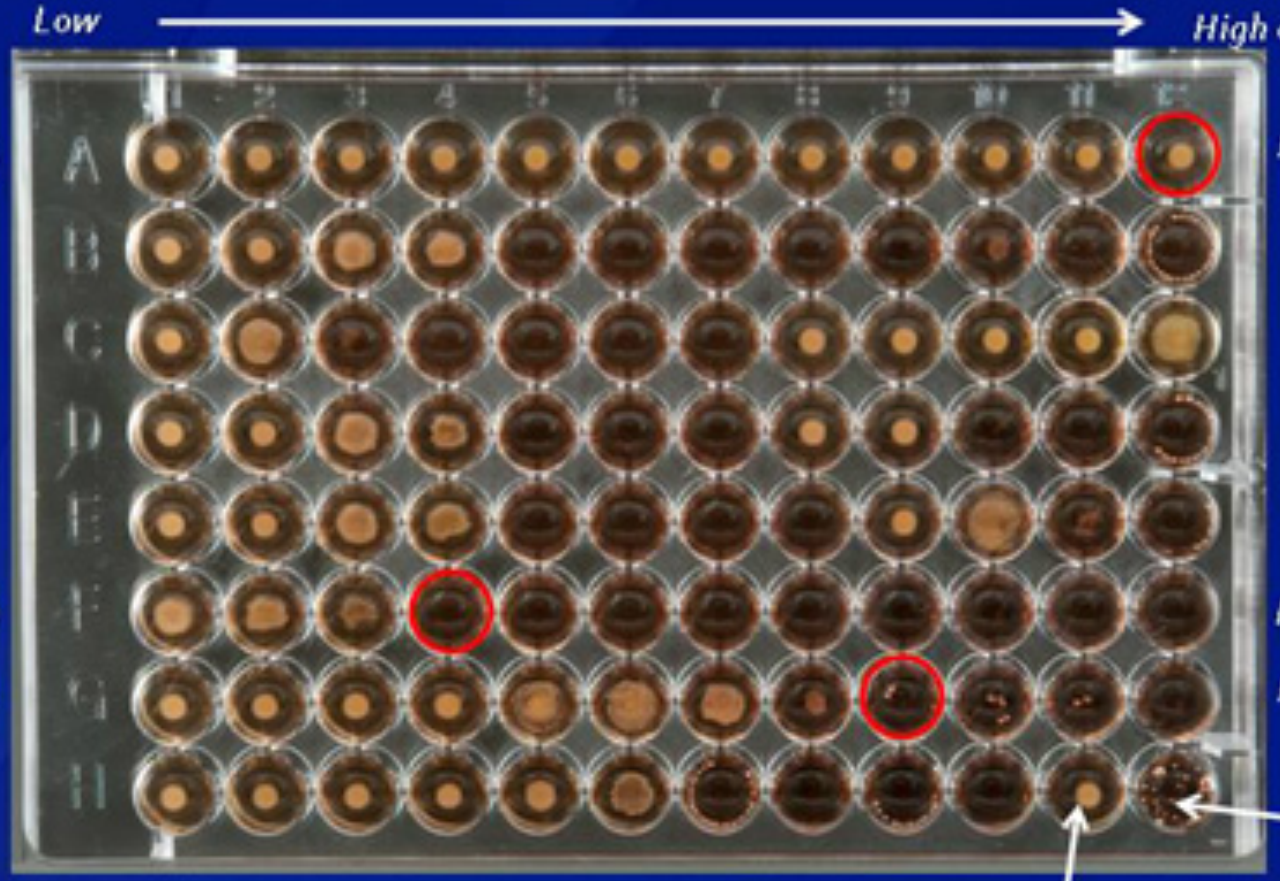
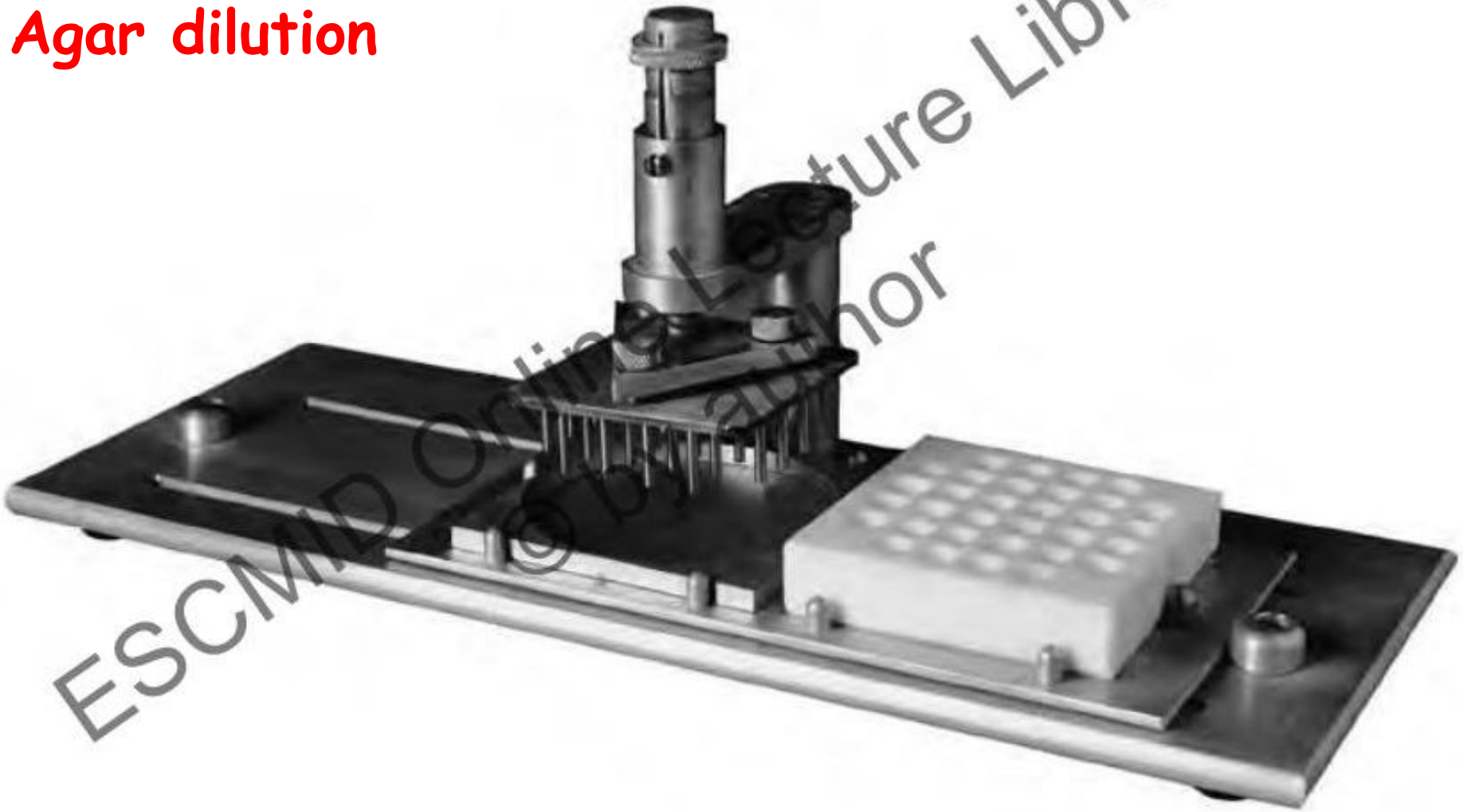


Photo courtesy of Dr. Lesley McGee, CDC

Steers replicator

Agar dilution



Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307-311.

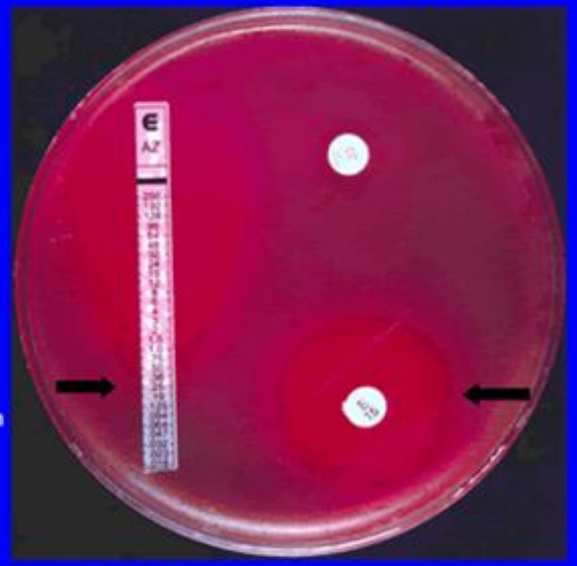
AGAR DILUTION

Control
(no antibiotic)

0.25 µg/ml

0.5 µg/ml

Antimicrobial Susceptibility Test E Test™ & Disk Diffusion



Minimum inhibitory concentration

Zone of inhibition of growth is larger than 26mm, meaning the bacteria is susceptible.

Photo courtesy of Dr. Richard Facklam, CDC

Automated Identification and antimicrobial susceptibility testing

Automated Identification and antimicrobial susceptibility testing

Media used for susceptibility testing

Broth dilution

Cation Adjust Mueller-Hinton broth (CAMHB)
(may be added with 2.5-5% lysed horse blood)

E-test, Agar dilution, Disk diffusion

Mueller-Hinton agar
(may be added with 5% sheep blood)

Haemophilus Test Medium (HTM)

GC agar base

Inoculum preparation

Direct suspension method

Any organism

Broth culture method

any non-fastidious organisms e.g.

Enterobacterales,

Non-ferment bacilli (NFB)

Inoculum preparation

- Use a sterile loop or cotton swab to pick colonies from an overnight culture on non-selective media. If possible, use several morphologically similar colonies to avoid selecting an atypical variant.
- Suspend in saline and mix to an even turbidity.
- Adjust the density of the suspension to 0.5 McFarland by adding saline or more bacteria. Preferably use a photometric device to measure the turbidity.
  - Exception: *Streptococcus pneumoniae* is suspended to 0.5 McFarland from a blood agar plate, but to 1.0 McFarland from a chocolate agar plate.

Inoculum

- The method requires an inoculum suspension equivalent to a 0.5 McFarland standard*.

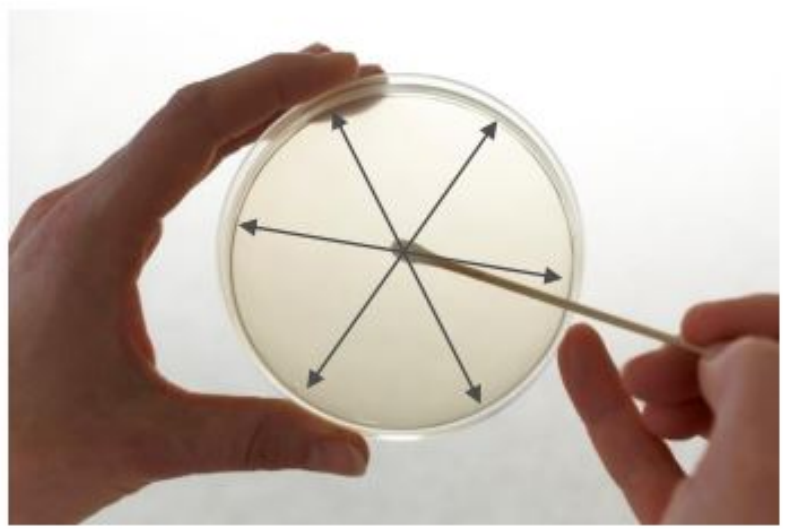
* Approximately corresponding to 1-2 x10^8 CFU/mL for *E. coli*.

Inoculation of plates

- Optimally, use the inoculum suspension within 15 minutes of preparation and always within 60 minutes.
- Make sure that agar plates are at room temperature prior to inoculation.
- Dip a sterile cotton swab into the suspension.
- For Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube to avoid over-inoculation.
- For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

Inoculation of plates

- Spread the inoculum evenly over the entire surface by swabbing in three directions or by using a plate rotator.
- For Gram-positive bacteria, take particular care to ensure that there are no gaps between streaks.
- When inoculating several agar plates with the same inoculum, dip the cotton swab into the suspension for each agar plate.



Summary of inoculation process

- Suspend isolated colonies from an overnight culture on a non-selective medium.

- Adjust to a density equivalent to McFarland 0.5, preferably with a photometric device. Optimally, use the inoculum within 15 minutes.

- Dip a sterile swab into the solution and remove excess fluid by turning the swab against the inside of the tube.

- Apply the inoculum with even strokes over the entire agar surface.

- Apply antibiotic disks within 15 minutes of inoculating the plate and start incubation within another 15 minutes

Application of antimicrobial disks

- Apply disks within 15 min of inoculation.
- Disks must be in close and even contact with the agar surface.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured.

The 15-15-15 minute rule

Follow these instructions for disk diffusion:

- Use the inoculum suspension optimally within **15 minutes** of preparation, and always within 60 minutes.
- Apply disks within **15 minutes** of inoculation.
- Incubate plates within **15 minutes** of disk application.

