



Basic methodology for disk diffusion and broth microdilution

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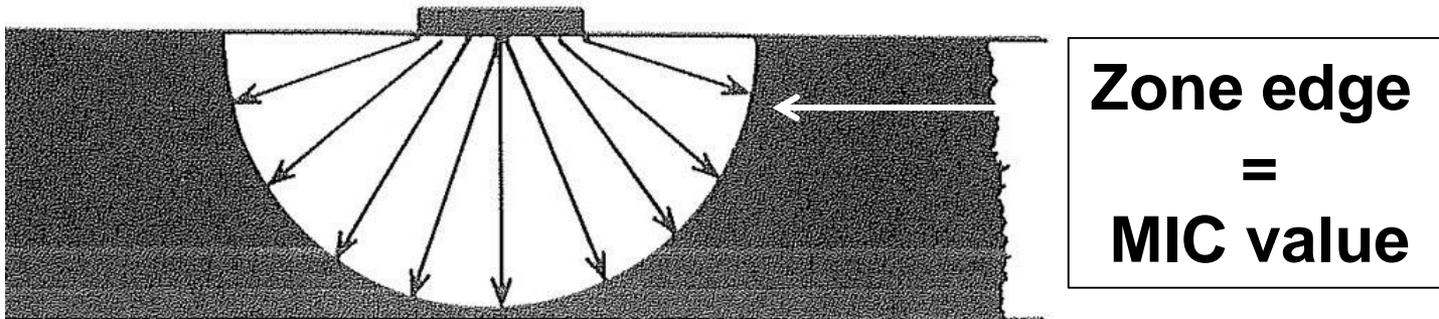
Standardisation of AST

- Results change with changed parameters.
 - It is crucial to adhere to the methodology to get reproducible and reliable results!
- Standardisation of:
 - Disk potency
 - Media
 - Type of media, supplements, pH, agar depth etc.
 - Inoculum
 - Incubation
 - Reading of results

The EUCAST disk diffusion method

Disk diffusion

- Radial diffusion of the antibiotic from a disk to form a concentration gradient in the agar
- Must be calibrated to MIC values



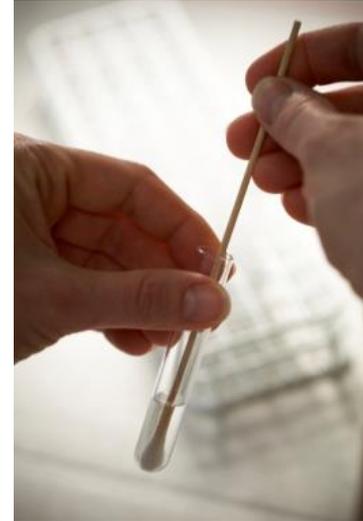
MH and MH-F media



- Non-fastidious organisms: Un-supplemented Mueller-Hinton (MH) agar
- Fastidious organisms: Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L β -NAD (MH-F)
- Agar depth: 4.0 ± 0.5 mm
- Test each new batch of MH agar powder to ensure that all zones are within EUCAST QC ranges

Inoculum preparation

- Inoculum: McF 0.5
 - Overnight culture on non-selective media
 - Pick several morphologically similar colonies to avoid selecting an atypical variant
 - It is recommended to use a photometric device to measure the density of the inoculum suspension



Inoculation of plates

- 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 and take particular care to ensure that there are no gaps between streaks.
- When using the same inoculum suspension for several plates, the cotton swab should be dipped in the inoculum suspension for each plate.

The more carefully the plates are streaked, the easier it is to read the zone diameters!



Application of disks and incubation

- Apply disks within 15 min of inoculation
 - Store disks at the temperature recommended by the manufacturer, protected from light and with a desiccant
- Incubate plates at 35°C for 16-20 h
 - Incubate MH plates in air
 - Incubate MH-F plates in 5% CO₂ (except for *Campylobacter*)
 - Do not exceed the incubation time!

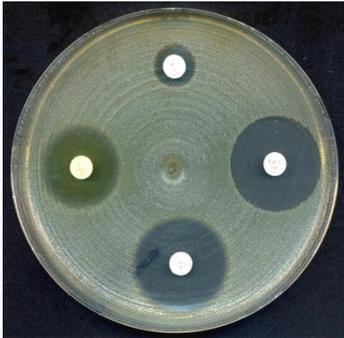
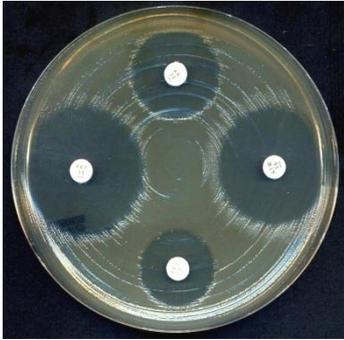


The 15-15-15 minute rule

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.

The growth should be confluent and evenly spread over the plate



Plates should look like this..

..and NOT like this!

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



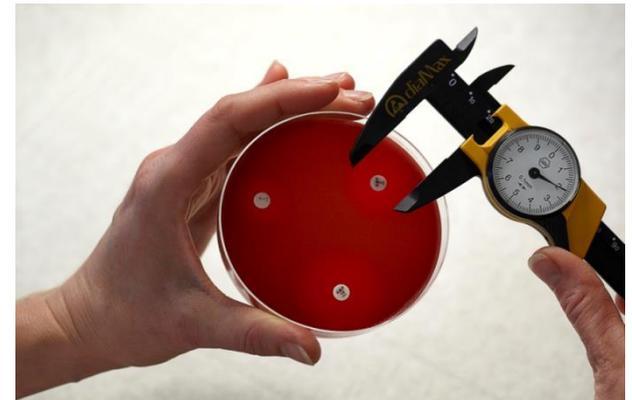
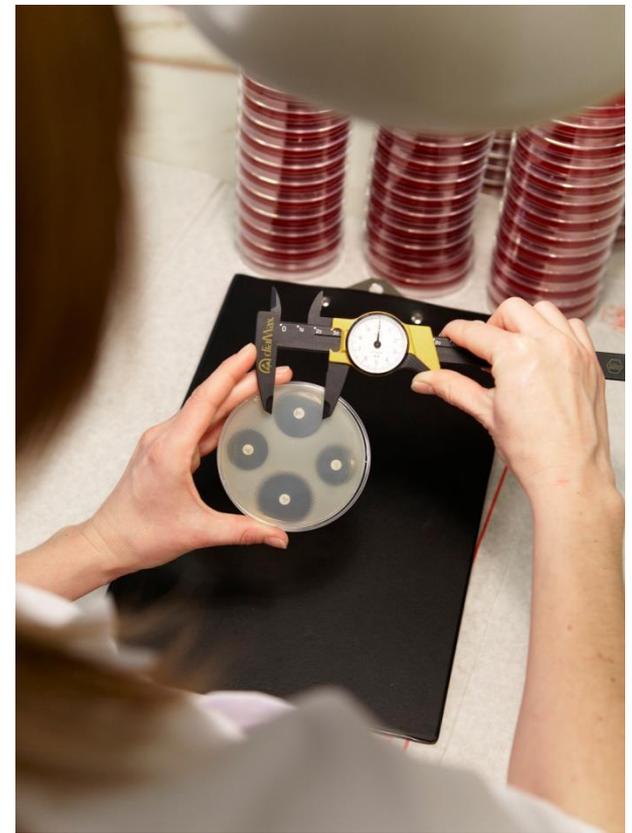
CoNS
Trimethoprim



S. pneumoniae
Rifampicin

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.

