



# Reading of broth microdilution MIC endpoints

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# BMD reading instructions

## **General reading instructions according to ISO Standard 20776-1:**

- Read the MIC as the lowest concentration of the agent that completely inhibits visible growth of the organism.
- Specific reading instructions are not stated in the ISO Standard, but referred to EUCAST (or CLSI) documents.
  - BMD EUCAST Reading Guide v. 5.0 (January 2024)

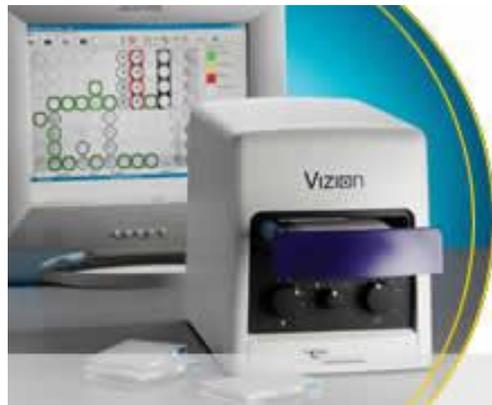
[https://www.eucast.org/ast\\_of\\_bacteria/mic\\_determination](https://www.eucast.org/ast_of_bacteria/mic_determination)

# Reading of BMD MICs

- Read MICs only for pure cultures and when there is sufficient growth (obvious button or definite turbidity) in the positive growth control.
- Check for purity:
  - Subculture from the growth-control well immediately after inoculation onto a non-selective agar plate OR
  - Perform disk diffusion from the same 0.5 McFarland suspension in parallel.
- Correct inoculum  $5 \times 10^5$  CFU/mL

# Reading of BMD MICs

- Results should be read manually.  
A mirror may be used to facilitate reading.
- The use of automated readers or camera systems must be calibrated to manual reading!