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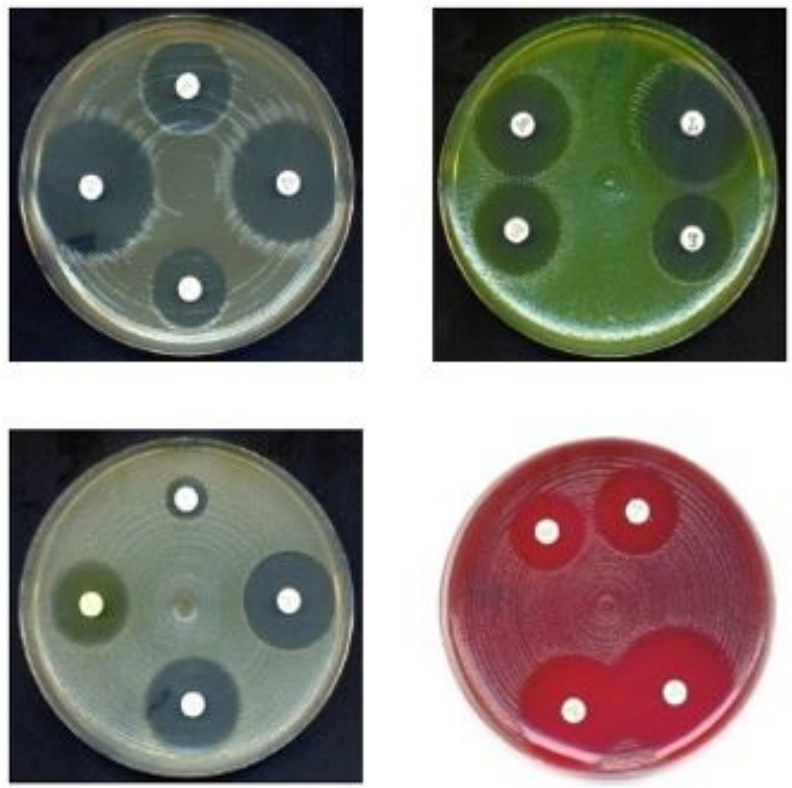
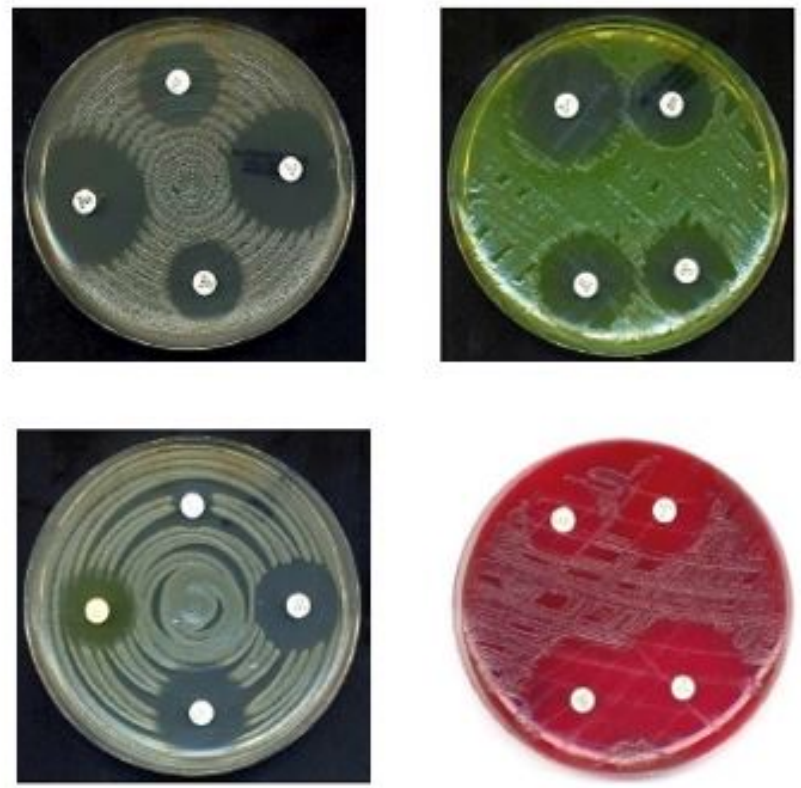
The growth should be confluent and evenly spread over the plate



Plates should look like this..



..and NOT like this!

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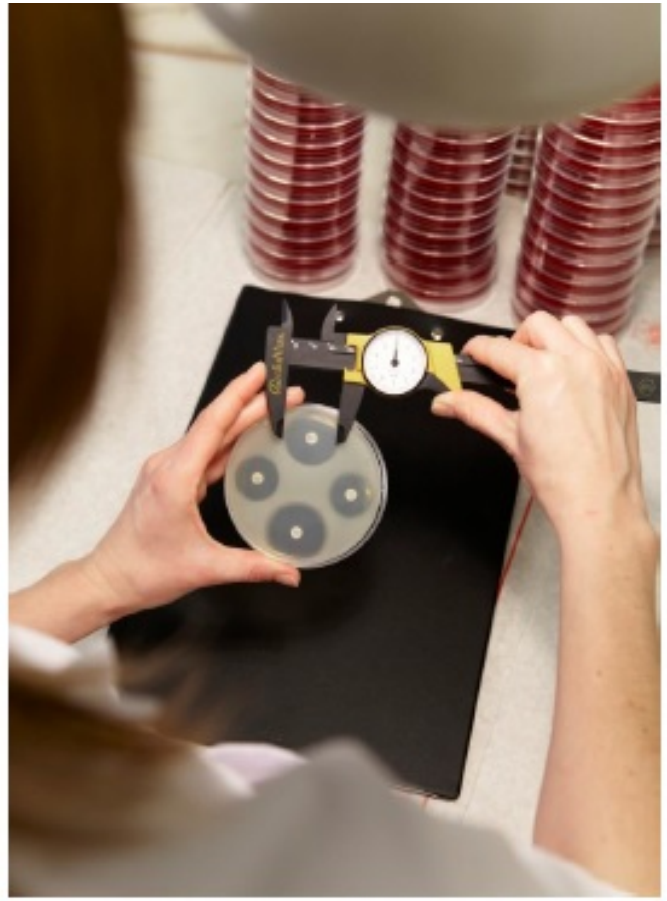
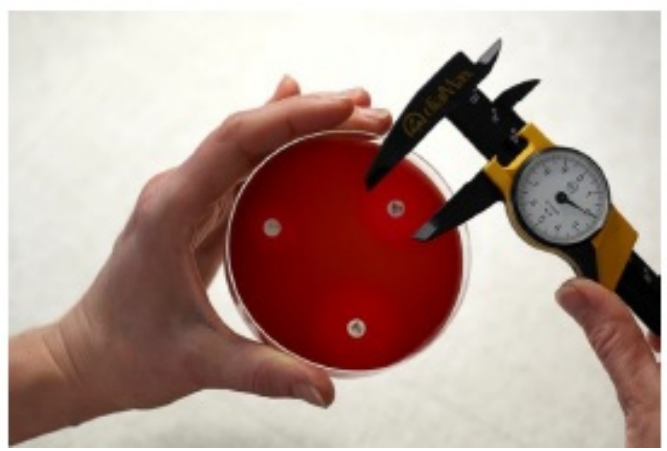
Reading zones

- Read **MH** plates from the back against a dark background illuminated with reflected light.



- Read **MH-F** plates from the front with the lid removed illuminated with reflected light.



Reading zones

- Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.

Examples:

E. coli
Ciprofloxacin

S. aureus
Erythromycin

CoNS
Trimethoprim

S. pneumoniae
Rifampicin

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Reading zones

- Do not use transmitted light (plate held up to light) or a magnifying glass, unless otherwise stated.
- Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
- Measure zone diameters to the nearest millimetre with a ruler or a calliper. If an automated zone reader is used, it must be calibrated to manual reading.
- In case of double zones, or distinct colonies within zones, check for purity and repeat the test if necessary. If cultures are pure, colonies within zones should be taken into account when measuring the diameter.

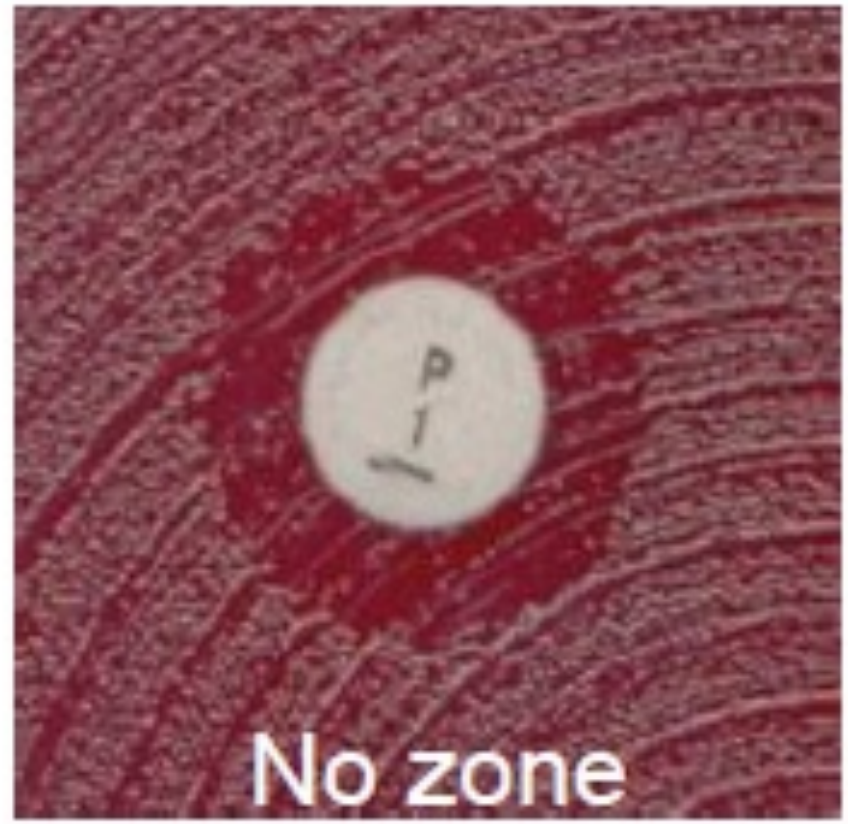
Colonies within zone

- In case of distinct colonies within zones, subculture the colonies, check purity and repeat test if necessary.

- Colonies that are not contaminations should be taken into account when reading zones.

E. coli with ESBL

H. influenzae with PBP mutations



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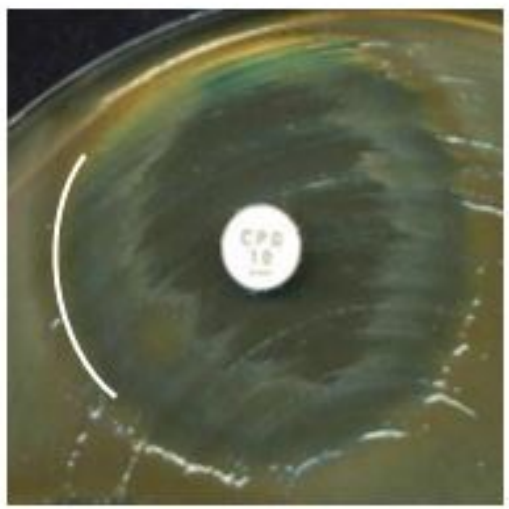
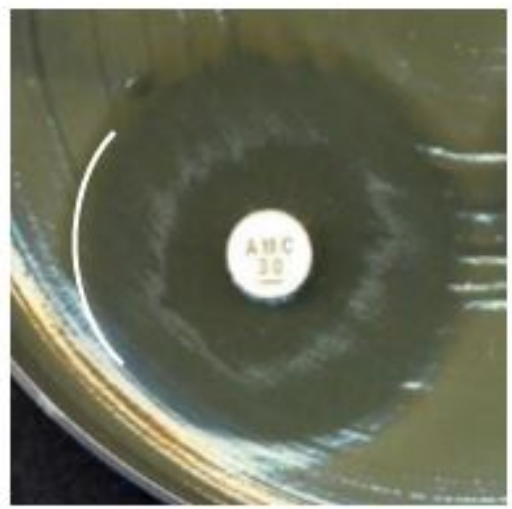
Swarming

- For *Proteus* spp., ignore swarming and read inhibition of growth.

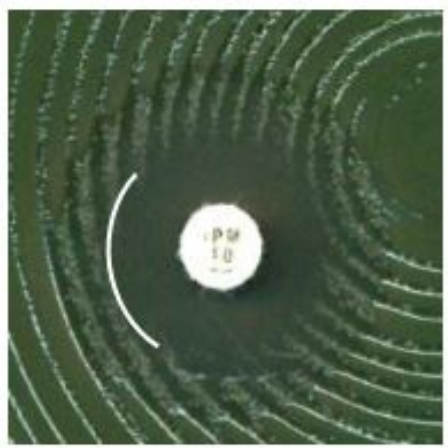




Double zones

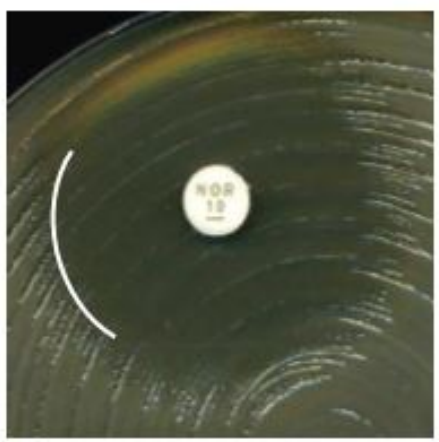
- In case of double zones, check for purity and repeat the test if necessary.

