EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Broth microdilution reference methodology

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ICARS - ILRI webinar series

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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:
  - Potency of antimicrobial agent/disk potency
  - Media
    - Type of media, supplements, pH, agar depth etc.
  - Inoculum
  - Incubation
  - Reading of results

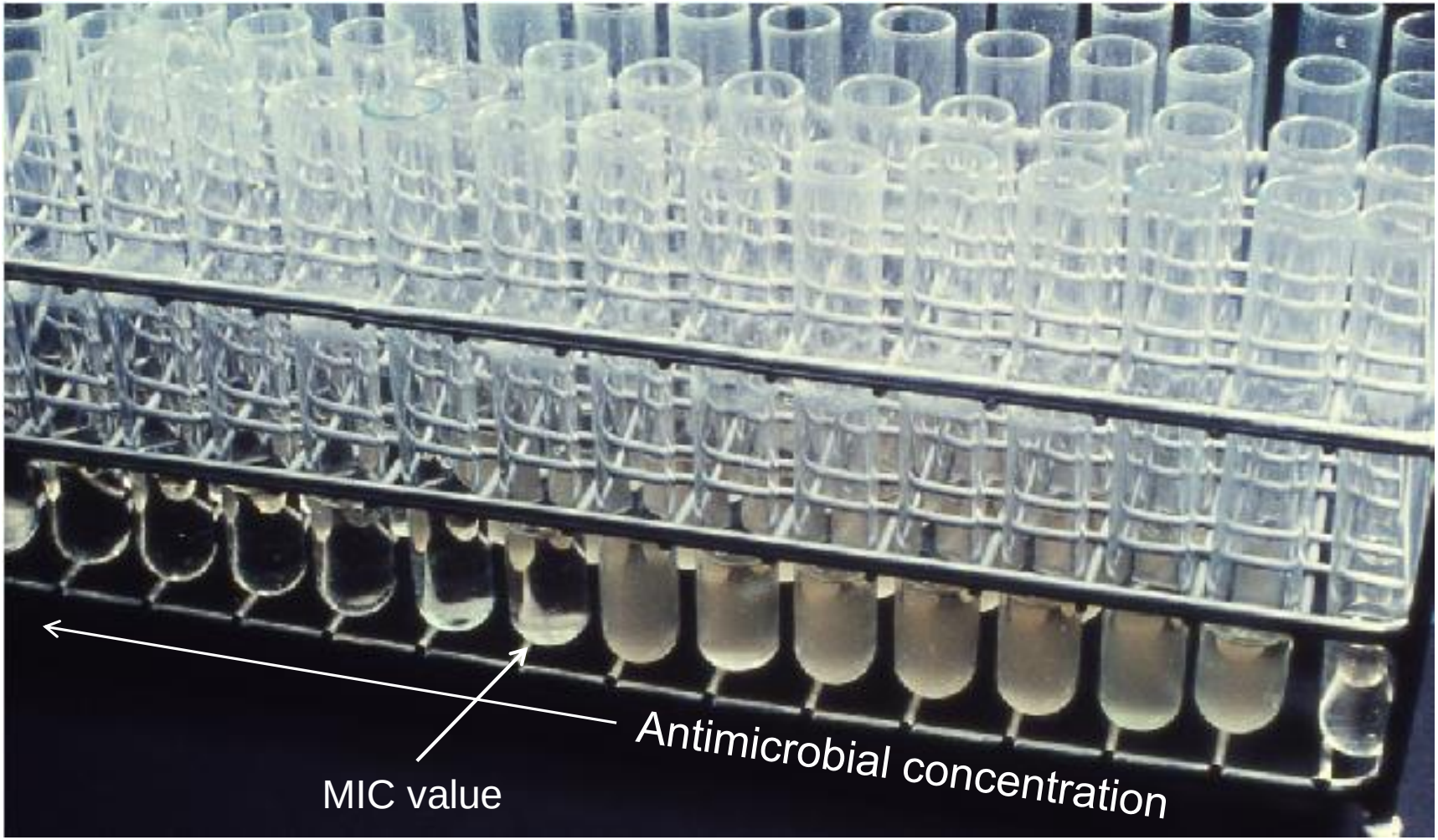
MIC – the gold standard

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 (≤200 µL)

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

Broth microdilution methodology

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

ISO 20776-1, 2019
EUCAST media for fastidious organisms

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

Broth microdilution (BMD)
ISO 20776-2, 2019

- Rapidly growing aerobic bacteria
- Fresh or frozen panels (<-60°C)
- Microdilution: ≤200 µL per well
- Standardisation of
  - Antimicrobial agents
    - Solvents, diluents, 2-fold dilutions
  - Inoculum
  - Incubation time and temperature
- Referral to EUCAST and CLSI documents for
  - Testing of fastidious organisms
  - Reading of MIC endpoints

EUCAST recommendations for BMD

- Perform BMD according to the ISO standard
  - Final inoculum of 5×10^5 CFU/mL
  - Sealed panels
  - Incubation in air, at 35°C for 16-20 h
    - 24 h incubation for glycopeptides

  Non-fastidious and fastidious organisms

- Commercial panels with freeze-dried antimicrobials are accepted by EUCAST if they produce expected results
  - BUT many commercial products include few dilutions, resulting in:
    - Truncated MICs in both the lower and upper end
    - Products difficult to verify and control