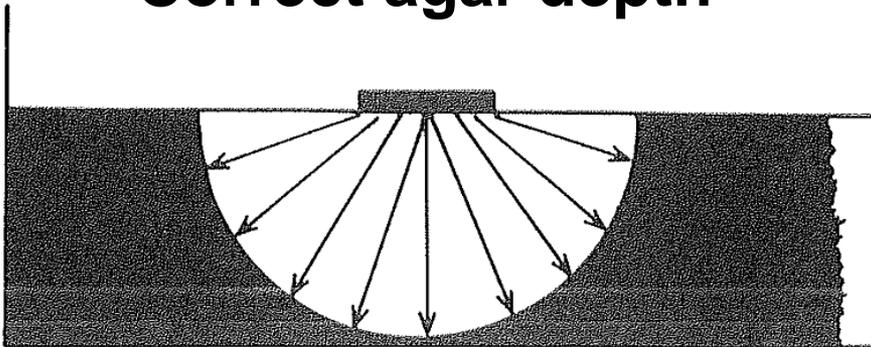


Questions

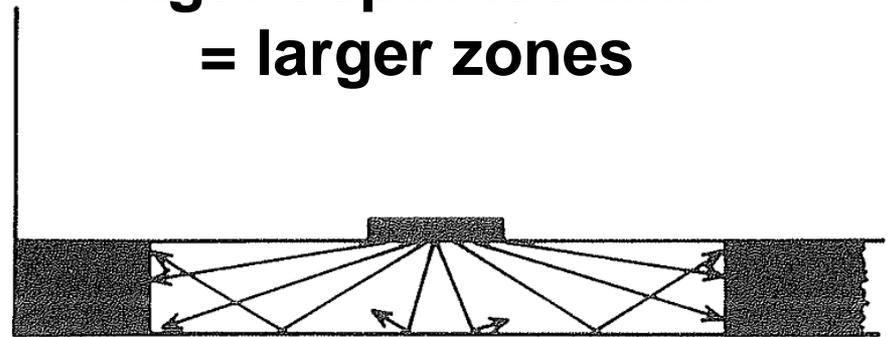
Why is the agar depth important?

- The diffusion of the antimicrobial agent depends on the agar depth:

Correct agar depth



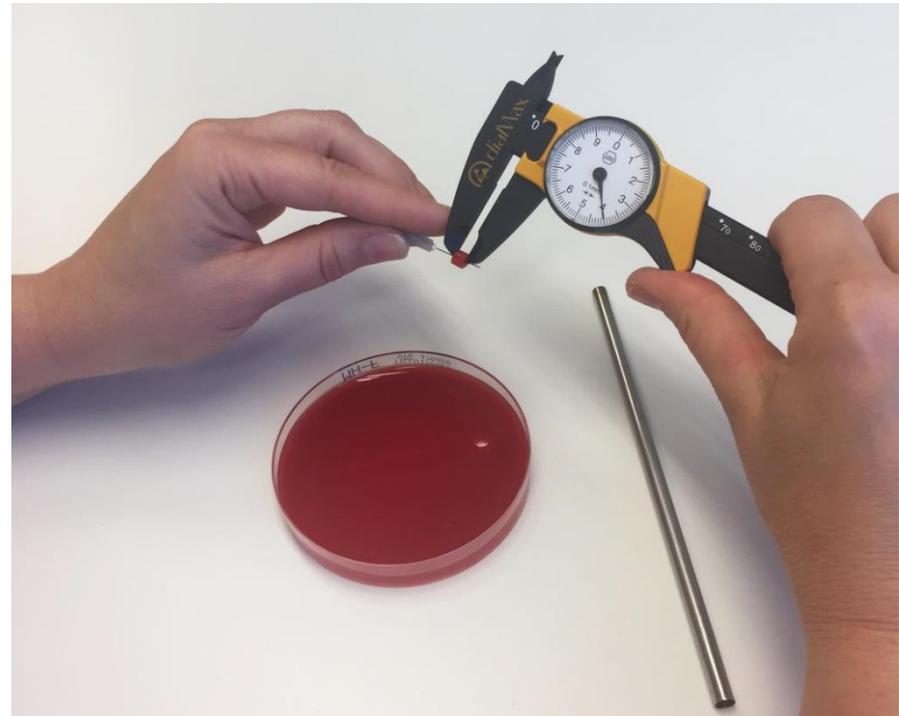
**Agar depth too thin
= larger zones**



- EUCAST QC criteria and zone diameter breakpoints are calibrated to an agar depth of 4.0 ± 0.5 mm.

Measuring agar depth

- Check the agar depth for all new lots of plates, both commercial and in-house produced.



Correct agar depth: 4.0 mm, with occasional variation between 3.5 and 4.5 mm!

Why do you recommend to dry MH-F plates before inoculation?

- Excess moisture may cause fuzzy zone edges and/or haze within zones.
 - No drops of water should be visible on the surface of the agar or inside the lid. This is often seen with plates stored in plastic bags or sealed containers.
 - If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min.



MH-F agar plates

Excess humidity and fuzzy zone edges



We often get poor growth of *Aerococcus* and *Corynebacteria*.

How can we improve that?

- For organisms that grow with small colonies
 - Use a wet cotton swab when inoculating plates
 - Streak plates slowly and carefully
- Limit the number of disks per plate
 - For very susceptible isolates, the number of disks per plate might have to be limited to two disks
- Investigate if poor growth is related to a specific media manufacturer

Can we use antimicrobial disks from one manufacturer and media from another?

- EUCAST recommendation: Results for QC strains should be within the acceptable limits and optimally close to the target values.