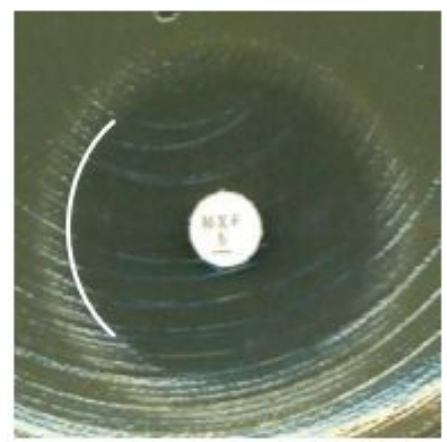
- If cultures are pure, read the inner zone.

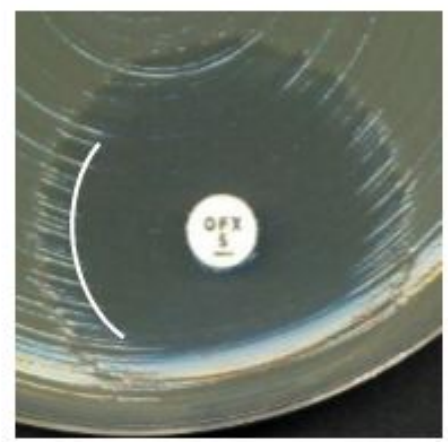


Fuzzy zone edges
Enterobacterales

- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is. Do not hold the plate up to light (transmitted light) or use a magnifying glass.







Fuzzy zone edges
Staphylococci

- Hold the plate against a dark background about 30 cm from the naked eye and estimate where the zone edge is. Do not hold the plate up to light (transmitted light) or use a magnifying glass.



Fuzzy zone edges
S. pneumoniae

- Small colonies that are visible when the plate is hold about 30 cm from the naked eye should be taken into account when reading zones.
- The presence of small colonies close to the zone edge may be related to excess humidity in the MH-F media, and may be reduced by drying the plates prior to use.

β-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.
- β-haemolysis is usually free from growth.

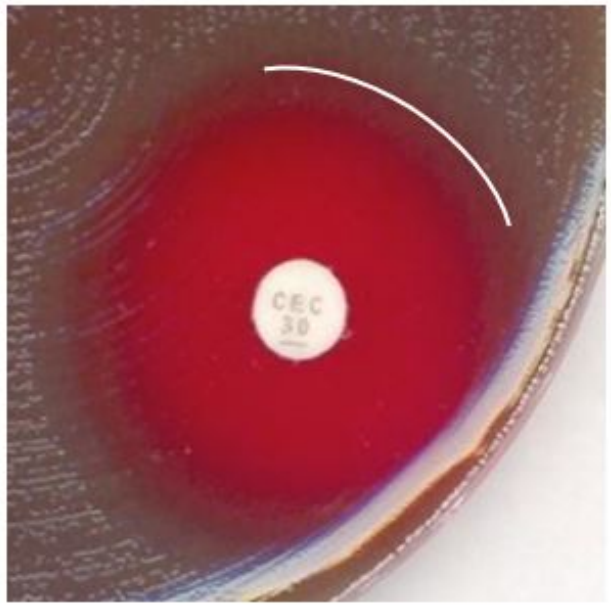
S. pyogenes

Streptococcus group C

α-haemolysis

- Tilt the plate back and forth to better differentiate between haemolysis and growth.

There is usually growth in the whole area of α-haemolysis.



For some organisms, there is additional α-haemolysis without growth. Tilt the plate to differentiate between haemolysis and growth.

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Specific reading instructions

- *Enterobacterales* with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid
- *Enterobacterales* and temocillin
- *Enterobacterales* and mecillinam
- *E. coli* and fosfomycin
- Trimethoprim and trimethoprim-sulfamethoxazole in general
- *Stenotrophomonas maltophilia, Achromobacter xylosoxidans* and *Burkholderia pseudomallei* with trimethoprim-sulfamethoxazole
- *Aeromonas* spp. and trimethoprim-sulfamethoxazole
- Enterococci and vancomycin
- *S. aureus* and benzylpenicillin
- Detection of inducible clindamycin resistance in staphylococci and streptococci
- *H. influenzae* and beta-lactam agents

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Enterobacterales with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid

- Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars. The inner zone is not seen with some batches of agar and when the outer zone is read there is no difference between batches.

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E. coli and mecillinam

- Ignore isolated colonies within the inhibition zone.

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E. coli and fosfomycin

- Ignore isolated colonies within the inhibition zone and read the outer zone edge.

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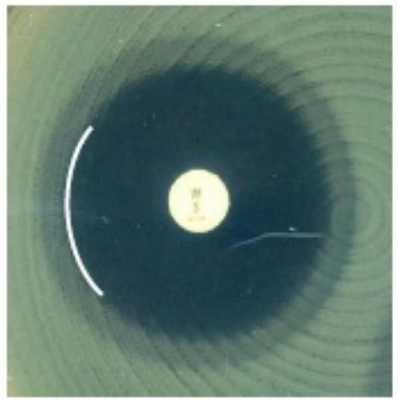
Trimethoprim and trimethoprim-sulfamethoxazole

- Follow the instructions for reading and read inner zones when double zones appear (see examples below).

- Ignore haze or faint growth up to the disk within a zone with otherwise clear zone edge.



E. coli

CoNS

Moraxella

Haemophilus

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Stenotrophomonas maltophilia and trimethoprim-sulfamethoxazole

- An isolate showing any sign of inhibition zone ≥ the susceptible breakpoint should be reported susceptible. Note that there may be substantial growth within zones.

Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone = Resistant

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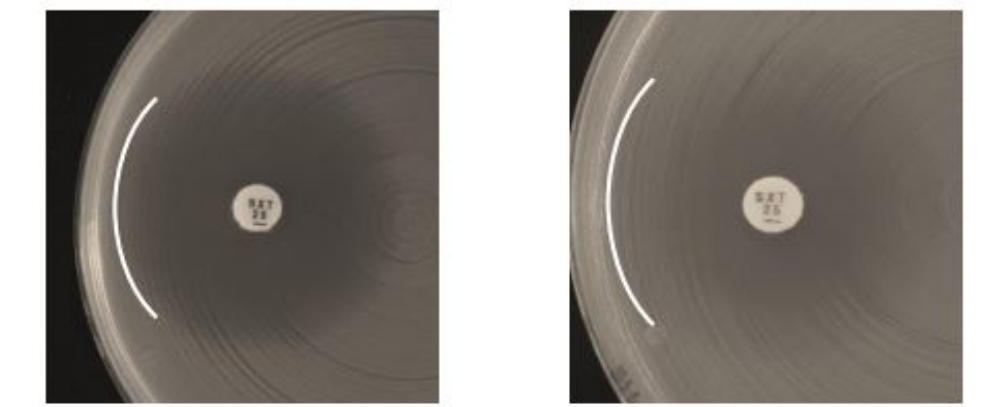
A. xylosoxidans with trimethoprim-sulfamethoxazole

- Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
  - Read the outer zone edge and interpret according to the breakpoints.
- If there is growth up to the disk and no sign of inhibition zone, report resistant.

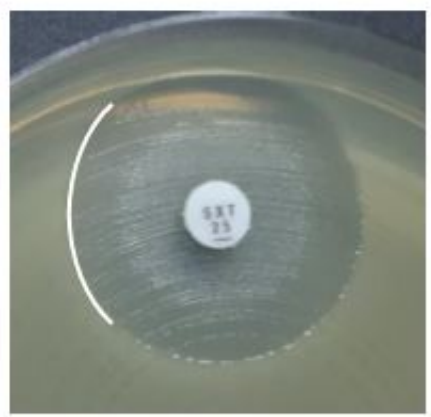


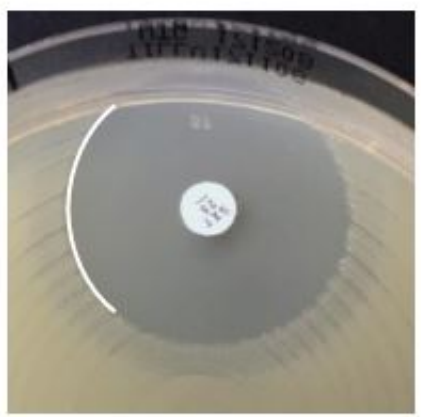
An outer zone can be seen

Growth up to the disk

Aeromonas spp. and trimethoprim-sulfamethoxazole

- Read the obvious zone edge and disregard haze or growth within the inhibition zone.
- If there is an obvious inner zone edge, read the inhibition zone as the inner zone.





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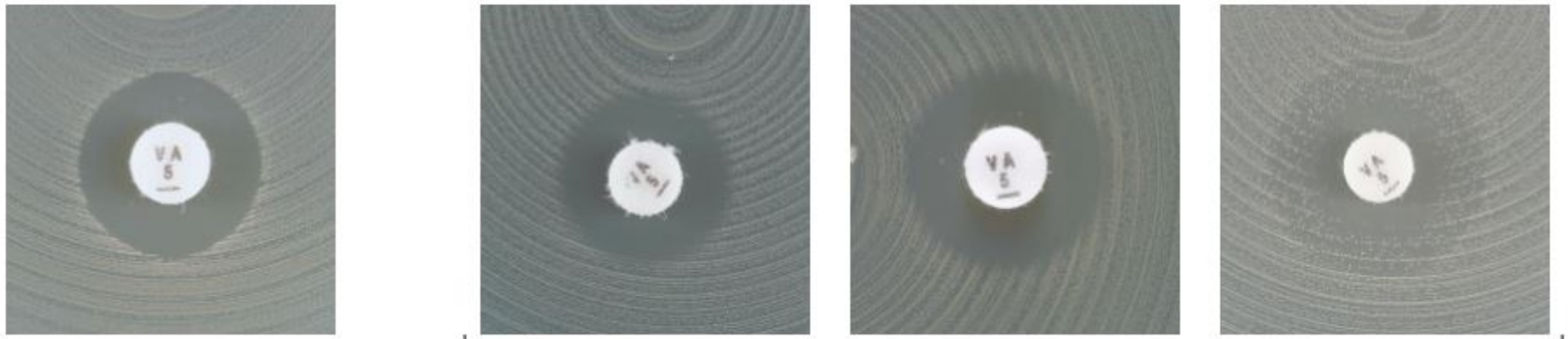
Enterococci and vancomycin

- Examine from the front of the plate with transmitted light (plate held up to light).
  - Vancomycin-susceptible enterococci exhibit sharp zone edges and do not exhibit colonies in the inhibition zone.
  - If the zone edge is fuzzy, if colonies grow within the zone or if you are uncertain, investigate further even if the zone diameter is ≥ 12 mm.
  - Isolates must not be reported susceptible before 24 h incubation.



non-VRE

VRE

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S. aureus and benzylpenicillin

- Examine from the front of the plate with transmitted light (plate held up to light).
  - Disk diffusion is more reliable than MIC for detection of penicillinase producers, provided the zone diameter is measured AND the zone edge closely inspected.
  - Penicillinase-producing *S. aureus* exhibits sharp zone edges with the 1 unit benzylpenicillin disk. If the zone edge is sharp, report as resistant even if the zone diameter is ≥ 26 mm.

S. aureus with sharp zone edge and zone diameter ≥ 26 mm = Resistant

S. aureus with fuzzy zone edge and zone diameter ≥ 26 mm = Susceptible

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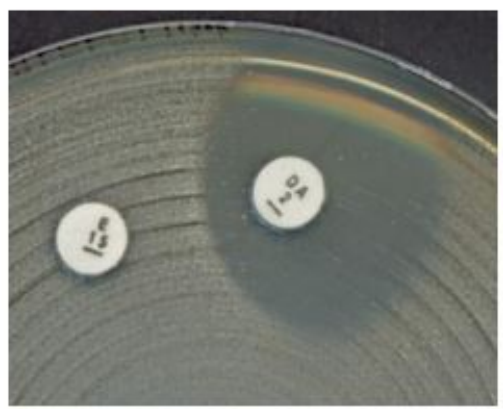
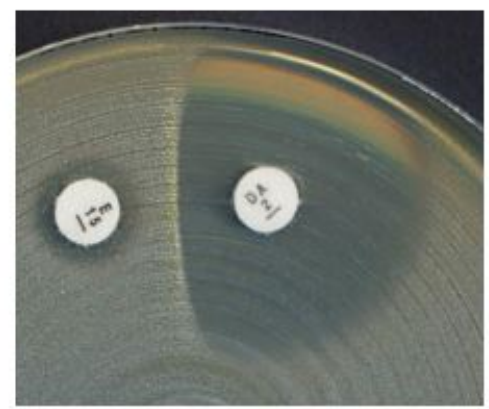
Detection of inducible clindamycin resistance in staphylococci

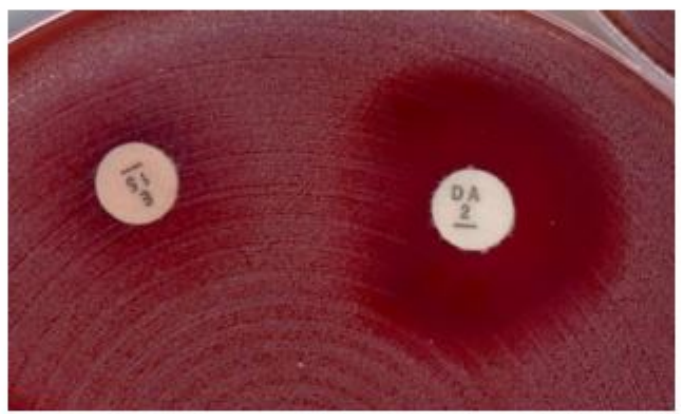
- Inducible clindamycin resistance can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place the erythromycin and clindamycin disks **12-20 mm apart** (edge to edge) and look for antagonism (the D phenomenon).