EUCAST media for BMD

- Broth media
  - Non-fastidious organisms
    - Un-supplemented Mueller-Hinton broth (ISO 20776-1)
  - Fastidious organisms
    - MH-F broth: Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L β-NAD

Preparation of MH-F broth

- Prepare and autoclave cation-adjusted MHB according to the manufacturer's instructions, but with 100 mL less deionized water per litre to allow for the addition of lysed horse blood.
- Cool medium to 42-45°C.
- Aseptically add 100 mL 50% lysed horse blood and 1 mL β-NAD stock solution per litre medium and mix well.
- Dispense MH-F broth in sterile containers with screw caps.
- Store MH-F broth at 4-8°C.
  - Storage conditions and shelf life should be determined as part of the laboratory quality assurance programme. A shelf life of 3 months can be expected.

https://www.eucast.org/ast_of_bacteria/media_preparation

Preparation of 50% lysed horse blood for MH-F broth

- Aseptically dilute mechanically defibrinated horse blood with an equal amount of sterile deionized water.
- Freeze the blood at -20°C overnight and thaw. Repeat the cycle until the cells are completely lysed (three cycles is usually sufficient but the ISO standard 20776-1 suggests that up to seven cycles may be required).
- Clarify the 50% lysed horse blood by centrifugation and discard the pellet. A clear solution is essential for reading. Repeating the centrifugation may improve the clarity of the solution.
- The stock solution may be stored at -20°C in aliquots and defrosted as required. Do not refreeze unused solution.

https://www.eucast.org/ast_of_bacteria/media_preparation

Preparation of inoculum

- Final inoculum of 5×10^5 CFU/mL
- Start with McF 0.5 (~ 1-2 x 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
Proteus, Morganella and *Providencia* spp.	0.5	10 µL (to reduce problems with swarming)
Haemophilus influenzae	0.5	50 µL
Streptococcus spp. and other fastidious Gram-positive cocci	0.5	100 µL

Check manufacturer's recommendations for commercial plates!

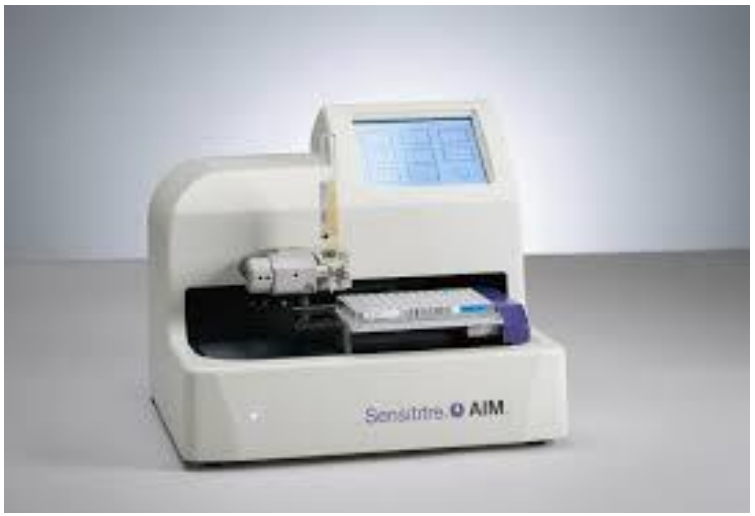
Control of inoculum / Viable counts

- Remove 10 µL from the growth control well immediately after inoculation
- Dilute in 10 mL of broth or saline
- Spread 100 µL of this dilution over the surface of a suitable agar plate, which is then incubated overnight
- 20-80 colonies are expected from an acceptable test suspension

ISO 20776-1, 2019

Inoculation of BMD plates

- Manually, preferably with a multi-channel pipette
- Using a dispensing robot



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, micro-dilution 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 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.

Growth appearance

- Growth appears as turbidity or as a deposit of cells at the bottom of the well (i.e. as a pellet). 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.