E. coli ATCC 25922

Ceftazidime-avibactam 10-4 µg BD disk			
BBL MH	Bio-Rad MH	bMx MH	Oxoid MH
28	27	27	25
28	27	28	26
27	27	27	25
28	27	26	25
27	28	27	25
27	27	26	27
27	27	27	25
27	26	26	26
28	28	27	26
28	28	27	26
28	28	28	26
29	28	27	27
27	27	27	25
28	26	26	25
26	27	25	25

Ceftazidime-avibactam 10-4 µg Oxoid disk			
BBL MH	Bio-Rad MH	bMx MH	Oxoid MH
29	28	28	26
29	28	29	27
28	27	27	26
29	28	28	26
29	28	28	26
28	28	28	27
28	28	27	26
28	27	28	27
28	28	27	27
29	28	27	26
28	28	28	27
29	29	28	27
28	28	28	26
29	28	28	26
27	27	26	26

Ceftazidime 10 µg Oxoid disk			
BBL MH	Bio-Rad MH	bMx MH	Oxoid MH
27	28	28	26
28	27	29	27
27	27	27	26
27	27	27	26
27	28	28	26
26	28	27	26
27	27	27	26
27	27	27	25
27	27	27	25
27	27	27	25
28	27	28	26
27	28	27	26
26	27	27	25
26	27	27	26
25	26	26	25

Mean	28	27	27	26
Target	27			
Range	24-30			

Mean	28	28	28	26
Target	27			
Range	24-30			

Mean	27	27	27	26
Target	26			
Range	23-29			

Disks from different manufacturers

Manufacturer	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Antimicrobial disk																		
Benzylpenicillin 1 unit					L	L			H	NA			NA		H			
Amoxicillin-clav. 20-10 µg	H	H		L			L	L					H	L				
Piperacillin-tazo. 30-6 µg							L	L	H				NA	L		H		H
Oxacillin 1 µg		H	L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg							NA						NA					
Cefoxitin 30 µg	H*		H		L		NA						L*	L		H		
Ceftazidime 10 µg							L	L					L	L*				
Meropenem 10 µg	H		H*				L				H		H	L*				
Ciprofloxacin 5 µg					L								H					L
Norfloxacin 10 µg							L		L				H*					
Pefloxacin 5 µg			L	L	L		NA		NA				H	H				
Gentamicin 10 µg					H				NA				H	H				H
Tobramycin 10 µg	NA		H										H*	H				
Erythromycin 15 µg			L		L		L		L				H		L*			
Tetracycline 30 µg			L		L*		L		L*					L	L		L	

Mean value within ± 1 mm of the target value

Mean value >1 mm but within ± 2 mm of the target value

Mean value >2 mm from target value but still within the QC range

Mean value out of the QC range

NA = Not Available

H = High, mean value > 1 mm above target

L = Low, mean value > 1 mm below target

* One or more readings out of QC range

How long can we use antimicrobial disks once the cartridges have been opened?

- Store disks according to manufacturer's instructions
 - Correct temperature, protected from light
 - Opened cartridges should also be stored with a desiccant
- Note that manufacturer's might have different recommendations for different disks
 - Some manufacturers recommend to store some disks at a lower temperature (freezer vs. fridge)
- Note that some disks are more sensitive to degradation than others and might have to be treated differently
 - E.g. carbapenems and amoxicillin-clavulanic acid

How long can we use antimicrobial disks once the cartridges have been opened?

- Allow disk cartridges to reach room temperature before opening
 - As soon as a disk cartridge is opened there is a risk of degradation of the agent due to humidity in the air
- Check if the manufacturer states how long an open cartridge can be used
- Set up a system in which disks are used frequent, or change to new cartridges at fixed intervals
- Perform QC daily or at least four times a week
 - Change to a new cartridge when there is a trend of decreasing zone diameters

How much variation is acceptable when reading zones from the same plate?

- When reading zones from the same plate, readings should optimally be within ± 1 mm of the mean value.
- QC ranges are most commonly based on a target value ± 3 mm
 - Set to allow for reasonable variation between manufacturers of disks and media, and for day-to-day variation in testing.

Reading exercise, Växjö laboratory

S. aureus

First occasion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
26	27	26	29	20	30
26	28	28	29	22	31
26	29	28	29	25	31
29	28	28	30	23	31
26	27	27	28	19	30
26	28	29	30	23	32
26	29	29	30	21	31
26	27	27	29	23	29
25	27	26	29	20	32
25	25	26	28	25	32
28	27	26	27	22	30

Mean

26

27

27

29

22

31

SD

1.2

1.1

1.2

0.9

2.0

1.0

> 1 mm above mean

> 1 mm below mean

Discussion

S. aureus and fuzzy zone edges

- 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.



Reading exercise, Växjö laboratory

S. aureus

First occasion

	Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacine	Rifampicin
	26	27	26	29	20	30
	26	28	28	29	22	31
	26	29	28	29	25	31
	29	28	28	30	23	31
	26	27	27	28	19	30
	26	28	29	30	23	32
	26	29	29	30	21	31
	26	27	27	29	23	29
	25	27	26	29	20	32
	25	25	26	28	25	32
	28	27	26	27	22	30
Mean	26	27	27	29	22	31
SD	1.2	1.1	1.2	0.9	2.0	1.0

> 1 mm above mean

> 1 mm below mean

Discussion

S. aureus and norfloxacin

- If cultures are pure, read the inner zone.
- There is almost always a double zone for *S. aureus* and norfloxacin.



Reading exercise, Växjö laboratory

S. aureus

Following analysis and discussion

Cefoxitin	Erythromycin	Clindamycin	Fusidic acid	Norfloxacin	Rifampicin
27	24	25	26	18	28
27	25	26	28	18	29
27	25	26	27	17	27
27	25	26	27	16	27
27	25	26	27	19	28
28	24	24	26	17	25
27	26	27	29	19	29
27	24	26	28	18	28
27	25	23	26	18	27
28	25	26	27	18	30
27	24	24	25	17	27

Mean

27

25

25

27

18

28

SD

0.4

0.6

1.2

1.1

0.9

1.3

> 1 mm above mean

> 1 mm below mean

Take-home message!

Standardisation of disk diffusion is crucial!

Factors affecting the zone size:

- Medium (agar depth, pH, supplements)
- Size of inoculum (McF 0.5)
- Age of inoculum (overnight culture)
- Streaking of plates (technique, speed, removing excess fluid)
- Antimicrobial disks (correct potency, storage and handling)
- Incubation environment (air/CO₂/microaerobic)
- Incubation time
- ...and the reported zone diameter also by reading!