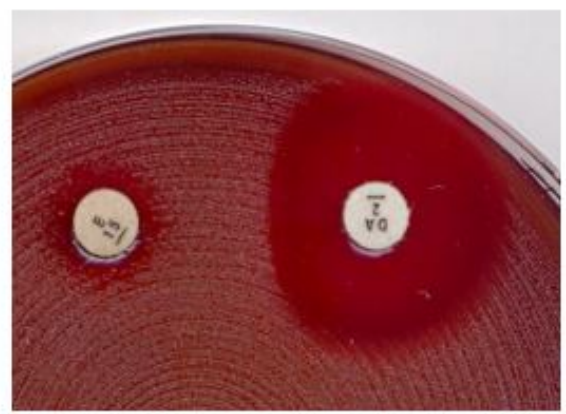


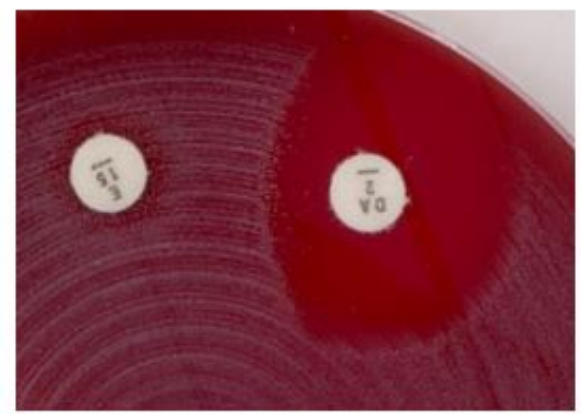


Detection of inducible clindamycin resistance in streptococci

- Inducible clindamycin resistance can be detected by antagonism of clindamycin activity and a macrolide agent.
- Place the erythromycin and clindamycin disks **12-16 mm apart** (edge to edge) and look for antagonism (the D phenomenon).







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Reading zones – exceptions (1)

Organism	Antimicrobial agent	Reading inhibition zones
Enterobacterales	Ampicillin Ampicillin-sulbactam Amoxicillin-clavulanic acid	Ignore fine growth that may appear as an inner zone on some batches of MH agar.
Enterobacterales	Temocillin	Ignore isolated colonies within the inhibition zone.
Enterobacterales	Mecillinam	Ignore isolated colonies within the inhibition zone.
E. coli	Fosfomycin	Ignore isolated colonies within the inhibition zone and read the outer zone edge.
Proteus spp.	Any	Ignore swarming.
S. maltophilia, *A. xylosoxidans* and *B. pseudomallei*	Trimethoprim-sulfamethoxazole	Ignore growth within the zone if any zone edge can be seen, even when growth within the zone is substantial.
S. aureus	Benzylpenicillin	Examine zone edge from the front of the plate with transmitted light (plate held up to light).

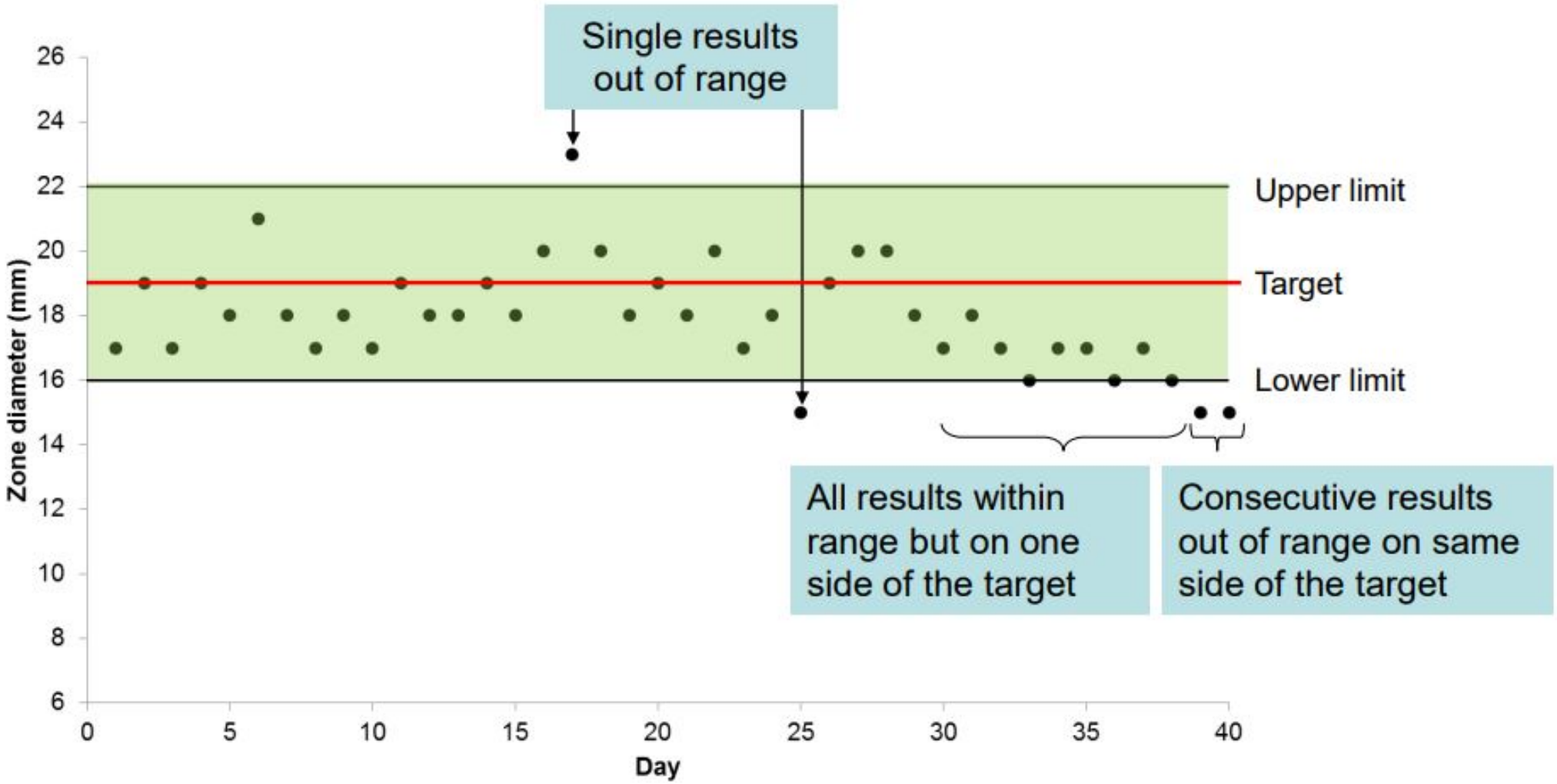
Reading zones – exceptions (2)

Organism	Antimicrobial agent	Reading inhibition zones
Staphylococci	Cefoxitin	Examine zones carefully to detect colonies within the inhibition zone.
Enterococcus spp.	Vancomycin	Examine zone edge from the front of the plate with transmitted light (plate held up to light).
Streptococcus spp.	Any	Read inhibition of growth and not the inhibition of haemolysis.
H. influenzae	Beta-lactam agents	Read the outer edge of zones where an otherwise clear inhibition zone contains an area of growth around the disk.
Aeromonas spp.	Trimethoprim-sulfamethoxazole	Read the obvious zone edge and disregard haze or growth within the inhibition zone
Any	Trimethoprim Trimethoprim-sulfamethoxazole	Ignore faint growth up to the disk and measure at the more obvious zone edge.

Use routine quality control strains to assess general performance

- Control tests should be set up and checked daily, or at least four times per week, for antibiotics which are part of routine panels.
- Control tests should always be read and evaluated before reporting results for clinical isolates.
- Each day that tests are set up, examine the results of the last 20 consecutive tests.
- Examine results for trends and for zones falling consistently above or below the target.
- If two or more of 20 tests are out of range investigation is required.

Monitoring test performance



Response to QC results out of range

- If two non-consecutive control zone diameters of 20 tests are outside the acceptable range – then report susceptibility test results and investigate.
- If two consecutive control zone diameters of 20 tests are outside the acceptable range – then investigate before reporting susceptibility test results. The tests may have to be repeated.
- If multiple disks (>2) are out of range on one day – then investigate before reporting susceptibility test results. The tests may have to be repeated.
- If resistance in a resistant control strain is not recognised – then suppress susceptibility test results, investigate and retest.

Factors that can effect outcome

Low pH

- too narrow zone: aminoglycosides, clindamycin, macrolides, quinolones
- too wide zone: penicillin, tetracyclines

High pH

Low calcium or magnesium ion

High calcium or magnesium ion

- too narrow zone: aminoglycosides, tetracyclines
- too wide zone: daptomycin

Factors that can effect outcome

Disk has lost potency causing? too narrow zone

Light inoculum => too wide zone

Heavy inoculum => too narrow zone

Thin media depth => too wide zone

Thick media depth => too narrow zone

Processing time (15-15-15 Rule)

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST reading guide for broth microdilution

EUCAST 2022 ver.4.0

B. Media for MIC determination by the broth microdilution method

Cation-adjusted Mueller-Hinton broth (MHB) and MHB supplemented with lysed horse blood and β-NAD (MH-F broth)

MH broth, un-supplemented cation-adjusted Mueller-Hinton broth, is used for testing of non-fastidious organisms according to the ISO standard 20776-1, 2019.

MH-F broth, cation-adjusted MH broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD, is used for testing *Streptococcus* spp. (including *S. pneumoniae*), *Haemophilus influenzae*, *Moraxella catarrhalis, Listeria monocytogenes*, *Campylobacter jejuni* and *coli*, *Pasteurella multocida*, *Corynebacterium* spp., *Aerococcus sanguinicola and urinae, Kingella kingae* and several other fastidious organisms.

Reading broth microdilution

Results are only valid when the following criteria are met:

- Sufficient growth, *i.e.* obvious button or definite turbidity, in the positive growth control.

- Pure culture
  - Check for purity by subculturing from the growth-control well immediately after inoculation onto a non-selective agar plate for simultaneous incubation.

- Correct inoculum 5 x 10^5 CFU/mL
  - Viable colony counts can be performed by removing 10 µL from the growth-control well or tube immediately after inoculation and diluting in 10 mL of saline. Mix and spread 100 µL onto a non-selective agar plate. After incubation, the number of colonies should be approximately 20-80.

Growth appearance

- Growth appears as turbidity or as a deposit of cells at the bottom of the well. The appearance of growth differs depending on the microorganism and the antimicrobial agent tested.

- For round-bottom wells, growth will most often appear as a button/pellet centered in the middle. For flat-bottom wells, growth may be scattered.

- Growth in antibiotic-containing wells may differ from growth seen in the positive growth control, even for pure cultures.

Reading MIC endpoints

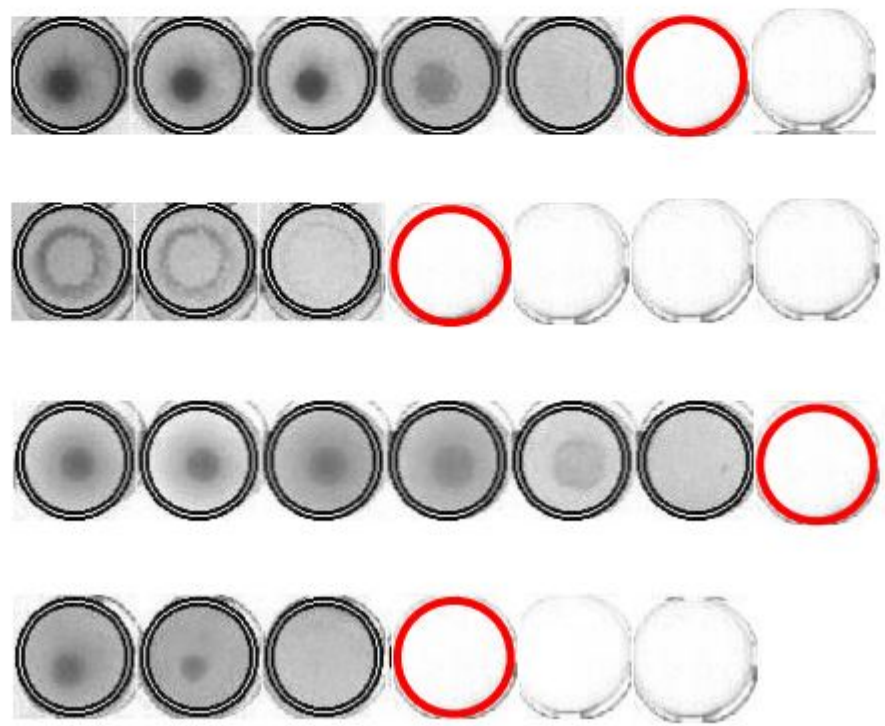
- Results should be read manually. The use of a mirror may facilitate reading.
- If an automated reader or camera system is used, it must be calibrated to manual reading.
- Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.

Trailing endpoints

- Most antimicrobial agent-organism combinations give distinct endpoints.
- Some agent-organism combinations may give trailing endpoints with a gradual fading of growth over 2 to 3 wells.
- Unless otherwise stated, endpoints should be read at complete inhibition of growth

Turbidity without pellet

- Haze or turbidity without a pellet is often seen for *Pseudomonas* spp. and *Acinetobacter* spp. This should be regarded as growth and the endpoint read at the first well with complete inhibition (clear broth).



Haemolysis

- For fastidious organisms tested in MH-F broth, haemolysis of the blood can be seen. This is often accompanied by turbidity or a deposit of growth (pellet).