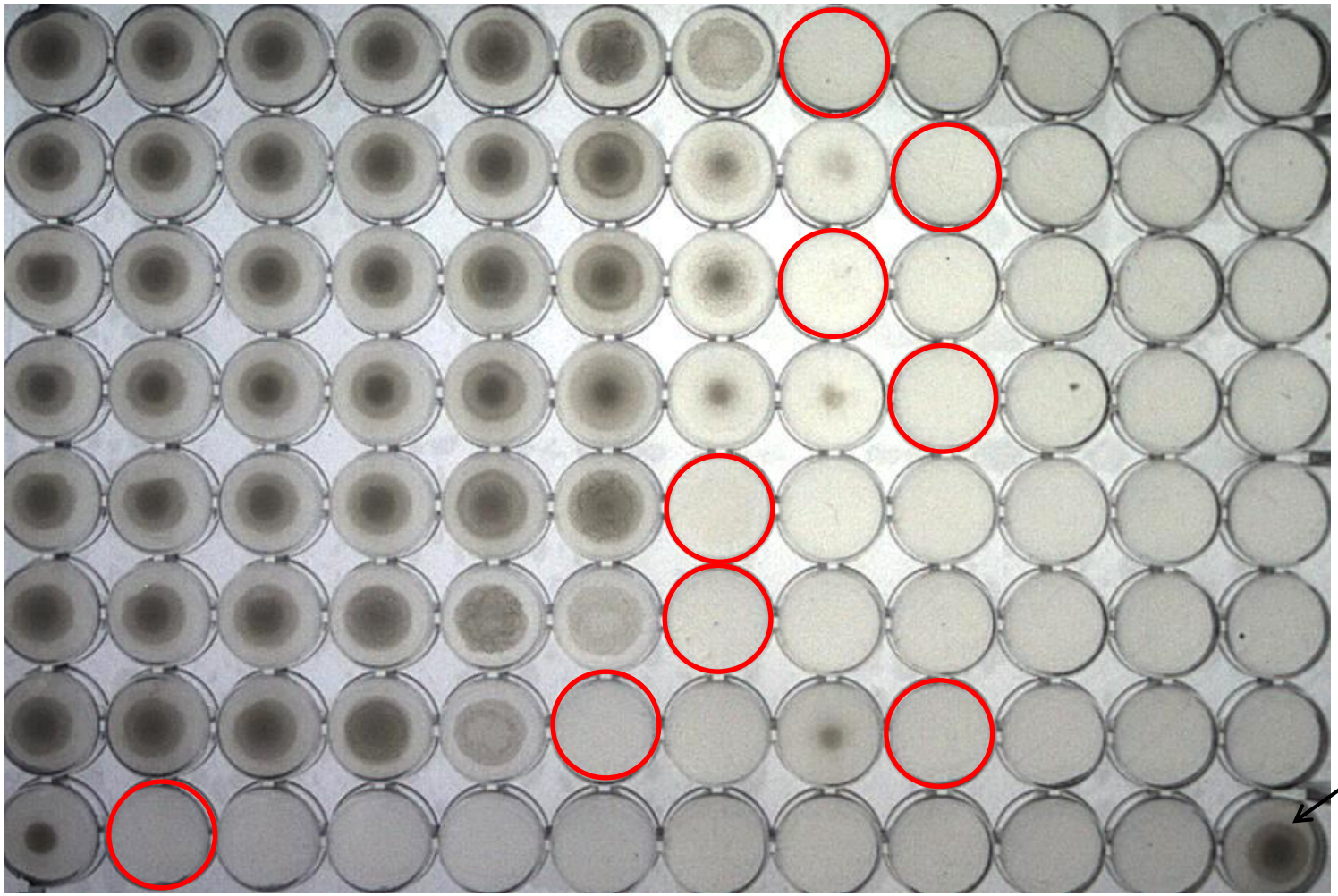
- Growth in antibiotic-containing wells may differ from growth seen in the positive growth control, even for pure cultures.

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.

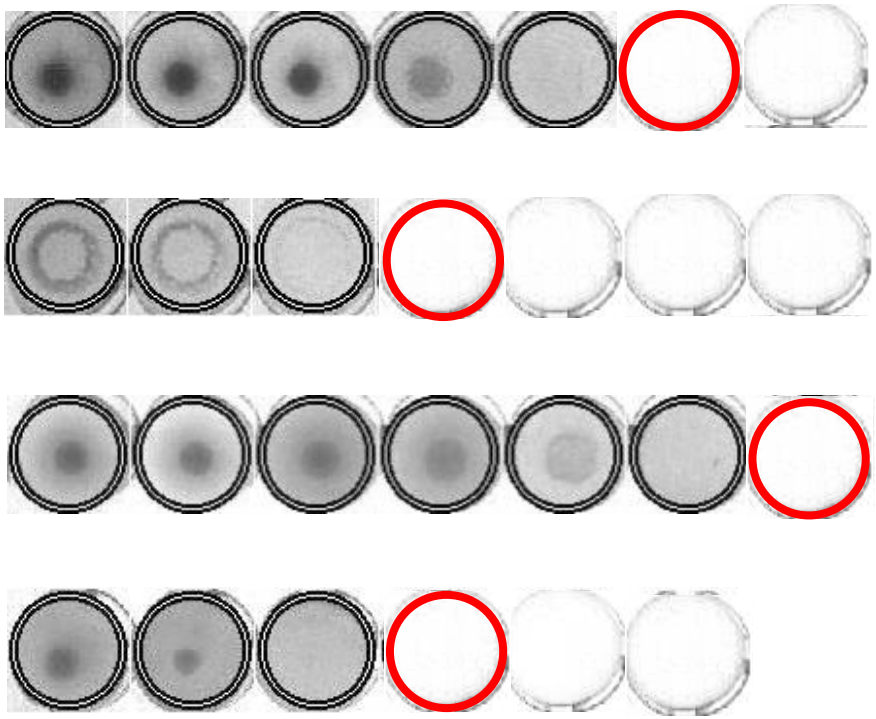
Reading of BMD panels

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.

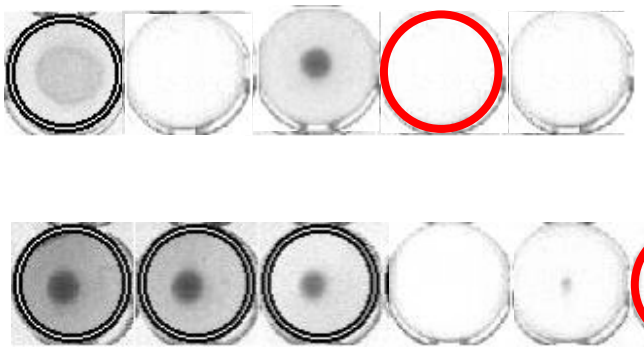
Skipped wells

- 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.