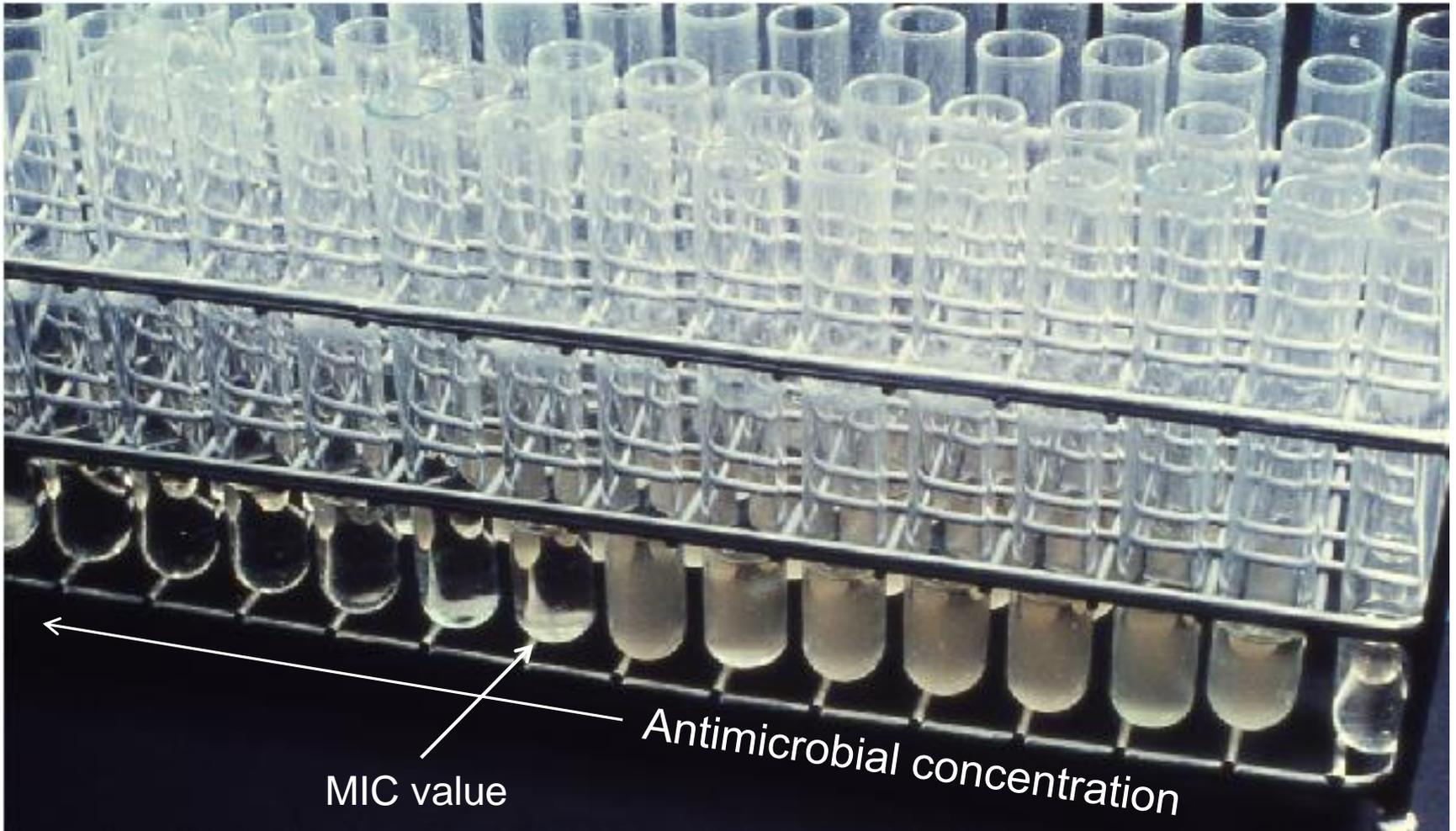
MIC determination with broth microdilution

MIC – the gold standard

Minimum Inhibitory Concentration

- “The lowest concentration that, under defined *in vitro* conditions, prevents visible growth of bacteria within a defined period of time” (ISO 20776-1).
- The MIC recorded as the lowest concentration of the agent that completely inhibits visible growth.

Broth macrodilution



Broth microdilution

Microtiter plates ($\leq 200 \mu\text{L}$)

SENSITITRE CUSTOM PLATE FORMAT

2011140914

Plate Code: **SEMHI2**

Date: **24-Jan-18**

	1	2	3	4	5	6	7	8	9	10	11	12
A	CIP 0.015	P/T4 0.008/4	DOX 0.12	AMP 0.25	AMOX 0.25	AUGC 0.25/2	FOT 0.015	FUR 0.25	AXO 0.015	SXT 0.06/1.19	MERO 0.03	IMI 0.25
B	CIP 0.03	P/T4 0.015/4	DOX 0.25	AMP 0.5	AMOX 0.5	AUGC 0.5/2	FOT 0.03	FUR 0.5	AXO 0.03	SXT 0.12/2.38	MERO 0.06	IMI 0.5
C	CIP 0.06	P/T4 0.03/4	DOX 0.5	AMP 1	AMOX 1	AUGC 1/2	FOT 0.06	FUR 1	AXO 0.06	SXT 0.25/4.75	MERO 0.12	IMI 1
D	CIP 0.12	P/T4 0.06/4	DOX 1	AMP 2	AMOX 2	AUGC 2/2	FOT 0.12	FUR 2	AXO 0.12	SXT 0.5/9.5	MERO 0.25	IMI 2
E	CIP 0.25	P/T4 0.12/4	DOX 2	AMP 4	AMOX 4	AUGC 4/2	FOT 0.25	FUR 4	AXO 0.25	SXT 1/19	MERO 0.5	IMI 4
F	CIP 0.5	P/T4 0.25/4	DOX 4	AMP 8	AMOX 8	AUGC 8/2	FOT 0.5	FUR 8	AXO 0.5	SXT 2/38	MERO 1	IMI 8
G	CIP 1	P/T4 0.5/4	P/T4 2/4	TET 0.12	TET 0.25	TET 0.5	FOT 1	FOT 4	AXO 1	SXT 4/76	MERO 2	MERO 8
H	CIP 2	P/T4 1/4	P/T4 4/4	TET 1	TET 2	TET 4	FOT 2	FOT 8	AXO 2	AXO 4	MERO 4	POS CON

ANTIMICROBICS

CIP	Ciprofloxacin
P/T4	Piperacillin / tazobactam constant 4
DOX	Doxycycline
AMP	Ampicillin
TET	Tetracycline
AMOX	Amoxicillin
AUGC	Amoxicillin / clavulanic acid constant 2
FOT	Cefotaxime
FUR	Cefuroxime
AXO	Ceftriaxone
SXT	Trimethoprim / sulfamethoxazole
MERO	Meropenem
IMI	Imipenem
POS	Positive Control

INTERNATIONAL
STANDARD

ISO
20776-1

Second edition
2019-06

**Susceptibility testing of infectious
agents and evaluation of performance
of antimicrobial susceptibility test
devices —**

Part 1:

**Broth micro-dilution reference
method for testing the in vitro activity
of antimicrobial agents against rapidly
growing aerobic bacteria involved in
infectious diseases**

BMD methodology

Inoculum	5 x 10 ⁵ CFU/mL
Media	MH broth (non-fastidious organisms) MH-F broth (Fastidious organisms)
Incubation	35°C in air (sealed panels) 16-20 h (24 h for glycopeptides)
Reading	Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye

Preparation of inoculum

- Final inoculum of 5×10^5 CFU/mL
- Start with McF 0.5 ($\sim 1-2 \times 10^8$ CFU/mL for *E. coli* ATCC 25922)
 - Dilutions depend on:
 - Volume of broth in wells (Fresh or freeze-dried panels)
 - Volume to be dispensed in each well