- Haemolysis with turbidity or pellet should be regarded as growth when determining endpoints.

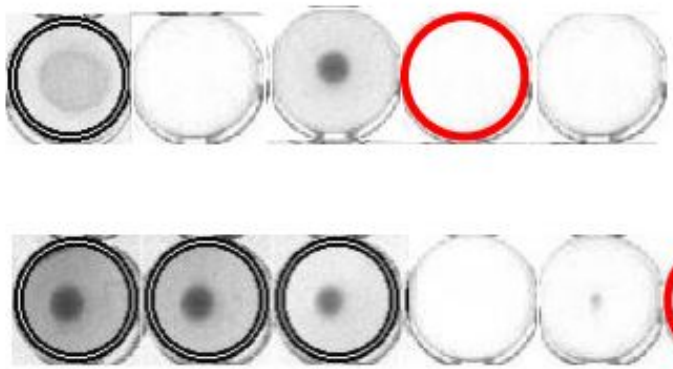
Skipped wells

- Occasionally a skip may be seen, *i.e.* a well showing no growth bordered by wells showing growth. There are several possible explanations including incorrect inoculation, contaminations, heterogenous resistance etc.

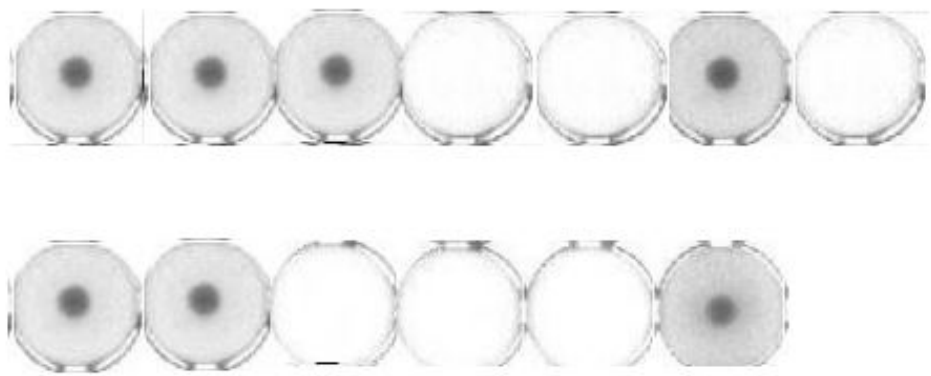
- When a single skipped well occurs, retest the isolate or read the highest MIC value to avoid reporting isolates as false susceptible.

- Do not report results for antimicrobial agents for which there is more than one skipped well.

Examples skipped wells



Retest or read the highest MIC value!



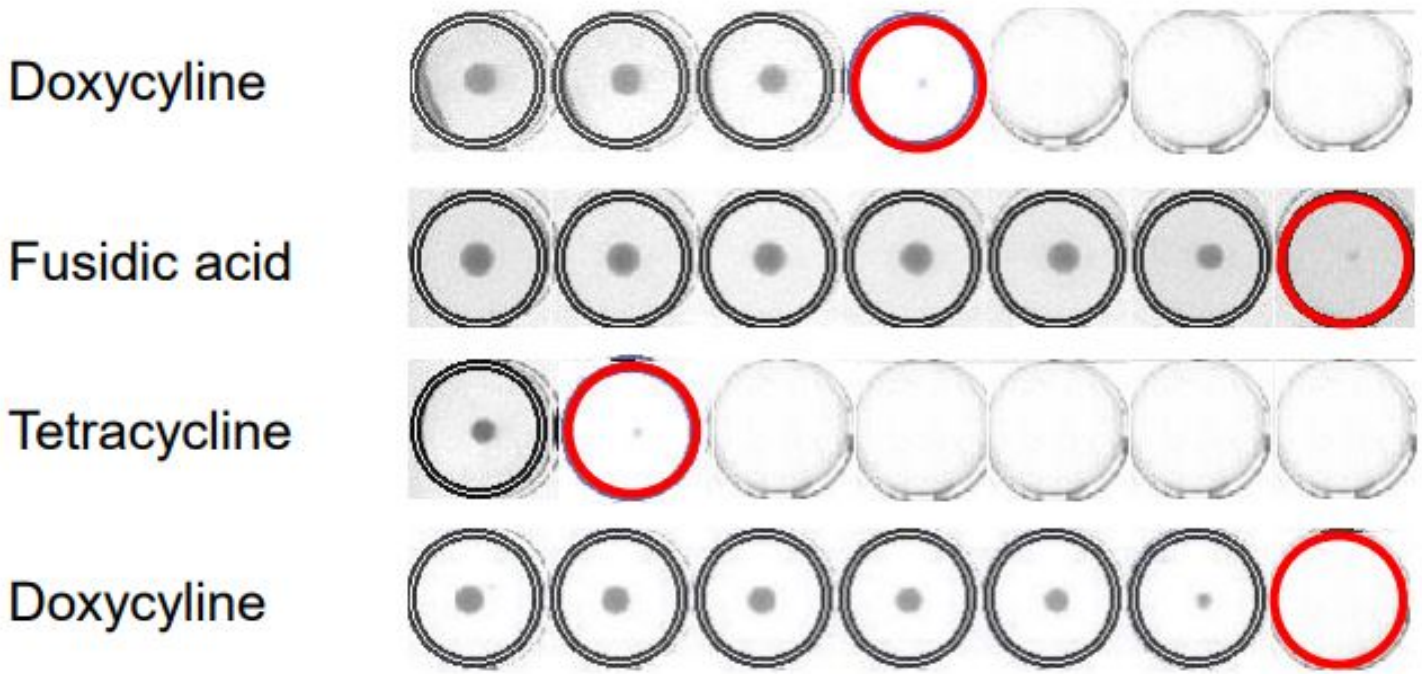
Results invalid!

Specific reading instructions

- The following antimicrobial agents require specific reading instructions:
  - Bacteriostatic antimicrobial agents, both with Gram-positive and Gram-negative organisms
  - Trimethoprim and trimethoprim-sulfamethoxazole
  - Cefiderocol

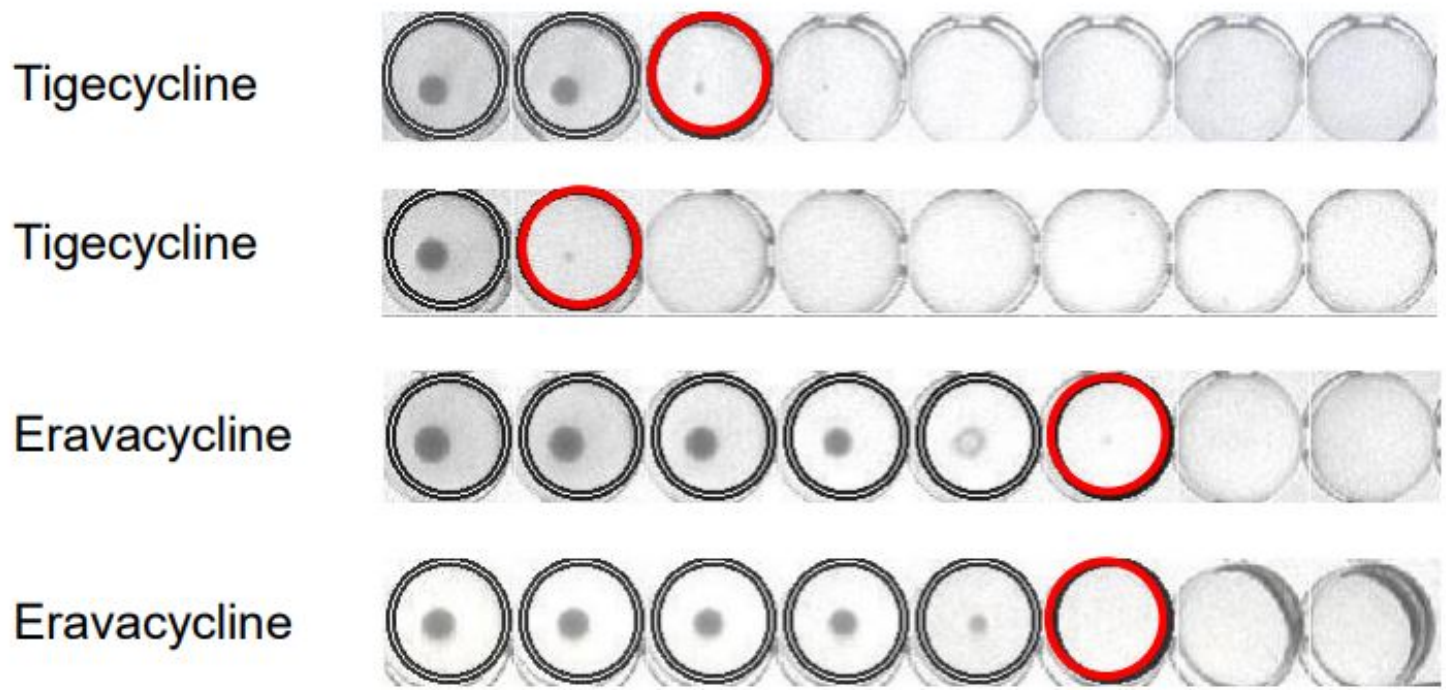
Gram-positive cocci with bacteriostatic antimicrobial agents

- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.



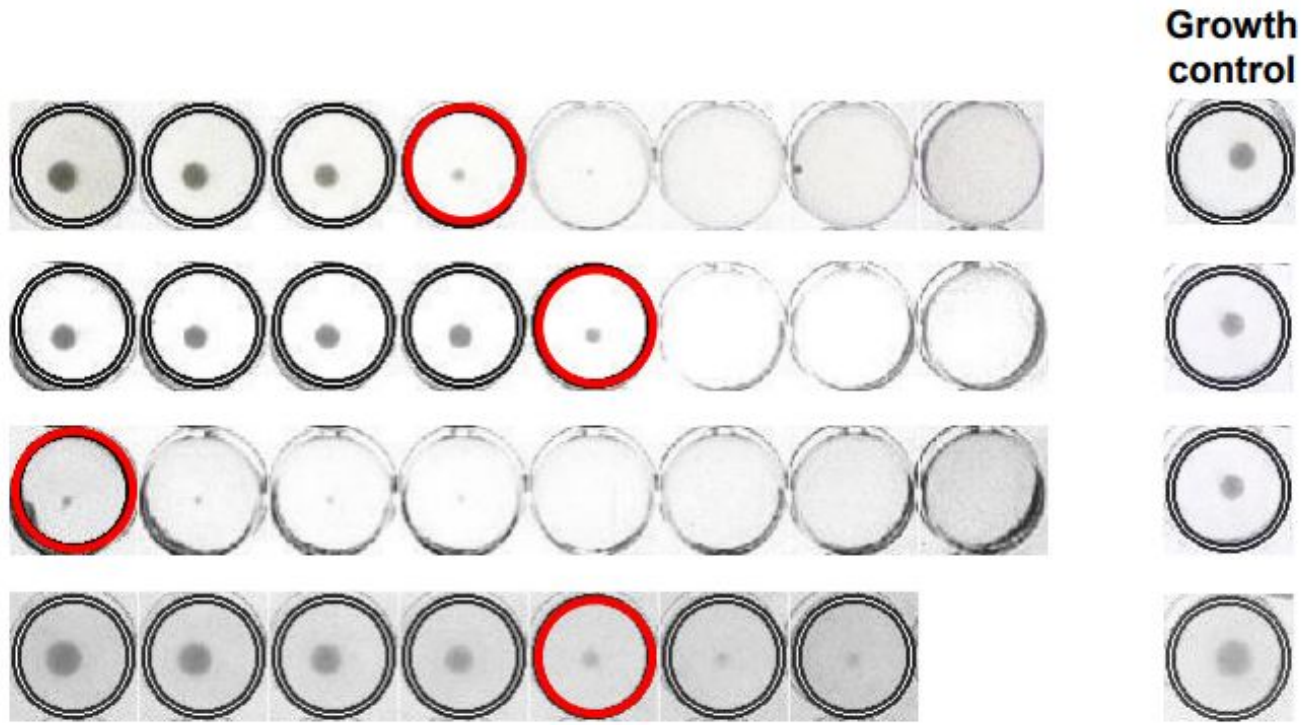
Gram-negative organisms with bacteriostatic antimicrobial agents

- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.



Trimethoprim and trimethoprim-sulfamethoxazole

Read the MIC at the lowest concentration that inhibits ≥80 % of growth as compared to the growth control.



Organism	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	Yes (24 h)
S. lugdunensis	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	No
S. epidermidis	No	Yes (24 h)	Yes (24 h)	Yes (16-18 h)	No
S. pseudintermedius	No	No	Yes (24 h)	Yes (16-18 h)	No
S. schleiferi	No	No	Yes (24 h)	Yes (16-18 h)	No
Staphylococcus spp. (not listed above or not identified to the species level)	No	Yes[a] (24 h)	Yes[a] (24 h)	No	No

CLSI-M100, 31st ed., 2021

Oxacillin MIC: For all species

Oxacillin disk diffusion: Only for *S. epidermidis, S. schleiferi, S. pseudintermedius*

Oxacillin salt agar: Only for *S. aureus*

Cefoxitin MIC: Only for *S. aureus* and *S. lugdunensis*

Cefoxitin disk diffusion: For all species EXCEPT *S. pseudintermedius* and *S. schleiferi*

Detecting *mecA*-Mediated Resistance Using Oxacillin

Isolates that test as *mecA* positive should be reported as methicillin or oxacillin (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.