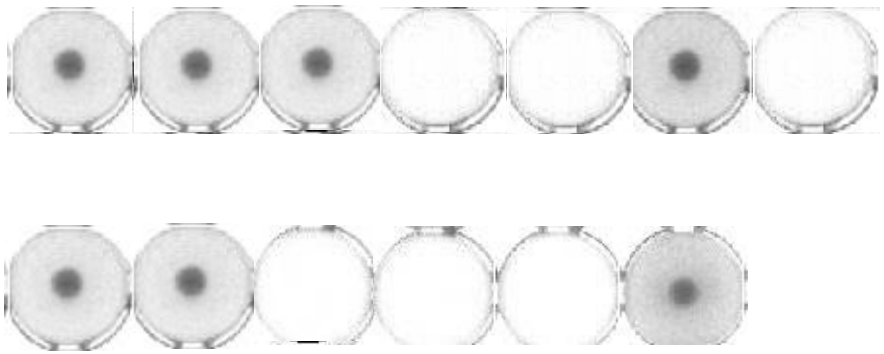
- When a single skipped well occurs, retest the isolate or read the highest MIC value to avoid reporting isolates as false susceptible.

- 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!



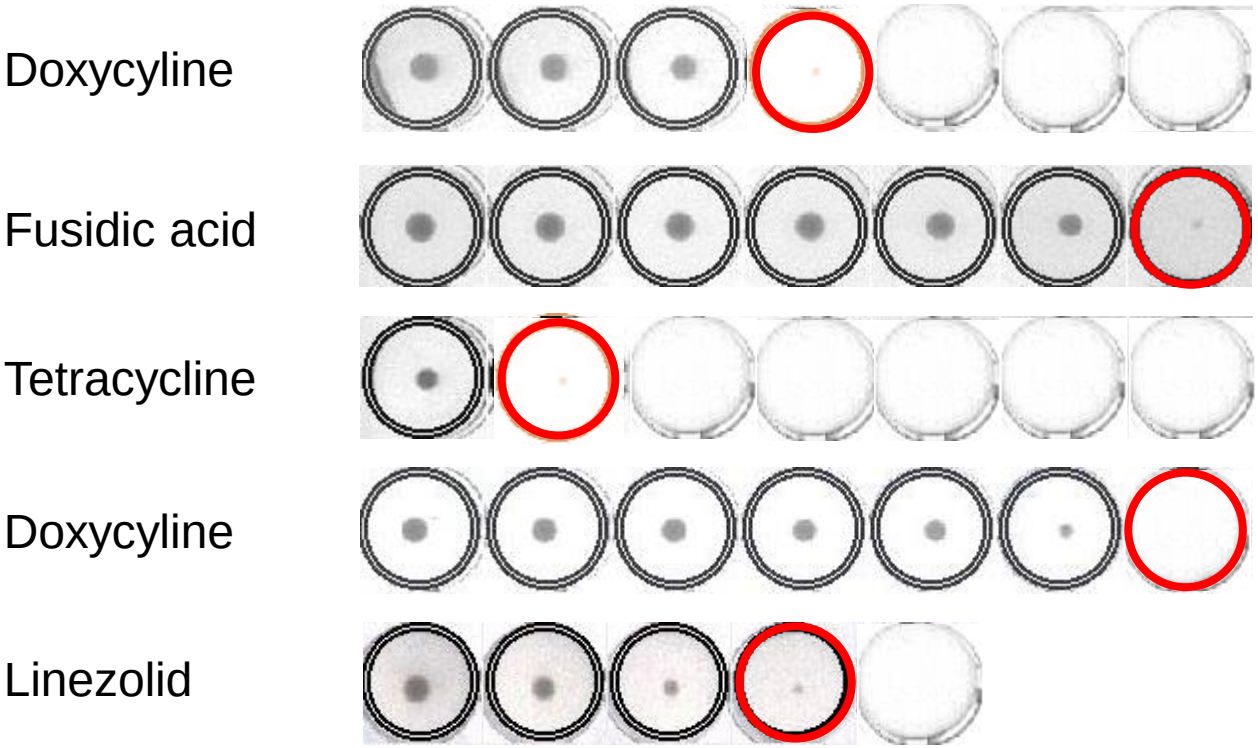
Results invalid!