Preparation of inoculum

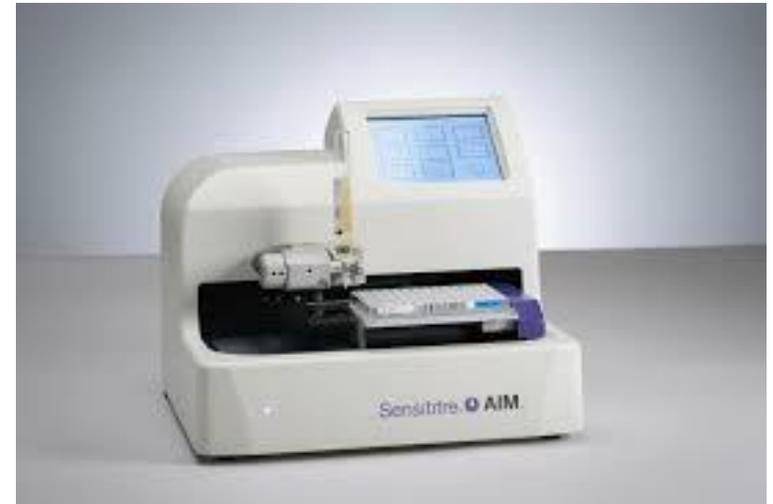
- Example for freeze-dried Sensititre panels with 100 µl dispensing (reconstitution) volume

Organism	McF	Transfer volume to 11 mL broth
Non-fastidious Gram-positive and Gram-negative bacteria	0.5	50 µL
<i>Proteus</i> , <i>Morganella</i> and <i>Providencia</i> spp.	0.5	10 µL (to reduce problems with swarming)
<i>Haemophilus influenzae</i>	0.5	50 µL
<i>Streptococcus</i> spp. and other fastidious Gram-positive cocci	0.5	100 µL

Check manufacturer's recommendations for commercial plates!

Inoculation of BMD plates

- Manually, preferably with a multi-channel pipette
- Using a dispensing robot
- A check of the purity and the size of the inoculum can be performed from the growth control well before incubating the plates.



AIM: part of the Sensititre system from Thermo Scientific

Incubation

- Seal panels with a tight lid or adhesive seal to avoid evaporation
- To avoid uneven heating, microdilution trays should not be stacked more than four high
 - By placing an empty tray at the top of each stack, condensation on the inside of the adhesive cover is reduced, which facilitates reading.
- Incubate at 35°C in air for 16-20 h
 - Glycopeptides should be read after 24 h incubation
 - Prolonged incubation (40-48 h) is only allowed when specified in the EUCAST Breakpoint Table
 - *Corynebacterium*, *Aerococcus*, *Kingella kingae* and *Campylobacter*

Reading BMD 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.

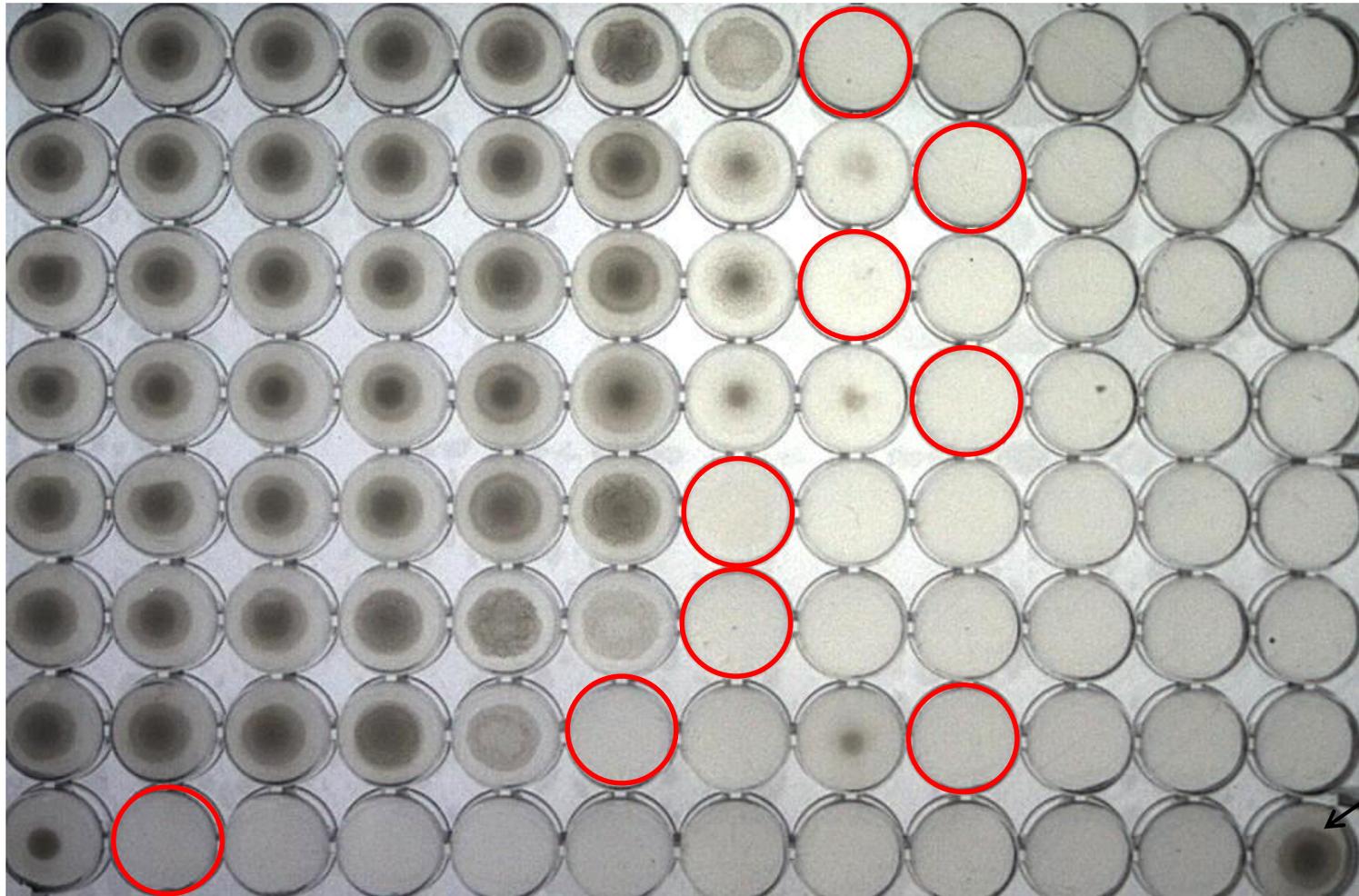


Reading BMD endpoints

- Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.
- Read MICs only when there is sufficient growth, *i.e.* obvious button or definite turbidity, in the positive growth control.