Because of the rare occurrence of methicillin (oxacillin)-resistance mechanisms other than *mecA*, isolates that test as *mecA* negative but for which the oxacillin MICs are resistant (MIC ≥ 4 µg/mL) should be reported as methicillin (oxacillin) resistant.

CLSI-M100, 30th ed., 2020

Enterobacterales (MH: G/D)

ESBLs Screening

- For *E. coli*, *Klebsiella pneumoniae* and *K. oxytoca*

Cefpodoxime	≤ 17 mm (17/18-20/21)	≥ 4 μg/ml
Ceftazidime	≤ 22 mm (17/18-20/21)	≥1 μg/ml
Cefotaxime	≤ 27 mm (22/23-25/26)	≥1 μg/ml
Ceftriaxone	≤ 25 mm (19/20-22/23)	≥1 μg/ml
Aztreonam	≤ 27 mm (17/18-20/21)	≥1 μg/ml

- For *Proteus mirabilis*

Cefpodoxime	≤ 22 mm	≥ 1 μg/ml
Ceftazidime	≤ 22 mm	≥ 1 μg/ml
Cefotaxime	≤ 27 mm	≥ 1 μg/ml

Enterobacterales (MH; G/D)

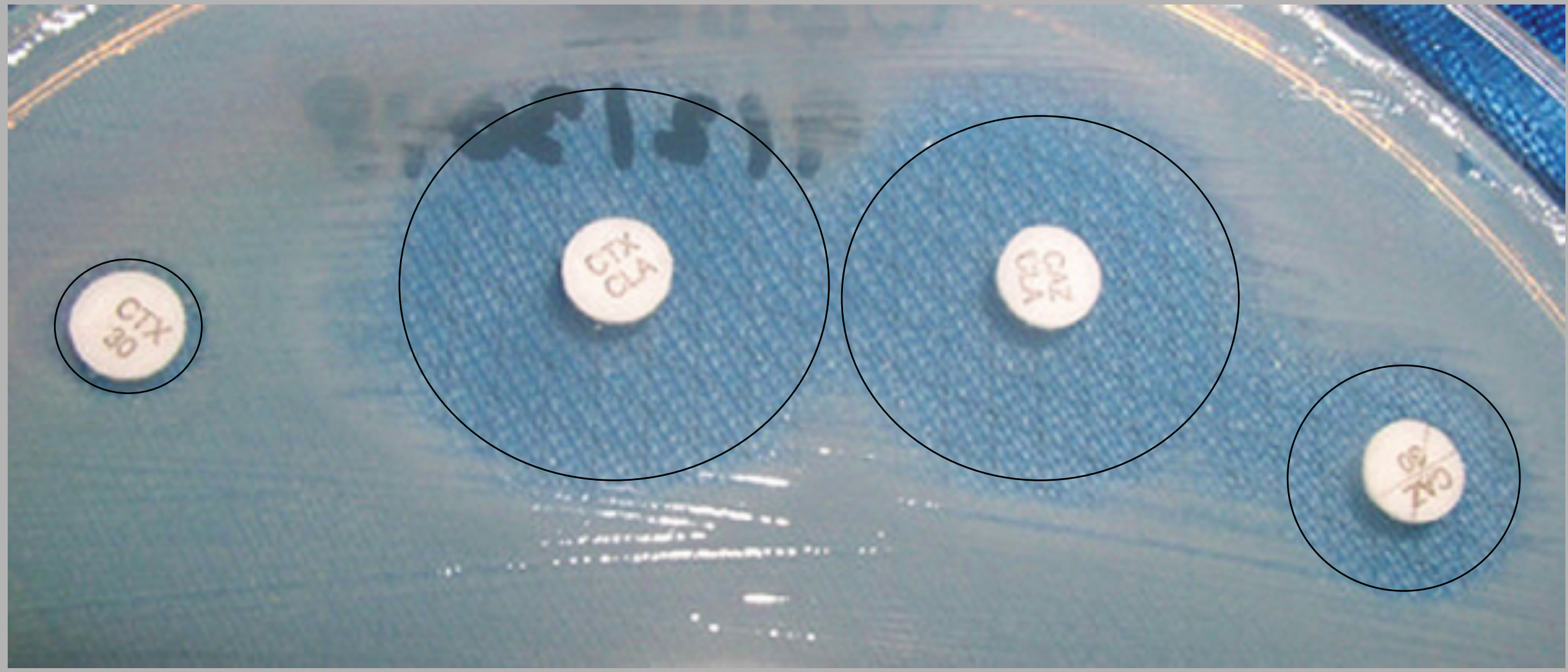
ESBLs confirmation

CTX vs CTX-Clavulanic acid

CAZ vs CAZ-Clavulanic acid

≥ 5 mm increase indicates ESBLs production
(or ≤ 3 twofold concentration decrease in MIC)

ESBL confirmatory test



Detection of carbapenemases

Screening for carbapenem resistance

- ❑ Susceptibility testing : CLSI, EUCAST guidelines
- ❑ Selective media

Confirmatory methods for detection of carbapenemase production

- ❑ Phenotypic detection
  - Modified Hodge test
  - Carbapenemase inhibition:
    clavulanic acid, boronic acid; EDTA, dipicolinic acid
  - Carba NP test
  - Modified carbapenem inactivation method (mCIM): 2017
    (± eCIM for metallo-beta-lactamase)
- ❑ Nucleic acid amplification testing
  - Conventional, real-time PCR
  - DNA microarrays
  - Gene sequencing detection
- ❑ Carbapenem hydrolysis (UV spectrophotometry, MALDI-TOF)

Carbapenemase screening in *Enterobacteriaceae (2013)*

	Disk diffusion	MIC-broth dilution
: Ertapenem	19-21 mm	2 μg/ml
: Meropenem	16-21 mm	2-4 μg/ml
: Imipenem	-	2-4 μg/ml

Positive screening test

+

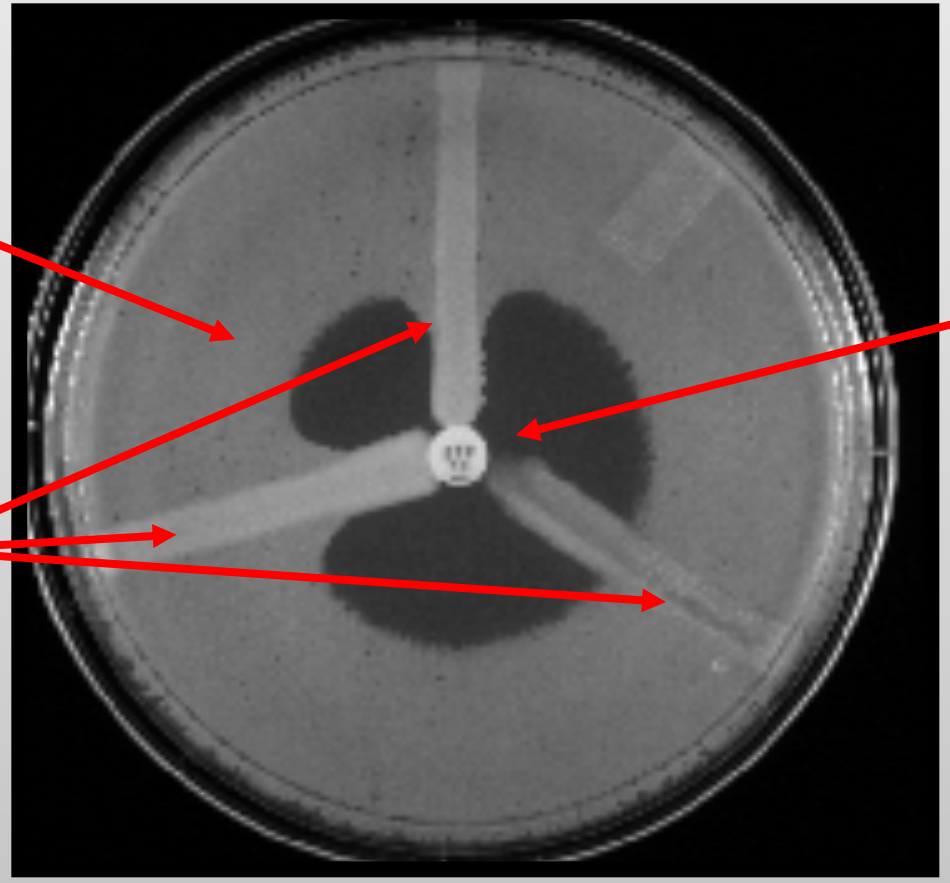
resistance to at least one of 3rd generation cephalosporins

↓

Confirmatory test

Modified Hodge Test (MHT)

Carbapenem Inactivation Assay



Disadvantage : time-consuming, and cannot distinguish the carbapenemase type

Anderson KF et al. Evaluation of methods to identify KPC in *Enterobacteriaceae*. JCM 2007; 45: 2723 – 2725

Direct Methods for Detection of Carbapenemase Activity

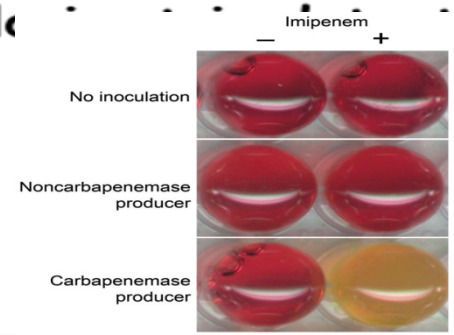
Colorimetric assays; The Carba NP test

100% sensitivity and specificity

Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*

Patrice Nordmann, Laurent Poirel, and Laurent Dortet

To rapidly identify carbapenemase producers in *Enterobacteriaceae*, we developed the Carba NP test. The test uses isolated bacterial colonies and is based on in vitro hydrolysis of a carbapenem, imipenem. It was 100% sensitive and specific compared with molecular-based techniques. This rapid (<2 hours), inexpensive technique may be implemented in any laboratory.



Patrice Nordmann, et al. Emerging Infectious Diseases, September 2012

Modified carbapenem inactivation method (mCIM)

mCIM Only or in Conjunction With eCIM
For epidemiological or infection control purposes. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for mCIM **positive and/or eCIM results**. mCIM **with or without eCIM** testing is not currently recommended for routine use. • **mCIM is used for detecting carbapenemases in *Enterobacteriaceae* and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-β-lactamases from serine carbapenemases in *Enterobacteriaceae*.** • **mCIM can be performed alone; however, eCIM must be performed together with mCIM.** • **eCIM is only valid if mCIM is positive.**

Modified carbapenem inactivation method (mCIM)

1 loop (1 µl) of suspected colony

suspended

2 ml of Tryptic soy broth (TSB)

1 disc of meropenem (10 µg)

Incubated at 35±2 °C in ambient air for 4 hrs (±15 mins)

Modified carbapenem inactivation method (mCIM)

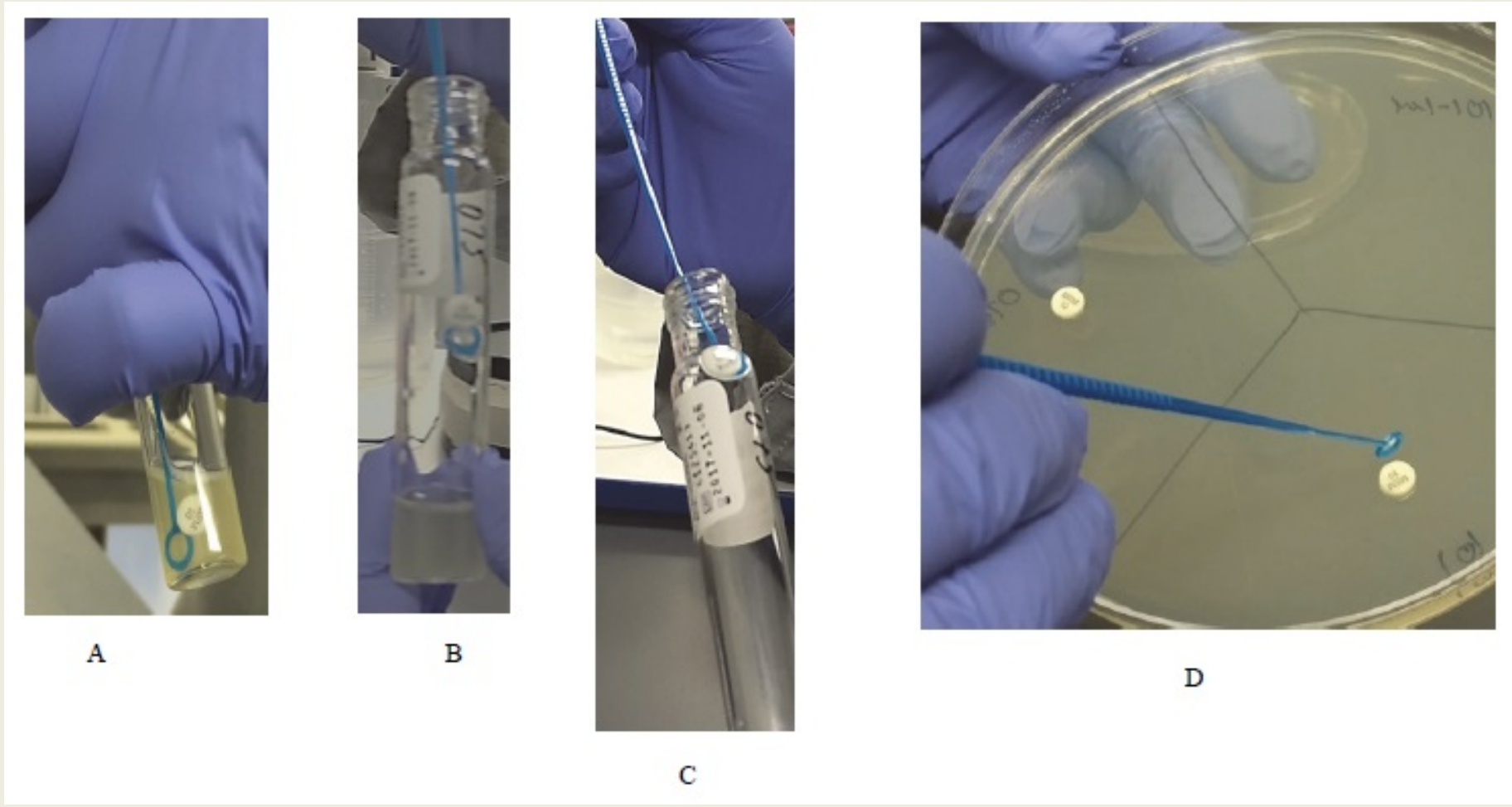
just before for 4 hrs (±15 mins)