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

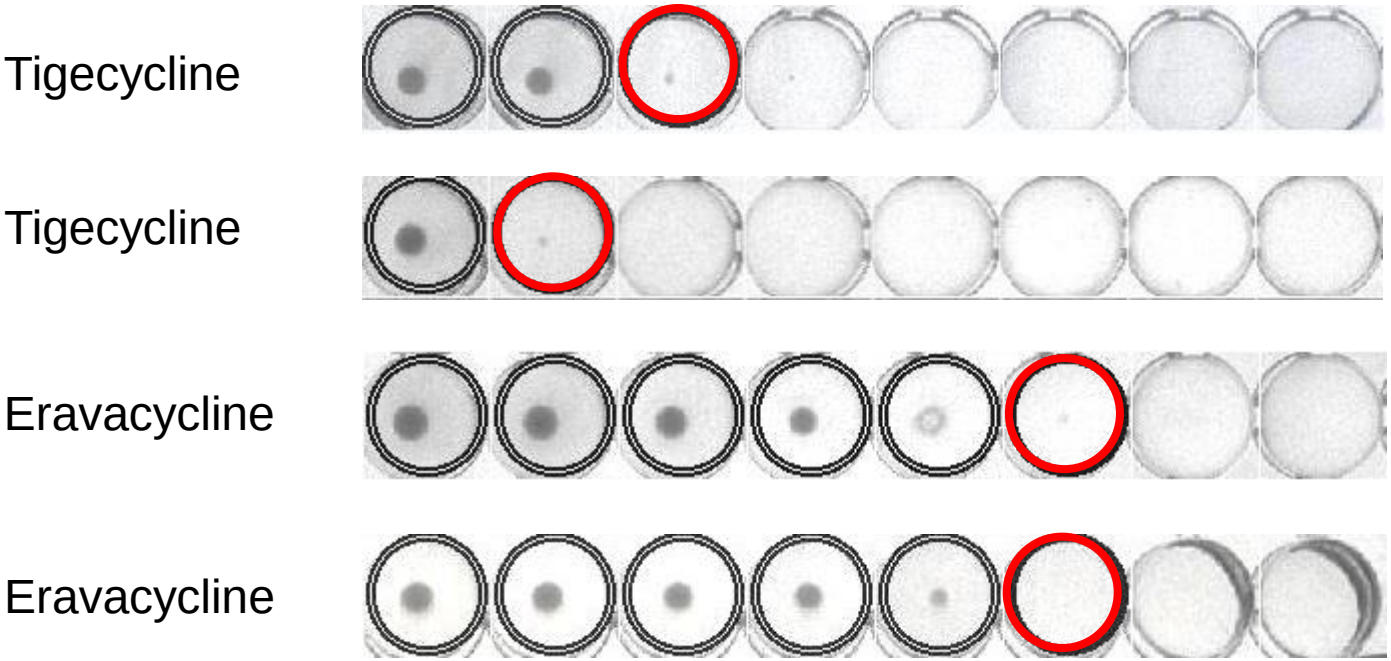
Gram-positive cocci with bacteriostatic antimicrobial agents

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



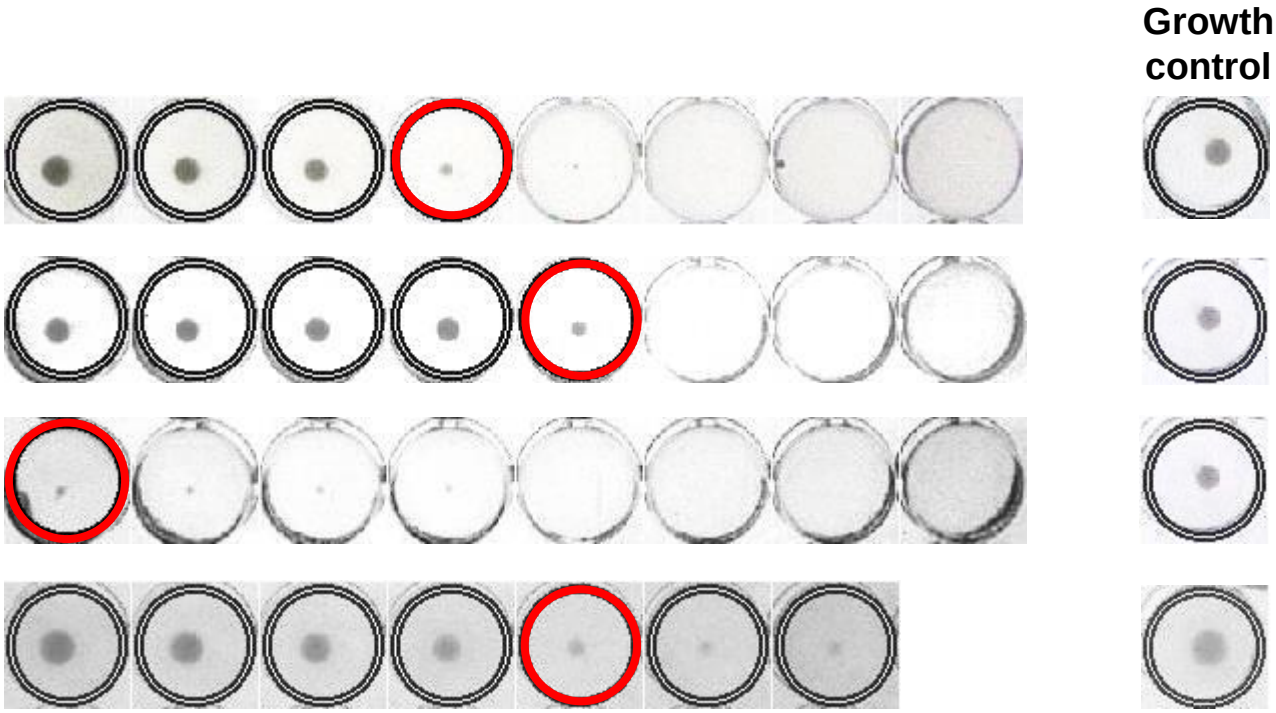
Gram-negative organisms with bacteriostatic antimicrobial agents

- 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.