Reading of BMD panels

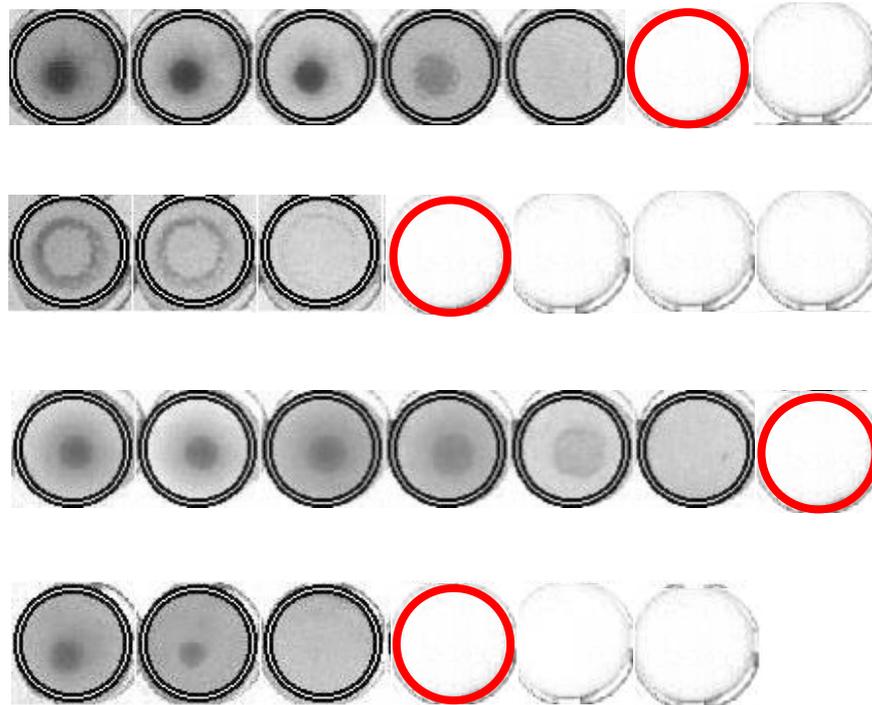
Unless otherwise stated, endpoints should be read at complete inhibition of growth.



Antimicrobial concentration \longrightarrow

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

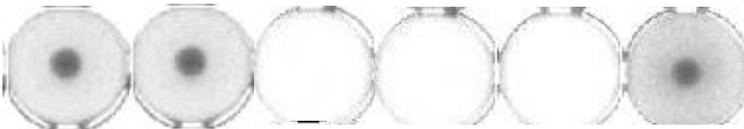
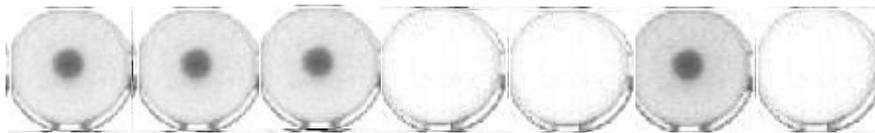
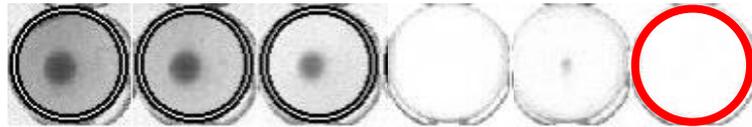
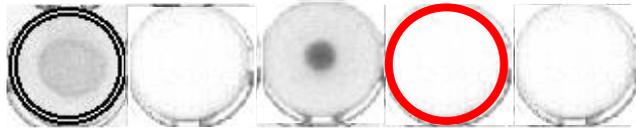
- For fastidious organisms tested in MH-F broth, haemolysis of the blood can be seen. This is often accompanied by turbidity or a deposit of growth (pellet).
- Haemolysis with turbidity or pellet should be regarded as growth when determining endpoints.



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.
- 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!

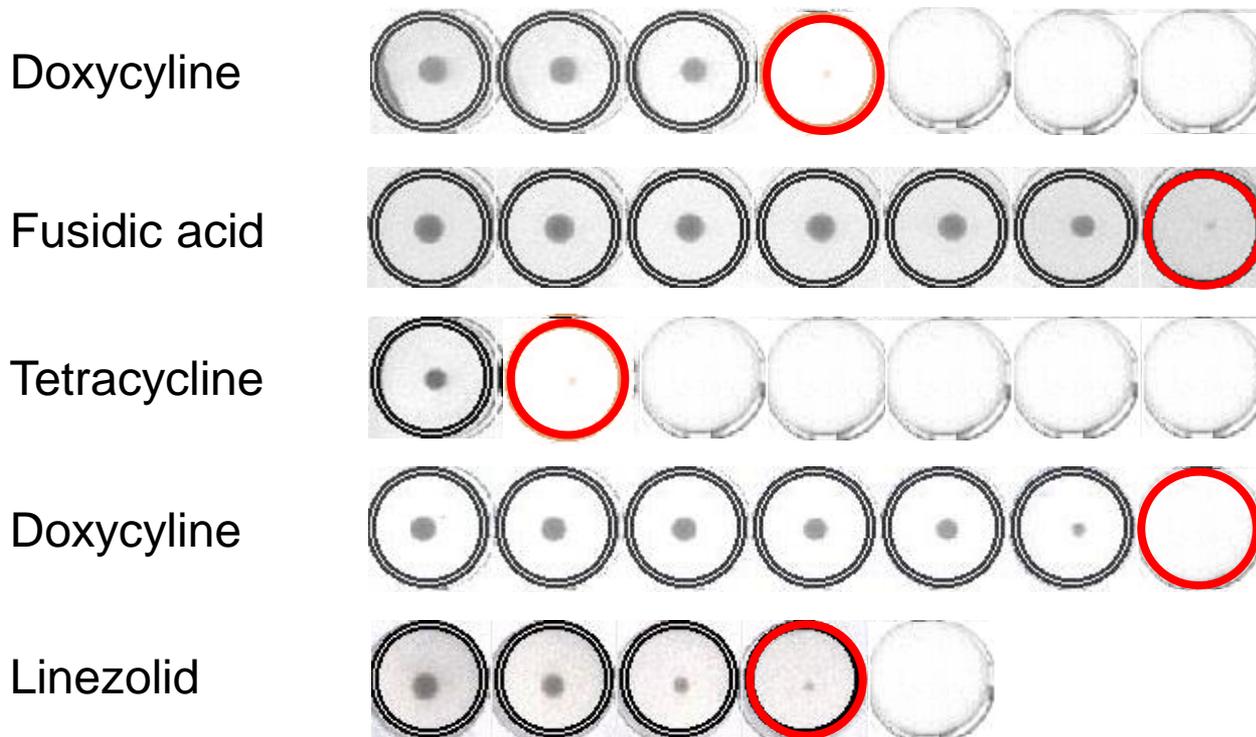
Results invalid!