Spread MHA with *E.coli* (ATCC 25922)

(similar protocol as regular susceptibility test)

Modified carbapenem inactivation method (mCIM)

After 4 hr ± 15 mins of incubation



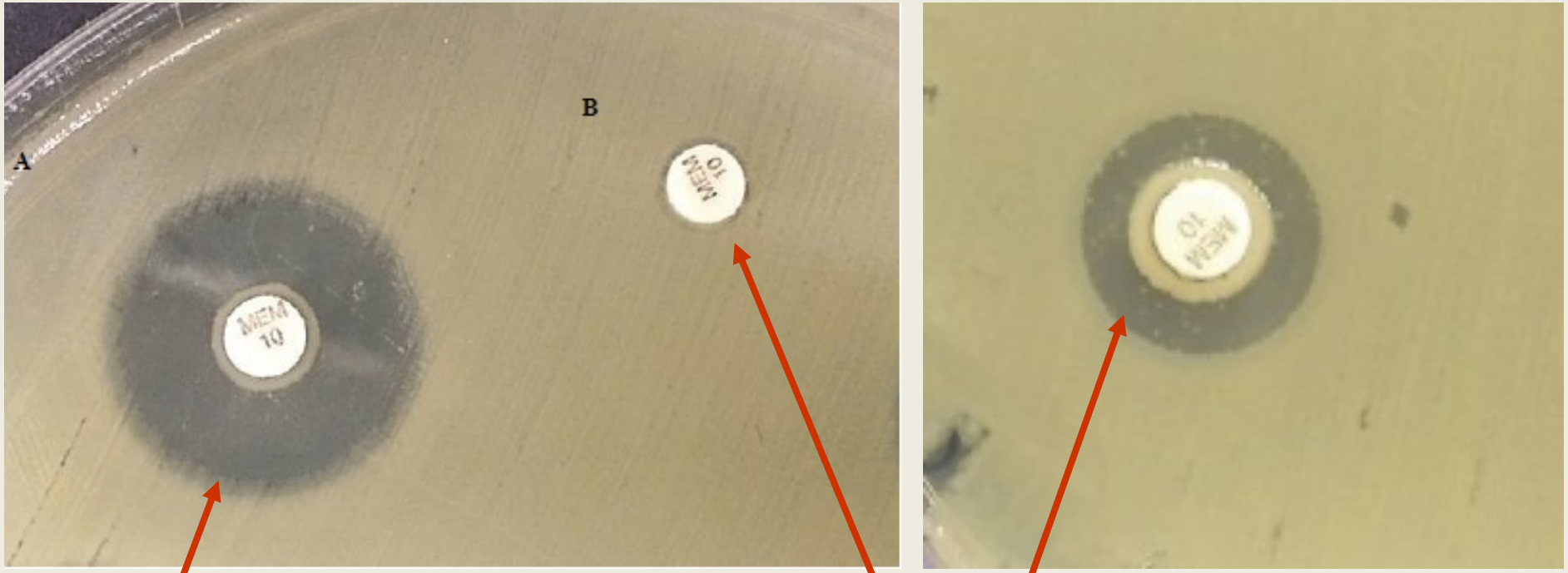
A

B

C

D

Modified carbapenem inactivation method (mCIM)



Negative
(≥19 mm)

Positive
(6-15 mm) or
(16-18 mm with colonies inside the zone)

eCIM

1. For each isolate, label a second 2-mL TSB tube for the eCIM test.
2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA.
3. Follow steps 1 through 9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel.
4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E.coli* ATCC® 25922 indicator strain.

NOTE: Additional QC is needed for the eCIM test (see QC recommendations).

Quality control strains for mCIM and eCIM

QC Strain	Organism Characteristic	Expected Result
K. pneumoniae ATCC® BAA-1705™	KPC positive Serine carbapenemase producer	mCIM positive eCIM negative
K. pneumoniae ATCC® BAA-1706™	Carbapenemase negative	mCIM negative
K. pneumoniae ATCC® BAA-2146™*	NDM positive Metallo-β-lactamase producer	mCIM positive eCIM positive

* eCIM positive control; to be set up only when the eCIM test is performed.

eCIM interpretation

eCIM – Interpret only when mCIM test is positive

- Metallo-β-lactamase positive:
  - A ≥5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C).
  - If the test isolate produces a metallo-β-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter when compared to the mCIM zone diameter.
- Metallo-β-lactamase negative:
  - A ≤4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D).
  - If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤4 mm) increase in zone diameter in the presence of EDTA compared to the mCIM zone diameter.

mCIM & eCIM report

mCIM Only or in Conjunction With eCIM		
mCIM Only		
mCIM Result	**eCIM Result**	**Report**
Negative	Not set up	Carbapenemase not detected
Positive	Not set up	Carbapenemase detected
Indeterminate	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*
mCIM and eCIM Combination Test		
mCIM Result	**eCIM Result**	**Report**
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected
Positive	Positive	Metallo-β-lactamase detected
Indeterminate	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*

* If indeterminate results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, CarbaNP), a test for carbapenemase genes or send isolate to a referral laboratory for further testing.

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Type: research-article

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Volume 00, Number 00, 2018

DOI: 10.1089/mdr.2018.0080

EPIDEMIOLOGY

Carbapenemase-Producing Carbapenem-Resistant *Enterobacteriaceae* from Bangkok, Thailand, and Their Detection by the Carba NP and Modified Carbapenem Inactivation Method Tests

Warawut Laolerd,[1] Yukihiro Akeda,[2–4] Likit Preeyanont,[5] Panan Ratthawongjirakul,[6] and Pitak Santanirand[1]

Aim: The purpose of the study was to determine the epidemiology of carbapenemase genes among carbapenem-resistant *Enterobacteriaceae* and evaluate the Carba NP and modified carbapenem inactivation method (mCIM) tests in their detection.

Materials and Methods: A total of 287 nonduplicated *Enterobacteriaceae* isolates, which were at least resistant to one of the carbapenems, were identified and detected for carbapenemase genes by multiplex PCR covering bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$. All positive genes were then sequenced. These isolates were phenotypically tested for the production of carbapenemases by mCIM and Carba NP tests to evaluate the efficacy of these methods.

Results: Seven species of carbapenem-resistant isolates mainly *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* were detected. Of these isolates, three families of carbapenemase genes, including bla_{NDM} ($bla_{NDM\text{-}1}$, $_{-4}$, $_{-5}$, $_{-9}$), bla_{OXA} ($bla_{OXA\text{-}48}$, $_{-181}$, $_{-232}$), and $bla_{IMP\text{-}14}$, were found. Of these, 223 (77.70%) carried at least one of the carbapenemase genes. The bla_{NDM} was detected in 160/223 (71.75%) isolates, of which 153/160 (95.63%) were the $bla_{NDM\text{-}1}$. Three types of the $bla_{OXA\text{-}48\text{-}like}$ group, $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}181}$, and $bla_{OXA\text{-}232}$, were found, 91/104 (87.5%) harbored the $bla_{OXA\text{-}232}$. In addition, 25.11% (56/223) of the carbapenemase-producing isolates harbored a combination of bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$. Phenotypic detection methods, mCIM and Carba NP, showed 100% sensitivity and specificity to bla_{NDM}, $bla_{IMP\text{-}14}$, and $bla_{OXA\text{-}48}$, while the mCIM was positive in all $bla_{OXA\text{-}181}$ and $bla_{OXA\text{-}232}$ isolates, only 12.5% (1/8) and 28.95% (11/38), respectively, were detected by the Carba NP test.

Conclusions: This study revealed a unique prevalence of carbapenemase genes in Bangkok, Thailand, as well as demonstrated the efficacy and limitation of phenotypic detection methods of carbapenemase in the area where $bla_{NDM\text{-}1}$ and $bla_{OXA\text{-}232}$ were predominant.

TABLE 1. DISTRIBUTION OF CARBAPENEMASE GENES AMONG CARBAPENEMASE-PRODUCING CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* ISOLATES BY MULTIPLEX PCR (N=223)

Organisms	bla_{NDM}, n *(%)*	$bla_{OXA\text{-}48\text{-}like}$, n *(%)*	bla_{IMP}, n *(%)*	bla_{VIM}	bla_{KPC}	bla_{NDM} *and* $bla_{OXA\text{-}48\text{-}like}$, n *(%)*	n *(%)*
Klebsiella pneumoniae	62 (27.80)	49 (21.97)	2 (0.90)	0	0	55 (24.66)	168 (75.34)
Escherichia coli	22 (9.87)	4 (1.79)	0	0	0	1 (0.45)	27 (12.11)
Enterobacter cloacae	9 (4.04)	3 (1.35)	5 (2.24)	0	0	0	17 (7.62)
Enterobacter aerogenes	1 (0.45)	0	0	0	0	0	1 (0.45)
Citrobacter freundii	5 (2.24)	0	0	0	0	0	5 (2.24)
Citrobacter koseri	1 (0.45)	0	0	0	0	0	1 (0.45)
Providencia rettgeri	4 (1.79)	0	0	0	0	0	4 (1.79)
Total	104 (46.64)	56 (25.11)	7 (3.14)	0	0	56 (25.11)	223 (100)

TABLE 2. IDENTIFICATION OF CARBAPENEMASE GENES AMONG CARBAPENEMASE-PRODUCING CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* ISOLATES CONTAINING RESISTANT GENES (N=223)

PCR	*SQ*	*KP*, n (%)	*EC*, n (%)	*ET*, n (%)	*OT*, n (%)	*Total*, n (%)
bla_{OXA-48} (56)	bla_{OXA-48}	9 (4.04)	1 (0.45)	0	0	10 (4.48)
	$bla_{OXA-181}$	5 (2.24)	3 (1.35)	0	0	8 (3.59)
	$bla_{OXA-232}$	35 (15.70)	0	3 (1.35)	0	38 (17.04)
bla_{IMP} (7)	bla_{IMP-14}	2 (0.90)	0	5 (2.24)	0	7 (3.14)
bla_{NDM} (104)	bla_{NDM-1}	58 (26.01)	20 (8.97)	10 (4.48)	9 (4.04)	97 (43.50)
	bla_{NDM-4}	2 (0.90)	0	0	0	2 (0.90)
	bla_{NDM-5}	1 (0.45)	2 (0.90)	0	1 (0.45)	4 (1.79)
	bla_{NDM-9}	1 (0.45)	0	0	0	1 (0.45)
bla_{NDM} and bla_{OXA-48} (56)	bla_{NDM-1} & $bla_{OXA-181}$	2 (0.90)	1 (0.45)	0	0	3 (1.35)
	bla_{NDM-1} & $bla_{OXA-232}$	53 (23.77)	0	0	0	53 (23.77)
	TOTAL	168 (75.34)	27 (12.11)	18 (8.07)	10 (4.48)	223 (100)

PCR, performed by simplex PCR; SQ, genotype by sequencing of PCR products; KP, *Klebsiella pneumoniae*; EC, *Escherichia coli*; ET, *Enterobacter* spp. (one isolate of *Enterobacter aerogenes*=bla_{NDM-1}, nine isolates of *Enterobacter cloacae*); OT, other organisms (four isolates of *Providencia rettgeri*=bla_{NDM-1}, one isolate of *Citrobacter koseri*=bla_{NDM-1}, four isolates of *Citrobacter freundii*, bla_{NDM-1}, and one isolate of *C. freundii*=bla_{NDM-5}).