Summary of 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, 16-20 h (sealed panels)
Reading	Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye

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search term

AST of bacteria

... 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**.

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search term

AST of bacteria

... 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 fosfomycin where agar dilution is used)
Medium: Mueller-Hinton broth
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1ºC, 18±2h
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: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1ºC, 18±2h
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.

Break

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	AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
E. coli ATCC 25922	4	≤0.06	0.12	0.25	≤0.015	≤0.25	0.008	0.5	0.12	≤0.03	≤0.015	8	4
E. coli ATCC 25922	4	≤0.06	0.06	0.5	≤0.015	≤0.25	0.008	0.5	0.12	≤0.03	≤0.015	8	4
E. coli ATCC 25922	8	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	16	4
E. coli ATCC 25922	8	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
E. coli ATCC 25922	4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
E. coli ATCC 25922	4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	8	2
E. coli ATCC 25922	4	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	≤0.015	16	2
E. coli ATCC 25922	4	≤0.06	0.06	0.25	≤0.015	0.5	0.008	0.5	0.25	≤0.03	0.03	8	4
E. coli ATCC 25922	4	≤0.06	0.12	0.25	≤0.015	0.5	0.008	0.5	0.12	≤0.03	0.03	8	4
E. coli ATCC 25922	4	≤0.06	0.12	0.25	≤0.015	0.5	-	0.5	0.25	≤0.03	0.03	8	4
Target	**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**
Range	**2-8**	**0.015-0.12**	**0.03-0.12**	**0.06-0.5**	**).004-0.01**	**0.25-2**	**).004-0.01**	**0.25-1**	**0.06-0.5**	**0.008-0.060**	**.008-0.06**	**4-16**	**1-4**

QC strain	AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
P. aeruginosa ATCC 27853	>32	1	8	1	0.25	2	4	0.5	2	1	0.25	>128	2
P. aeruginosa ATCC 27853	>32	2	>8	1	0.25	2	2	0.5	1	1	0.25	>128	2
P. aeruginosa ATCC 27853	>32	1	>8	2	0.25	2	4	1	2	1	0.25	>128	4
P. aeruginosa ATCC 27853	>32	-	8	1	0.25	2	2	1	2	1	0.25	>128	2
P. aeruginosa ATCC 27853	>32	2	8	2	0.25	2	2	1	2	1	0.25	>128	2
P. aeruginosa ATCC 27853	>32	2	>8	2	0.25	1	4	1	2	1	0.25	>128	8
P. aeruginosa ATCC 27853	>32	2	>8	-	0.25	1	4	1	4	1	0.5	>128	-
P. aeruginosa ATCC 27853	>32	1	>8	2	0.25	1	4	1	1	1	0.25	>128	-
P. aeruginosa ATCC 27853	>32	2	>8	2	0.25	1	4	1	2	1	0.5	>128	4
P. aeruginosa ATCC 27853	>32	2	>8	2	0.25	1	>4	1	2	1	0.5	>128	2
Target		**1-2**		**2**	**0.25-0.5**	**1-2**		**1**	**2**	**1-2**	**0.25-0.5**		**2-4**
Range		**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

Enterobacterales panel

QC strain	AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
K. pneumoniae ATCC 700603	>32	1	4	>16	0.25	2	0.06	8	0.12/0.5	1	0.06	64	16
K. pneumoniae ATCC 700603	>32	0.5	2	16	0.5	1	0.06	4	0.25	1	0.03	128	8
K. pneumoniae ATCC 700603	>32	0.5	2	>16	0.5	1	0.06	8	0.12/0.5	0.5	0.03	64	8
K. pneumoniae ATCC 700603	>32	0.5/2	2	16	0.5	1	0.06	4	0.25	0.5	0.03	128	8
K. pneumoniae ATCC 700603	>32	1	2	>16	0.5	0.5	0.03	8	0.12/0.5	1	0.03	64	8
K. pneumoniae ATCC 700603	>32	1	2	16	0.5	1	0.06	4	0.25	1	0.03	64	8
K. pneumoniae ATCC 700603	>32	0.5	2	>16	0.5	1	0.06	4	0.25	1	0.03	64	8
K. pneumoniae ATCC 700603	>32	1	2	>16	0.5	1	0.06	4	0.12	1	0.03	64	8
K. pneumoniae ATCC 700603	>32	1	2	>16	0.5	≤0.25	0.06	4	0.12	1	0.03	128	16
K. pneumoniae ATCC 700603	>32	0.5	2	>16	0.5	0.5	0.06	4	0.25	1	0.03	64	16
Target													**16**
Range													**8-32**