Specific reading instructions related to trailing endpoints

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

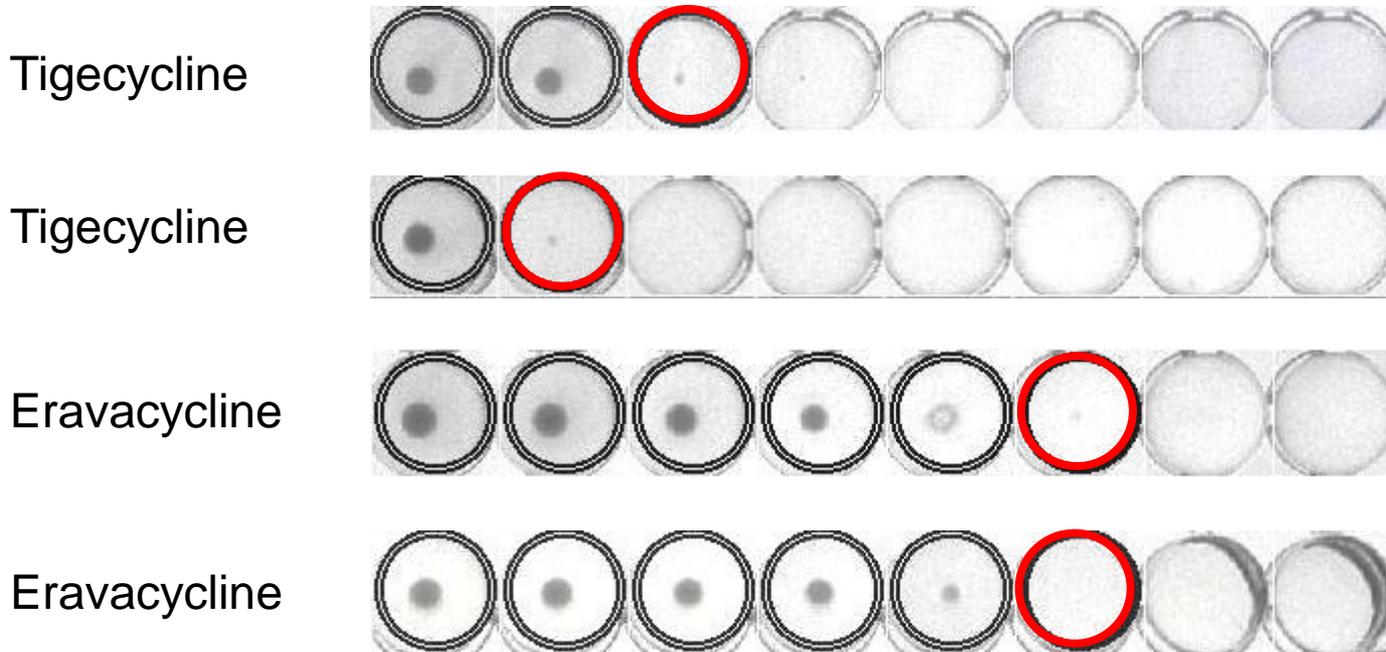
Gram-positive cocci with bacteriostatic antimicrobial agents

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



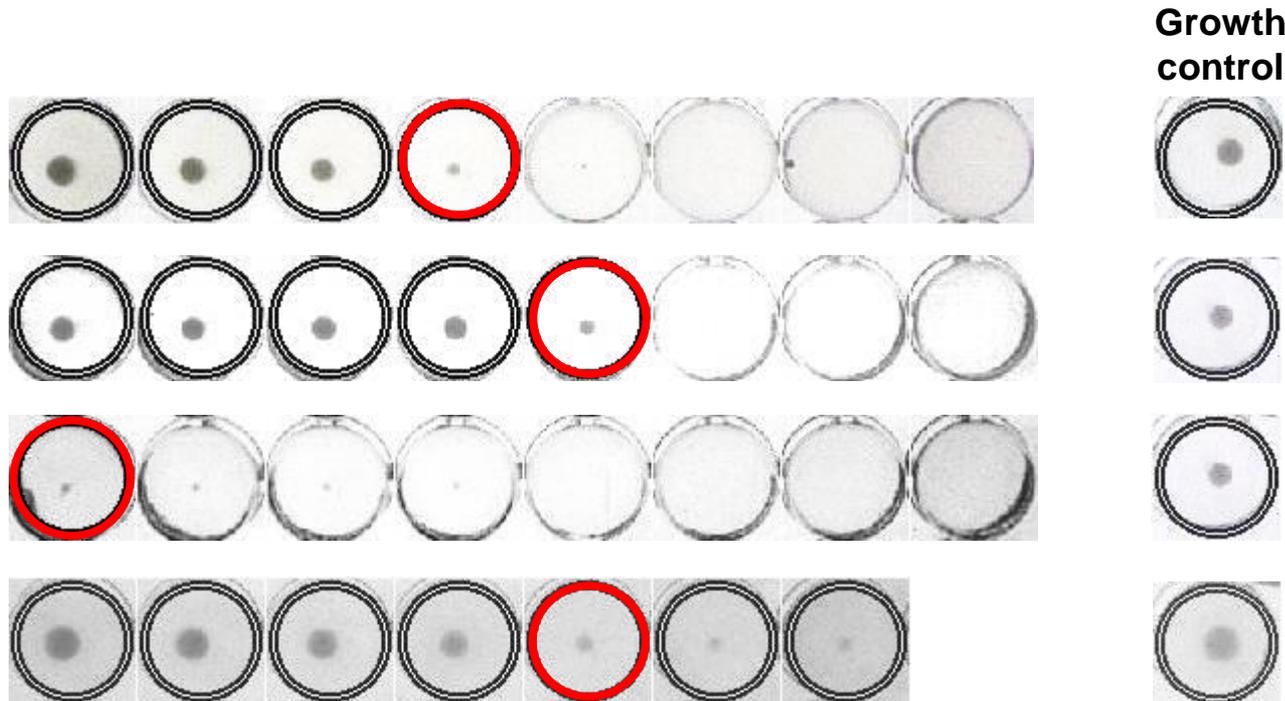
Gram-negative organisms with bacteriostatic antimicrobial agents

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



Trimethoprim and trimethoprim-sulfamethoxazole

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



Quality control

- Reference methods don't automatically give the correct results just because they are reference methods.
- For reproducible and reliable results, methodology must be strictly adhered to.
- Quality control must be performed for all materials used (BMD panels, broth etc).
 - Commercial BMD panels must often be tested with several strains to cover all agents

Example of QC of BMD panels

Enterobacterales panel

QC strain

E. coli ATCC 25922
 E. coli ATCC 25922

AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
4	≤0.06	0.12	0.25	≤0.015	≤0.25	0.008	0.5	0.12	≤0.03	≤0.015	8	4
4	≤0.06	0.06	0.5	≤0.015	≤0.25	0.008	0.5	0.12	≤0.03	≤0.015	8	4
8	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	16	4
8	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
4	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	16	2
4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.25	≤0.03	0.03	8	4
4	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	0.03	8	4
4	≤0.06	0.12	0.25	≤0.015	0.5	-	0.5	0.25	≤0.03	0.03	8	4