The efficacy of mCIM and the Carba NP test on detection of carbapenemase genes (N=223)

Carbapenemase gene	mCIM Positive	Carba NP Positive
bla$_{OXA-48}$ (10)	10	10
bla$_{OXA-181}$ (8)	8	1
bla$_{OXA-232}$ (38)	38	11
bla$_{IMP-14}$ (7)	7	7
bla$_{NDM-1}$ (97)	97	97
bla$_{NDM-4}$ (2)	2	2
bla$_{NDM-5}$ (4)	4	4
bla$_{NDM-9}$ (1)	1	1
bla$_{NDM-1}$ and*bla*$_{OXA-181}$ (3)	3	3
bla$_{NDM-1}$ and *bla*$_{OXA-232}$ (53)	53	53
Total (223)	223	189

Table 2A: *Enterobacterales*

- Colistin and polymyxin B MIC breakpoints, warning, reporting comments, and reference

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
LIPOPEPTIDES (36) WARNING: Clinical and PK-PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended. (37) Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.											
O	Colistin or polymyxin B		– –	– –	– –	– –	– –	– –	≤2^ ≤2	≥4 ≥4	(38) Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[8]). (39) Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[8]). (40) When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. (41) For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

- **Colistin:** broth microdilution / CBDE (Colistin broth disk elution) / CAT (Colistin agar test)
- **Polymyxin B:** broth microdilution

General facts about colistin and polymyxin B

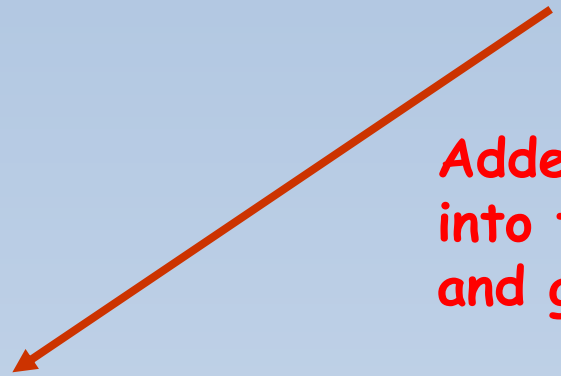
- They have limited clinical efficacy, even if an intermediate result is obtained.
- Alternative agents are strongly preferred.
- They should be used in combination of one or more active antimicrobial agents.
- For colistin, broth microdilution, colistin broth disk elution (CBDE) and colistin agar test (CAT) are acceptable.***
- For polymyxin B, only the broth microdilution are acceptable.
- Disk diffusion and gradient diffusion methods (E-test) should not be performed.
- Colistin and polymyxin B are considered equivalent agents.
- MICs obtained from testing colistin predict MICs to polymyxin B and vice versa.

***The CBDE and CAT methods were evaluated for *Acinetobacter* spp. by CLSI and found to yield inaccurate results. Therefore, these 2 methods can be used for Enterobacterales and *Pseudomonas aeruginosa* only, NOT for *Acinetobacter* spp.

Tests for colistin resistance for Enterobaterales and *Pseudomonas aeruginosa*

Colistin broth disk elution (CBDE) method

Cation adjusted Mueller Hinton broth (CAMHB) (4 tubes x 10 ml each)
: lebelling as PC (positive control), 1, 2, and 4 µg/ml)



Added 1, 2, and 4 disks of colistin (10 µg)
into tube lebelling as 1, 2, and 4 µg/ml, respectively,
and gently vortex of each tube

Incubated the tubes at RT for 30-60 mins

Colistin broth disk elution (CBDE) method (cont.)

Preparing the bacterial suspension at 0.5 McFarland turbidity
(used this adjusted suspension within 15 mins after preparation)

Added 50 µl of the bacterial suspension
into each tube (PC, 1, 2, and 4 µg/ml, respectively),
and then vortex the suspension

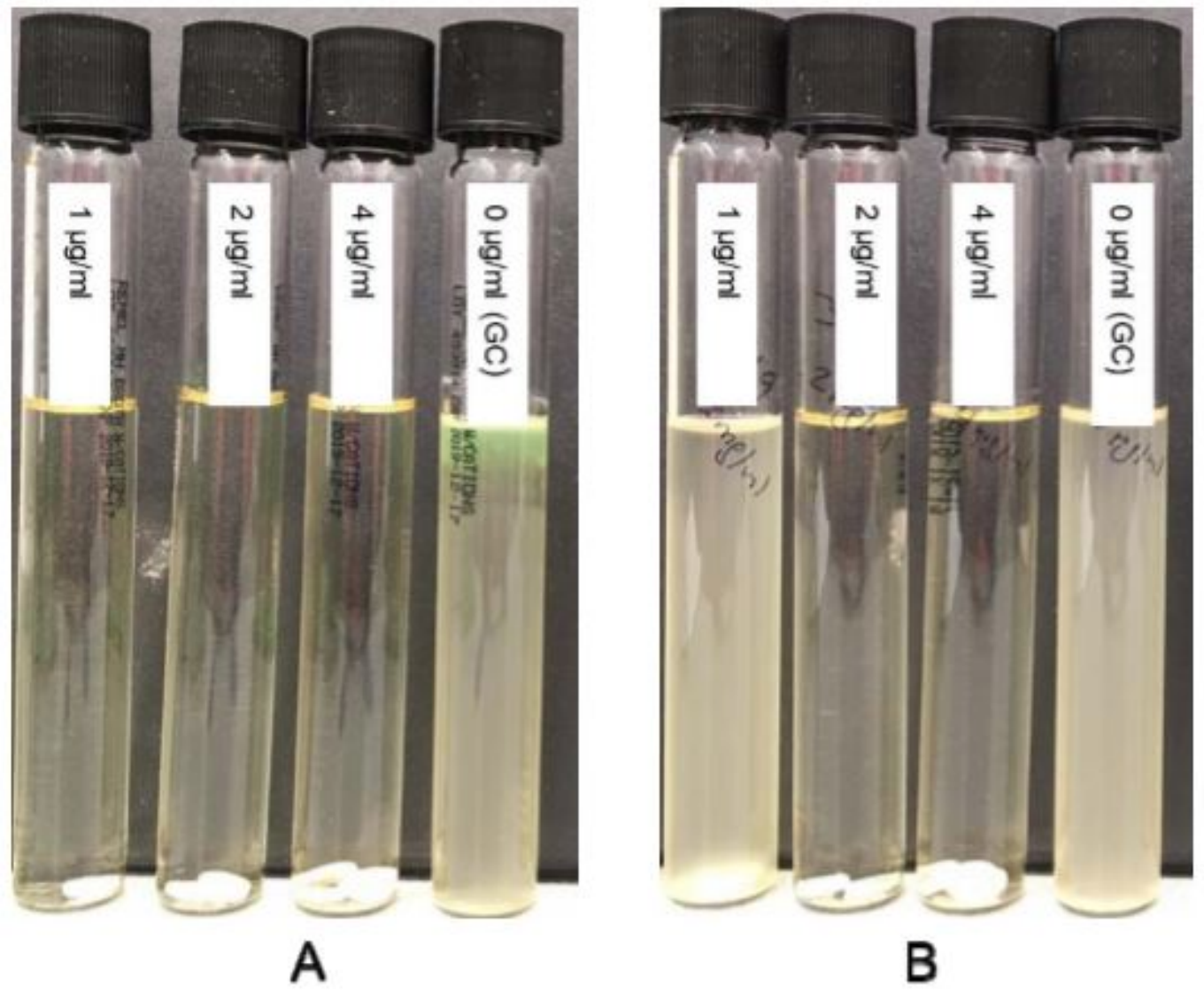
=> Final bacterial concentration approximately 7.5×10^5 CFU/ml

Incubated at 33-35 °C in ambient air for 16-20 hrs

Note: Performing a purity check by using 10 µl loop

Colistin broth disk elution (CBDE) method (cont.)

Examples of results



MIC of organism A ≤ 1 µg/ml

MIC of organism B = 2 µg/ml

Colistin broth disk elution (CBDE) method (cont.)

Interpretation of results (for Enterobacterales and *Pseudomonas aeruginosa*)

MIC ≤ 2 µg/ml read as Intermediate

MIC ≥ 4 µg/ml read as Resistant

If there is an inconsistent growth pattern (eg. No growth at 2 µg/ml but growth at 1 and 4 µg/ml => repeat the test

An inconsistent growth pattern may be the result of

: contamination at higher dilution
: heteroresistance
: improper concentration of antimicrobial agent in the tube
: error inoculating the tube

Terminology used in susceptibility testing

Susceptible (S)

Susceptible-dose dependent (SDD)

Intermediate (I) / (I^)

Resistant (R)

Nonsusceptible (NS)

Terminology used in susceptibility testing

Susceptible (S)

… used for organisms that are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy.

Intermediate (I)

… approach usually attainable blood and tissue levels and/or for which response rates may be lower than for susceptible isolates.

… also includes a buffer zone, to prevent small, uncontrolled technical factors from causing major discrepancies in interpretations

NOTE for 2022: An (I) with "^" indicates agents that have the potential to concentrate in the urine. It's for informational use only.

Terminology used in susceptibility testing

Susceptible-Dose Dependent (SDD)

"susceptibility of an isolate is dependent on the dosing regimen" If the test results are in the SDD category, it is necessary to use a dosing regimen (ie, higher doses, more frequent doses or both) that results in higher drug exposure than the regular doses for susceptible isolates. Consideration should be given to the maximum approved dosage regimen, because higher exposure gives the highest possibility of adequate coverage of an SDD isolate.

Susceptible dose dependent (SDD)-2022

Enterobacterales:	Cefepime, Piperacillin, Piperacillin-tazobactam
Staphylococcus aureus:	Ceftalorine
Enterococcus faecium:	Daptomycin (MIC only)

Terminology used in susceptibility testing

Nonsusceptible (NS)

… used for organisms that have only a susceptible interpretive category. A susceptible-only interpretive category may be applied to

1. agents for which no or rare resistant isolates have been encountered. (identification and susceptibility test results should be confirmed.)***

2. certain breakpoint which is limited to the reliability of the technique.

Nonsusceptible (NS) (1)

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

(5) Breakpoints for *Streptococcus* spp. β-hemolytic group are proposed based on population distributions of antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Sy available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
			S	I	R	S	I	R
PENICILLINS								
(6) An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for app against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is a surrogate for ampicillin, amoxicillin, amoxi cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For g a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.								
A	Penicillin or	10 units	≥24	–	–	≤0.12	–	–
A	ampicillin	10 µg	≥24	–	–	≤0.25	–	–
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
See comment (6).								
B	Cefepime or	30 µg	≥24	–	–	≤0.5	–	–
B	cefotaxime or	30 µg	≥24	–	–	≤0.5	–	–
B	ceftriaxone	30 µg	≥24	–	–	≤0.5	–	–
C	Ceftaroline	30 µg	≥26	–	–	≤0.5	–	–
CARBAPENEMS								

Nonsusceptible (NS) (2)

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
			S	I	R	S	I	R
PENICILLINS								
(5) For nonmeningitis isolates, a penicillin MIC of ≤ 0.06 µg/mL (or oxacillin zone ≥ 20 mm) can predict susceptibility to the following ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozi cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem.								
See general comment (4).								
A	Penicillin	1 µg oxacillin	≥20	–	–	–	–	–
A	Penicillin parenteral (nonmeningitis)	–	–	–	–	≤2	4	≥8
A	Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12

Terminology used in susceptibility testing

Nonsusceptible (NS)

≠

Not susceptible

↓

Intermediate and Resistant

Table 1A: Suggested grouping of agents

- Warning of antimicrobial agents should not be routinely reported for bacteria isolated from CSF.

=> agents administered by oral route only

=> 1st and 2nd generation cephalosporins

=> Cephamycins

=> Doripenem, Ertapenem, Imipenem

=> Clindamycin

=> Lefamulin

=> Macrolides

=> Tetracyclines

=> Fluoroquinolones

Appendix A

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Escherichia coli, *Klebsiella* ***pneumoniae***, ***K. oxytoca***, and *Proteus mirabilis*	**Cephems**	**Cephalosporin III/IV** – I/**SDD** or R			X
Salmonella and *Shigella* spp.[c]	**Cephems**	Cephalosporin III – I or R		X	
	Macrolides	**Azithromycin – NWT or R**		**X**	
	Fluoroquinolones	**Any** fluoroquinolone – I or R		X	
Acinetobacter baumannii **complex**	**Carbapenems**	**Any** carbapenem[d] – I or R			**X**
	Lipopeptides	Colistin/polymyxin **B** – R	**X**		
Pseudomonas aeruginosa	**β-lactam combination agents**	**Ceftolozane-tazobactam – I or R**		**X**	
	Carbapenems	Any carbapenem[d]– I or R			X
	Aminoglycosides	Amikacin **and** gentamicin and tobramycin – R			X
	Lipopeptides	Colistin/polymyxin **B** – R	**X**		
Stenotrophomonas maltophilia	**Folate pathway antagonists**	Trimethoprim-sulfamethoxazole – I or R			**X**

Antimicrobial intrinsic resistance of *Enterobacteriaceae* (CLSI, M100S-31st ed., 2022)

B1. Enterobacterales

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Citrobacter freundii	R	R	R		R	R	R						
Citrobacter koseri, *Citrobacter amalonaticus* group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to ß-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R						R[c]	
Klebsiella (formerly *Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, *Klebsiella oxytoca*, *Klebsiella variicola*	R			R									
Morganella morganii	R	R			R		R	[d]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[d]	R	R	R	R	
Proteus penneri	R				R		R	[d]	R	R	R	R	
Proteus vulgaris	R				R		R	[d]	R	R	R	R	
Providencia rettgeri	R	R			R			[d]	R	R	R	R	
Providencia stuartii	R	R			R			[d]	R	R	R	R	[e]
Raoultella spp.[f]	R			R									
Salmonella and *Shigella* spp.	There is no intrinsic resistance to ß-lactams in these organisms; refer to **WARNING** below for reporting.												
Serratia marcescens	R	R	R		R	R	R				R	R	
Yersinia enterocolitica	R	R		R	R								

C. amalonaticus **group** = *C. amalonaticus, C. farmer, C, sedlakii*

E. Cloacae **complex** = *E. asburiae, E. cloacae, E. hormaechei*

Raoultella **spp.** = *R. ornithinolytica, R. terrigena, R. planticola*

R[c] = applied to *H. alvei* and *H. paralvei*