QC strain	AMP	FEP	CTX	CAZ	CIP	COL	ERT	GEN	IMI	LEV	MER	NIT	PTZ
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	8	0.03	0.5	0.25	>4	0.03	16	4
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	4	0.03	1	0.25	>4	0.03	16	4
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	4
E. coli NCTC 13846	>32	0.12	0.25	0.25	>4	4	0.03	0.5	0.12	>4	0.03	16	4
E. coli NCTC 13846	>32	0.25	0.5	0.5	>4	4	0.03	0.5	0.25	>4	0.03	16	4
E. coli NCTC 13846	>32	0.25	0.5	0.5	>4	4	0.03	0.5	0.25	>4	0.03	16	4
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	≤0.015	16	4
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	2	0.03	≤0.25	0.12	>4	0.03	16	8
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	8
E. coli NCTC 13846	>32	0.25	0.25	0.5	>4	4	0.03	0.5	0.12	>4	0.03	16	4
Target						**4**							
Range						**(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

Caveats for BMD

- BMD can only be used for organisms that grow sufficiently well in broth (MH or MH-F broth)
  - Agar dilution is recommended for anaerobic bacteria and *N. gonorrhoeae* and will be recommended for *Nocardia* spp.
- Some agents cannot be tested with BMD due to poor reproducibility
  - Mecillinam and fosfomycin should be tested with agar dilution (ISO 20776-1)

Implementation of BMD

- ISO 20776-2 (2021): To guide manufacturers in the conduct of performance evaluation studies
- Testing scheme:
  - QC each day of testing
    - ≥95% within range
  - Reproducibility (10 isolates x 3)
    - MICs within ± one two-fold dilution of the mode/median for ≥ 95 % of the results
  - Clinical isolates (n=300)
    - Essential agreement and bias

ISO 20776-2: Data analysis clinical isolates

- Essential agreement (EA)
  - MIC within ± one two-fold dilution step from the reference MIC (≥ 90%)
- Bias
  - Whether the results that differ from the reference method are significantly skewed or predominantly in one direction (less than ± 30% bias)

Example of EA and bias analysis

PCG BMD

PCG Etest	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4
0.008									
0.016	7	1							
0.03	1	2	2	3					
0.06		1	10	9					
0.125				2	2				
0.25					11	6			
0.5					1	4	4	4	1
1						2	8	14	2
2								6	8
4									3

	No	%
>2 dilutions lower	1	0.9
2 dilutions lower	9	7.9
1 dilution lower	46	40.4
Identical	53	46.5
1 dilution higher	5	4.4
2 dilutions higher		
>2 dilutions higher		

EA = 93%
57.3% below, 4.4% above
Bias = - 52.9%

PCG BMD

PCG MTS	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4
0.008	3	1							
0.016	5	1							
0.03		1	4	5					
0.06		1	8	9					
0.125					8	5			
0.25					6	2	1		
0.5						3	7	9	
1						2	4	15	4
2									8
4									2

	No	%
>2 dilutions lower		
2 dilutions lower	25	21.9
1 dilution lower	57	50.0
Identical	29	25.4
1 dilution higher	3	2.6
2 dilutions higher		
>2 dilutions higher		

EA = 78 %
71.9% below, 2.6% above
Bias = - 69.3%

Implementation of an MIC method in a clinical laboratory

- No official EUCAST criteria
- Guidance based on EDL experience:
  - Relevant QC strains
    - At least three repeats per QC strain
      - Strains representing target organism
      - Beta-lactamase producing strains must be added for inhibitor-combination agents
      - Relevant resistant QC strains if available (e.g. *E. coli* NCTC 13846 for colistin)
    - Analyse vs. target and range in EUCAST QC tables

Use QC strains to practise reading of results!

Implementation of an MIC method in a clinical laboratory

- Guidance based on EDL experience:
  - Clinical isolates with known MICs
    - 25-100 isolates representing target organisms (depending on product and agents)
      - Wild-type isolates and isolates with elevated MICs
      - EUCAST/CCUG reference collections for *S. pneumoniae* and *P. aeruginosa*
      - UKNEQAS specimens
      - Resistant QC strains

EUCAST/CCUG reference collections

- To be used to evaluate or implement MIC methods in laboratories
- Can be ordered from CCUG, https://ccug.se/
- *S. pneumoniae*
  - 10 strains with varying levels of susceptibility to beta-lactam agents
  - Reference MIC values for beta-lactams and agents from other classes
- *P. aeruginosa*
  - 9 strains with varying levels of susceptibility to agents from different classes
  - Reference MIC values and genomic resistance profile (WGS)

https://www.eucast.org/ast_of_bacteria/strains_with_defined_susceptibility

Example *S. pneumoniae*

CCUG 74415

***Streptococcus pneumoniae* from sputum**

Animicrobial agent	**MIC (mg/L)**
Benzylpenicillin	0.5
Ampicillin	0.5
Amoxicillin	0.5
Amoxicillin-clavulanic acid[1]	0.25-0.5
Piperacillin-tazobactam[2]	0.5-1
Cefotaxime	1
Ceftriaxone	1
Cefuroxime	4
Imipenem	0.03
Meropenem	0.06
Levofloxacin	1
Moxifloxacin	0.125
Vancomycin	0.5
Azithromycin	>2
Clarithromycin	>2
Erythromycin	>2
Clindamycin	>2
Doxycycline	8
Tetracycline	>8
Linezolid	1
Rifampicin	0.03-0.06
Trimethoprim-sulfamethoxazole[3]	1