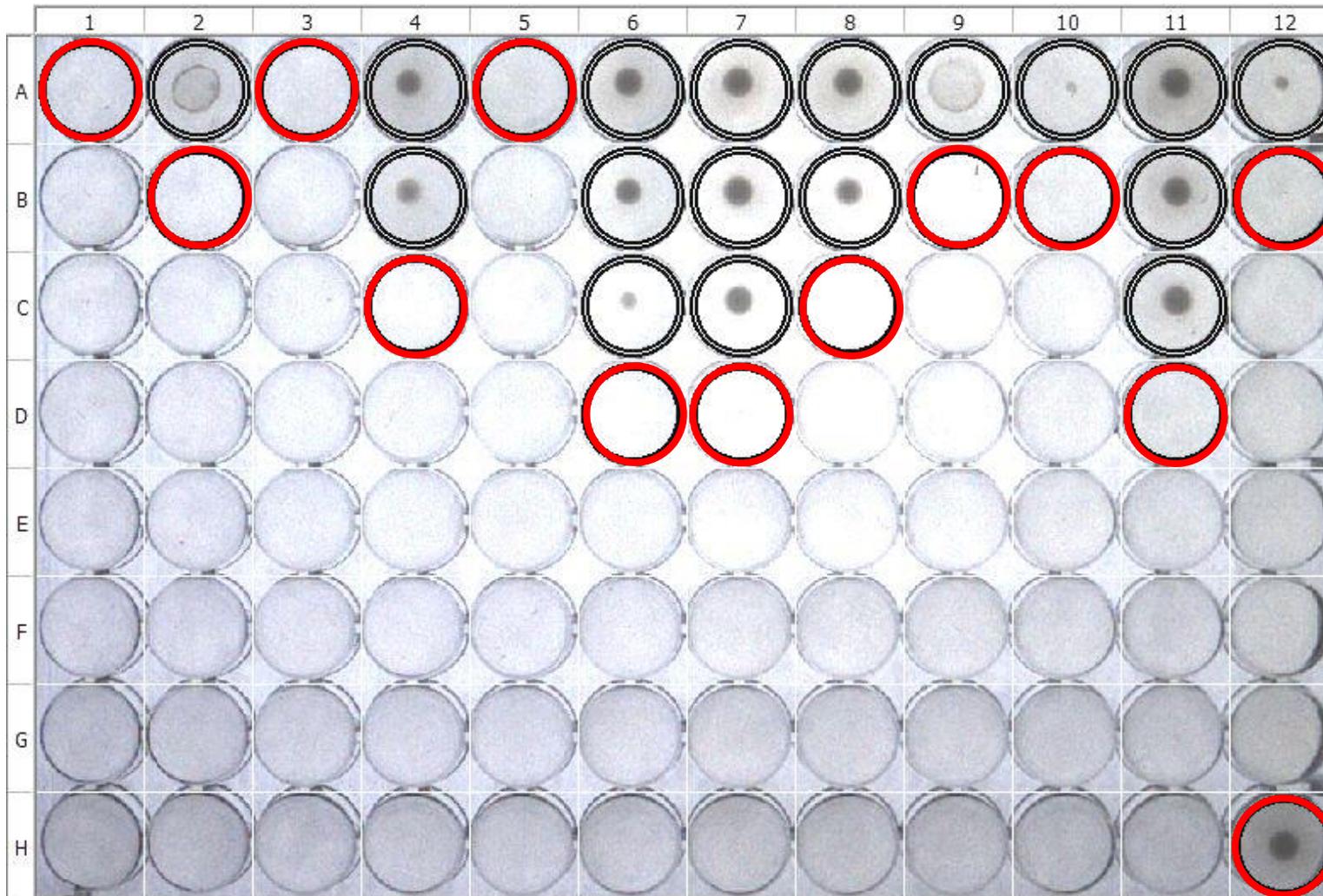
# Growth appearance

- Growth appears as turbidity or as a deposit of cells (pellet) 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.

# Plate format, Example

	1	2	3	4	5	6	7	8	9	10	11	12
A	FEP 0.06	P/T4 1/4	DOR 0.03	AMI 1	ETP 0.015	GEN 0.25	CIP 0.015	TOB 0.25	AXO 0.03	TAZ 0.12	LEVO 0.03	TGC 0.12
B	FEP 0.12	P/T4 2/4	DOR 0.06	AMI 2	ETP 0.03	GEN 0.5	CIP 0.03	TOB 0.5	AXO 0.06	TAZ 0.25	LEVO 0.06	TGC 0.25
C	FEP 0.25	P/T4 4/4	DOR 0.12	AMI 4	ETP 0.06	GEN 1	CIP 0.06	TOB 1	AXO 0.12	TAZ 0.5	LEVO 0.12	TGC 0.5
D	FEP 0.5	P/T4 8/4	DOR 0.25	AMI 8	ETP 0.12	GEN 2	CIP 0.12	TOB 2	AXO 0.25	TAZ 1	LEVO 0.25	TGC 1
E	FEP 1	P/T4 16/4	DOR 0.5	AMI 16	ETP 0.25	GEN 4	CIP 0.25	TOB 4	AXO 0.5	TAZ 2	LEVO 0.5	TGC 2
F	FEP 2	P/T4 32/4	DOR 1	AMI 32	ETP 0.5	GEN 8	CIP 0.5	TOB 8	AXO 1	TAZ 4	LEVO 1	TGC 4
G	FEP 4	P/T4 64/4	DOR 2	AMI 64	ETP 1	GEN 16	CIP 1	TOB 16	AXO 2	TAZ 8	LEVO 2	TGC 8
H	FEP 8	FEP 16	DOR 4	DOR 8	ETP 2	ETP 4	CIP 2	CIP 4	AXO 4	TAZ 16	LEVO 4	POS CON

# Reading of MICs *E. coli*



MIC  
(mg/L)