Target

Range

4 0.03-0.06 0.06 0.12-0.25 0.008 0.5-1 0.008 0.5 0.125-0.250.015-0.030.015-0.03 8 2
 2-8 0.015-0.12 0.03-0.12 0.06-0.5 0.004-0.015 0.25-2 0.004-0.015 0.25-1 0.06-0.5 0.008-0.060.008-0.06 4-16 1-4

QC strain

P. aeruginosa ATCC 27853
 P. aeruginosa ATCC 27853

AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
>32	1	8	1	0.25	2	4	0.5	2	1	0.25	>128	2
>32	2	>8	1	0.25	2	2	0.5	1	1	0.25	>128	2
>32	1	>8	2	0.25	2	4	1	2	1	0.25	>128	4
>32	-	8	1	0.25	2	2	1	2	1	0.25	>128	2
>32	2	8	2	0.25	2	2	1	2	1	0.25	>128	2
>32	2	>8	2	0.25	1	4	1	2	1	0.25	>128	8
>32	2	>8	-	0.25	1	4	1	4	1	0.5	>128	-
>32	1	>8	2	0.25	1	4	1	1	1	0.25	>128	-
>32	2	>8	2	0.25	1	4	1	2	1	0.5	>128	4
>32	2	>8	2	0.25	1	>4	1	2	1	0.5	>128	2

Target

Range

1-2 2 0.25-0.5 1-2 1 2 1-2 0.25-0.5 2-4
 0.5-4 1-4 0.125-1 0.5-4 0.5-2 1-4 0.5-4 0.125-1 1-8

On target
Upper limit
Lower limit
Out of range

Example of QC of BMD panels

Enterobacteriales panel

QC strain

K. pneumoniae ATCC 700603
 K. pneumoniae ATCC 700603

AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
>32	1	4	>16	0.25	2	0.06	8	0.12/0.5	1	0.06	64	16
>32	0.5	2	16	0.5	1	0.06	4	0.25	1	0.03	128	8
>32	0.5	2	>16	0.5	1	0.06	8	0.12/0.5	0.5	0.03	64	8
>32	0.5/2	2	16	0.5	1	0.06	4	0.25	0.5	0.03	128	8
>32	1	2	>16	0.5	0.5	0.03	8	0.12/0.5	1	0.03	64	8
>32	1	2	16	0.5	1	0.06	4	0.25	1	0.03	64	8
>32	0.5	2	>16	0.5	1	0.06	4	0.25	1	0.03	64	8
>32	1	2	>16	0.5	1	0.06	4	0.12	1	0.03	64	8
>32	1	2	>16	0.5	≤0.25	0.06	4	0.12	1	0.03	128	16
>32	0.5	2	>16	0.5	0.5	0.06	4	0.25	1	0.03	64	16

Target
Range

16
8-32

QC strain

E. coli NCTC 13846
 E. coli NCTC 13846

AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
>32	0.25	0.25	0.5	>4	8	0.03	0.5	0.25	>4	0.03	16	4
>32	0.25	0.25	0.5	>4	4	0.03	1	0.25	>4	0.03	16	4
>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	4
>32	0.12	0.25	0.25	>4	4	0.03	0.5	0.12	>4	0.03	16	4
>32	0.25	0.5	0.5	>4	4	0.03	0.5	0.25	>4	0.03	16	4
>32	0.25	0.5	0.5	>4	4	0.03	0.5	0.25	>4	0.03	16	4
>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	≤0.015	16	4
>32	0.25	0.25	0.5	>4	2	0.03	≤0.25	0.12	>4	0.03	16	8
>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	8
>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	4

Target
Range

4
(2-8)

On target
Upper limit
Lower limit
Out of range

Disk diffusion as control method

- Advantages of performing disk diffusion in parallel for at least some agents representing relevant antimicrobials:
 - Purity test
 - Control of the inoculum
 - A second method that can be used to interpret difficult BMD tests

AST of bacteria

Organization

Consultations

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Expert rules and expected phenotypes

Resistance mechanisms

Guidance documents

SOP

MIC and zone distributions and ECOFFs

AST of bacteria

Media preparation

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Quality Control

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Warnings!

MIC testing services from EUCAST



... Media preparation

Preparation of MH plates and broth

EUCAST antimicrobial susceptibility testing is based on MH agar and MH broth, without supplements for non-fastidious organisms and with supplements for fastidious organisms (Streptococci, Haemophilus influenzae, Campylobacter and others as indicated in the breakpoint tables). The plate and broth for fastidious organisms are named MH-F plates and MH-F broth.

[Preparation of agar plates and broth for EUCAST AST](#). Version 7, January 1, 2022.

For translations to other languages - see [Translations](#).

For previous versions of documents - see [Previous versions](#).

search term

AST of bacteria

- [Organization](#)
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- [EUCAST News](#)
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- [Clinical breakpoints and dosing](#)
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AST of bacteria

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... MIC determination

MIC determination of non-fastidious and fastidious organisms

The EUCAST recommendations for MIC determination for non-fastidious organisms are in complete agreement with the recommendations from the International Standards Organisation (+ ISO).

For fastidious organisms (streptococci including *S. pneumoniae*, *H. influenzae*, *Moraxella catharrhalis*, *Listeria monocytogenes*, *Pasteurella* spp, *Kingella kingae*, *Aerococcus* spp, *Campylobacter* spp, and others), EUCAST recommends the same methodology but with the use of MH-F broth (MH broth with lysed horse blood and beta-NAD), see EUCAST media preparation file.

[Media preparation](#) (agar and broth for non-fastidious and fastidious organisms) (v 6.0, 1 January, 2020).

[Broth microdilution - EUCAST reading guide v 4.0](#) (1 January 2022)

Guidance on cefiderocol MIC testing - see guidance document.

There are a number of commercially available surrogate MIC determination methods, such as commercial broth microdilution methods, gradient tests, semi-automated devices, etc. It

Information in EUCAST Breakpoint Tables

***Staphylococcus* spp.**

MIC determination (broth microdilution according to ISO standard 20776-1 except for fosfomicin where agar dilution is used)

Medium: Mueller-Hinton broth

Inoculum: 5×10^5 CFU/mL

Incubation: Sealed panels, air, $35 \pm 1^\circ\text{C}$, $18 \pm 2\text{h}$

Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.

Quality control: *Staphylococcus aureus* ATCC 29213. For agents not covered by this strain, see EUCAST QC Tables.

Streptococcus pneumoniae

MIC determination (broth microdilution according to ISO standard 20776-1)

Medium: Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β -NAD (MH-F broth)

Inoculum: 5×10^5 CFU/mL

Incubation: Sealed panels, air, $35 \pm 1^\circ\text{C}$, $18 \pm 2\text{h}$

Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.

Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

Questions and/or problem?

Contact us!

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