[1] Fixed 2 mg/L clavulanic acid

[2] Fixed 4 mg/L tazobactam

[3] Trimethoprim:sulfamethoxazole in the ratio 1:19

Example *P. aeruginosa*

CCUG 75455

***Pseudomonas aeruginosa* (ST244)**

Animicrobial agent	MIC (mg/L)	Main resistance mechanisms[a,d]	Strength of resistance genotype[b,d]	Correlation with phenotype[c,d]
Piperacillin-tazobactam[1]	>32	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Cefepime	32	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ceftazidime	>16	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ceftazidime-avibactam[2]	8-16	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	Moderate	Full correlation
Ceftolozane-tazobactam[1]	4	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	Moderate	Full correlation
Imipenem	32	Inactivation of OprD (K407X), AmpC overexpression (AmpD aa162InsPERIQGHCDIA)	High	Full correlation
Meropenem	32	Inactivation of OprD (K407X), AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Aztreonam	64	AmpC overexpression (AmpD aa162InsPERIQGHCDIA), MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted), PBP3 mutation (V465L)	High	Full correlation
Ciprofloxacin	1	MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted)	Moderate	Full correlation
Levofloxacin	4	MexXY overpexpression (mexZnt343Δ1), MexAB-OprM overexpression (NalD deleted)	Moderate	Full correlation
Amikacin	16	MexXY overpexpression (mexZnt343Δ1)	Moderate	Full correlation
Tobramycin	1	MexXY overpexpression (mexZnt343Δ1)	Weak	Full correlation
Colistin	Note[3]	LPS colistin resistance mutation (ParR E214K)	High	Full correlation

[1] Fixed 4 mg/L tazobactam

[2] Fixed 4 mg/L avibactam

[3] A reference MIC value could not be established due to poor reproducibility in repeated tests.

[a] List of resistance mechanisms/mutations

[b] Expected effect of the summatory of mutations (weak, moderate, high)

[c] Correlation between genotype and phenotype (full correlation, partial correlation, no correlation)

[d] Adapted from S. Cortes-lara et al Clin Microbiol Infect 2021; 27: 1631-1637

Example of an evaluation of gradient tests using the *S. pneumoniae* collection

- Gradient tests for 6 beta-lactam agents from two manufacturers (Etest and MTS) were tested on MH-F agar from two manufacturers (BBL and Oxoid).

Beta-lactam MIC values	Benzyl-penicilli		Ampicillin		Amoxicillin		Cefotaxime		Ceftriaxone		Meropenem		Total	%
	Etest	MTS	Etest	MTS	Etest	MTS	Etest	MTS	Etest	MTS	Etest	MTS		
2 dilutions lower			1	1		2			3	2			**9**	*4*
1 dilution lower	3	5	10	10	3	12	7	5	11	12			**78**	*33*
Identical	17	15	9	9	16	6	13	15	4	4	16	14	**138**	*58*
1 dilution higher					1				2	2	4	6	**15**	*6*
2 dilutions higher														
Total	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**240**	*100*

UKNEQAS specimens for MIC method implementation

UK NEQAS Microbiology

Antimicrobial susceptibility	Laboratory : **1209**
Distribution : **4835**	Page 2 of 9
Dispatch Date : 01-Feb-2021	

Specimen : 6193 *Escherichia coli* from urine

	ISO MIC mg/L		Result		Breakpoints	
Reference Lab	1	2	EUCAST	CLSI	EUCAST	CLSI
Amoxicillin-clavulanic acid	4	8	S	S	S≤32 R>32	S≤8 R≥32
Ampicillin	4	8	S	S	S≤8 R>8	S≤8 R≥32
Cephalexin	8	16	S	-	S≤16 R>16	
Cefuroxime (oral)	4	4	S	S	S≤8 R>8	S≤4 R≥32
Ciprofloxacin	0.015	0.03	S	S	S≤0.25 R>0.5	S≤0.25 R≥1
Fosfomycin (oral)	1	1	S	S	S≤8 R>8	S≤64 R≥256
Mecillinam (oral)	0.25	0.25	S	S	S≤8 R>8	S≤8 R≥32
Nitrofurantoin (oral)	16	16	S	S	S≤64 R>64	S≤32 R≥128
Trimethoprim (oral)	>64	>64	R	R	S≤4 R>4	S≤8 R≥16
Co-trimoxazole	>64	>64	R	R	S≤2 R>4	S≤2 R≥4

Example of implementation of BMD using UKNEQAS isolates

Escherichia coli **specimen 3253**

Antimicrobial agent	Reference MIC (mg/L)	MIC (mg/L)	Reference categorisation (SIR)	Categorisation (SIR)	Comments
Amikacin	8	16	S	I	Results close to breakpoint. MIC within ± 1 dilution.
Amoxicillin	≥128	>32	R	R	
Amoxicillin-clavulanic acid	16	16	R	R	
Ampicillin	≥128	>32	R	R	
Cefotaxime	32	>8	R	R	
Ceftazidime	64	>16	R	R	
Ceftriaxone	32	>4	R	R	
Cefuroxime	≥128	>16	R	R	
Ciprofloxacin	0.25	0.25	S	S	
Ertapenem	0.06	0.06	S	S	
Gentamicin	0,5-1	1	S	S	
Imipenem	0.25	0.25	S	S	
Meropenem	0.03	0.03	S	S	
Piperacillin-tazobactam	4	8	S	S	
Tobramycin	8-16	8	R	R	

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