FEP  
≤0.06

PTZ  
2

DOR  
≤0.03

AMI  
4

ERT  
≤0.015

GEN  
2

CIP  
0.12

TOB  
1

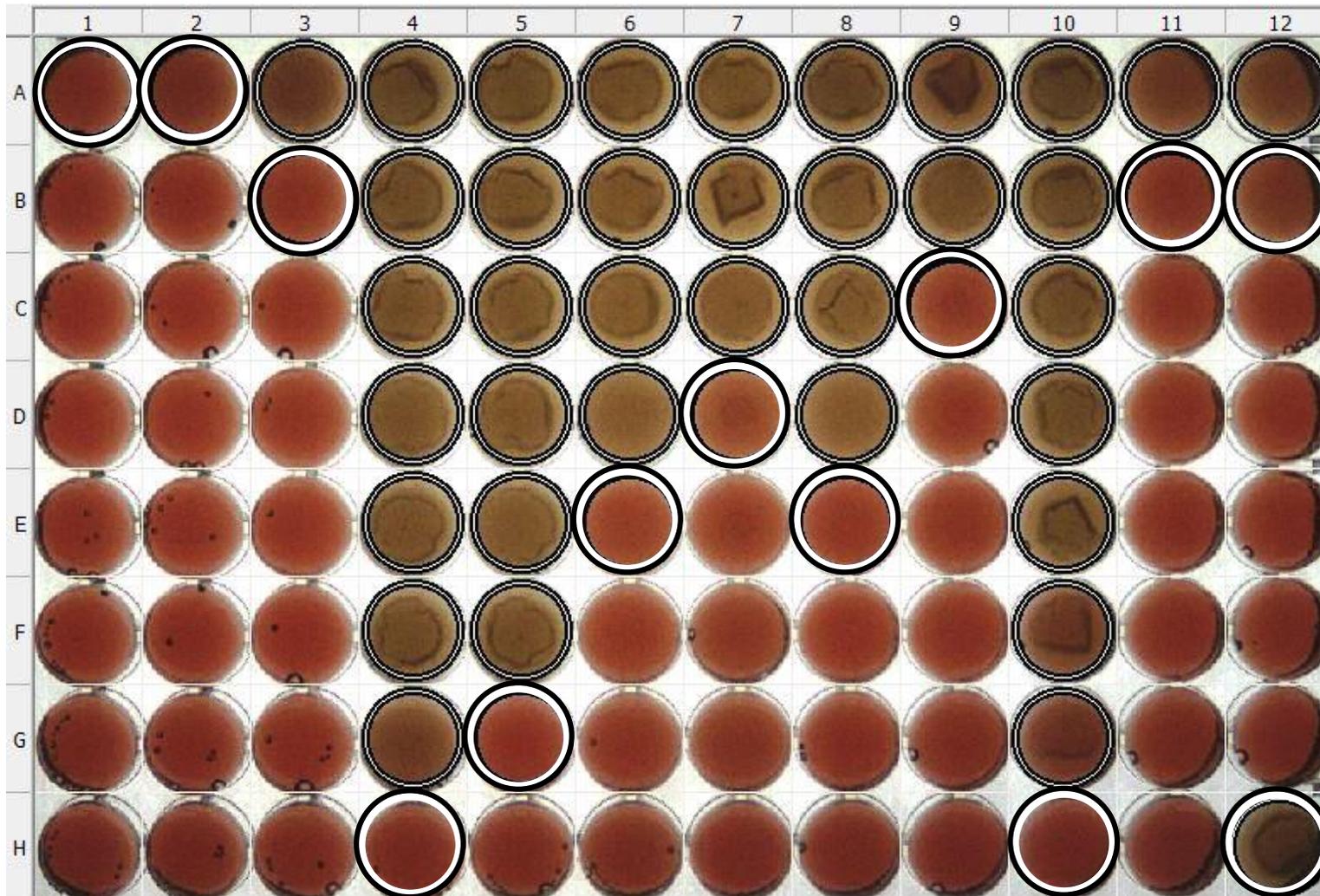
CRO  
0.06

CAZ  
0.25

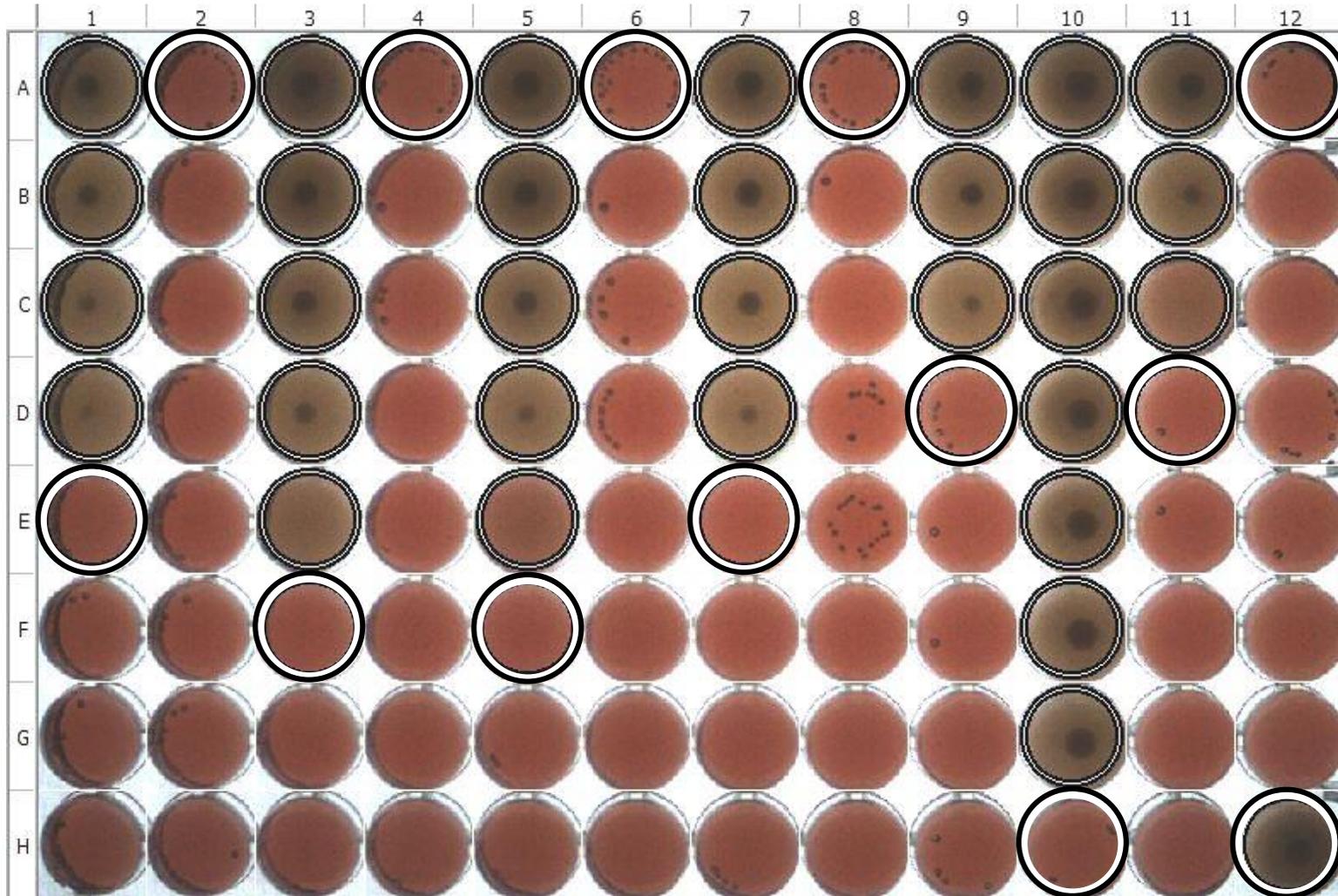
LEV  
0.25

TIG  
0.25

# Reading of MICs *H. influenzae*



# Reading of MICs *S. pneumoniae*



# **Specific reading instructions**

# Specific reading instructions

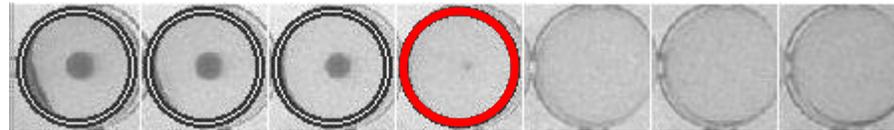
The following antimicrobial agents require specific reading instructions:

- Bacteriostatic antimicrobial agents with Gram-positive and Gram-negative organisms
  - For example erythromycin, clindamycin, tetracycline, tigecycline and linezolid.
- Trimethoprim and trimethoprim-sulfamethoxazole
- Cefiderocol
  - Specific testing conditions (iron-depleted MH broth) and specific reading instructions

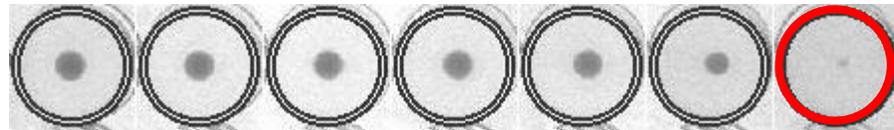
# Gram-positive cocci with bacteriostatic antimicrobial agents

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

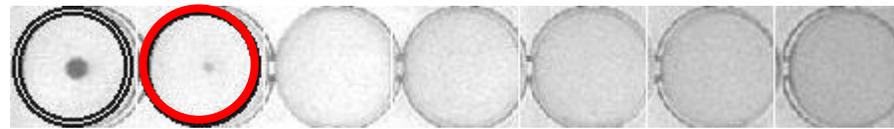
Doxycycline



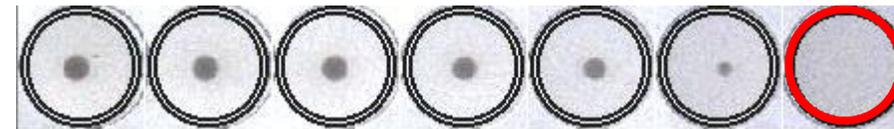
Fusidic acid



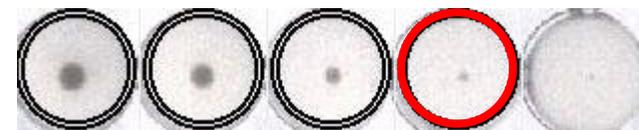
Tetracycline



Doxycycline



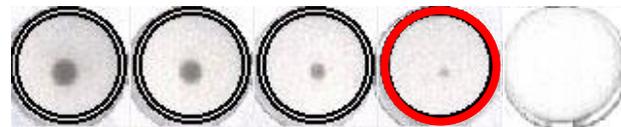
Linezolid



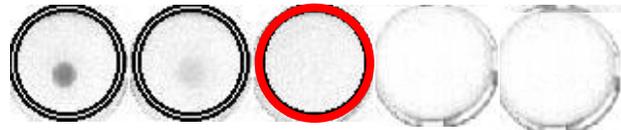
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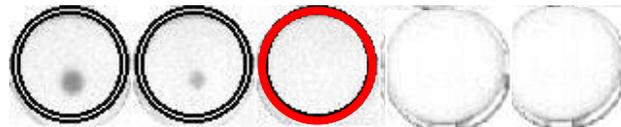
Linezolid



Linezolid



Tedizolid



Tedizolid



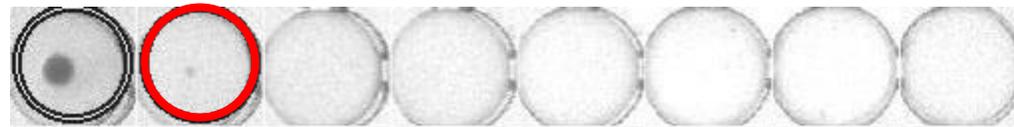
# Gram-negative organisms with bacteriostatic antimicrobial agents

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

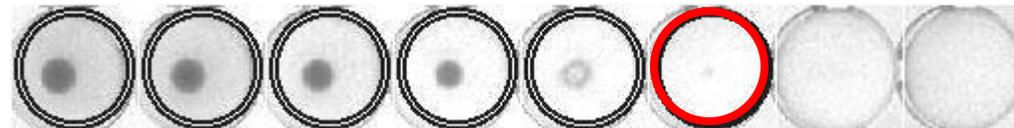
Tigecycline



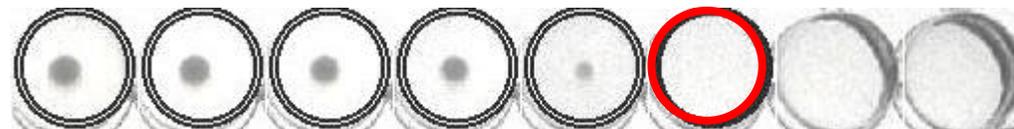
Tigecycline



Eravacycline

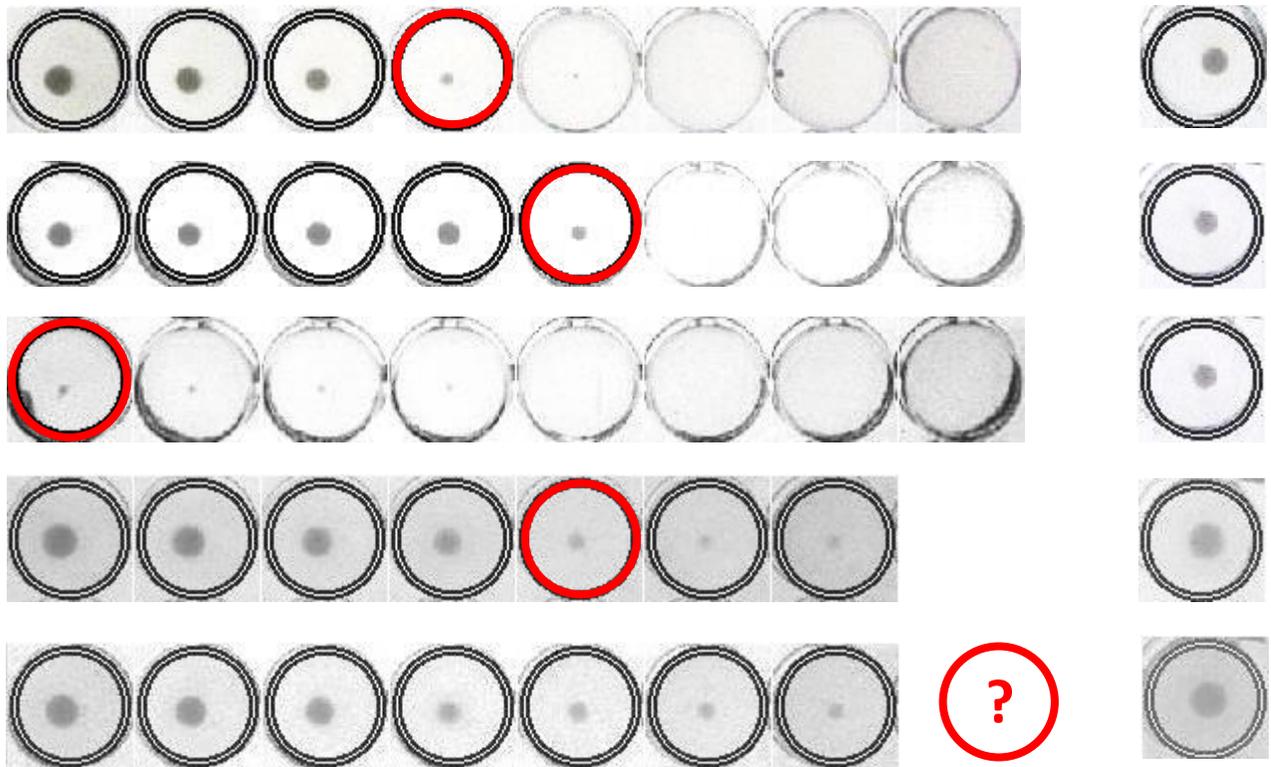


Eravacycline



# Trimethoprim and trimethoprim-sulfamethoxazole

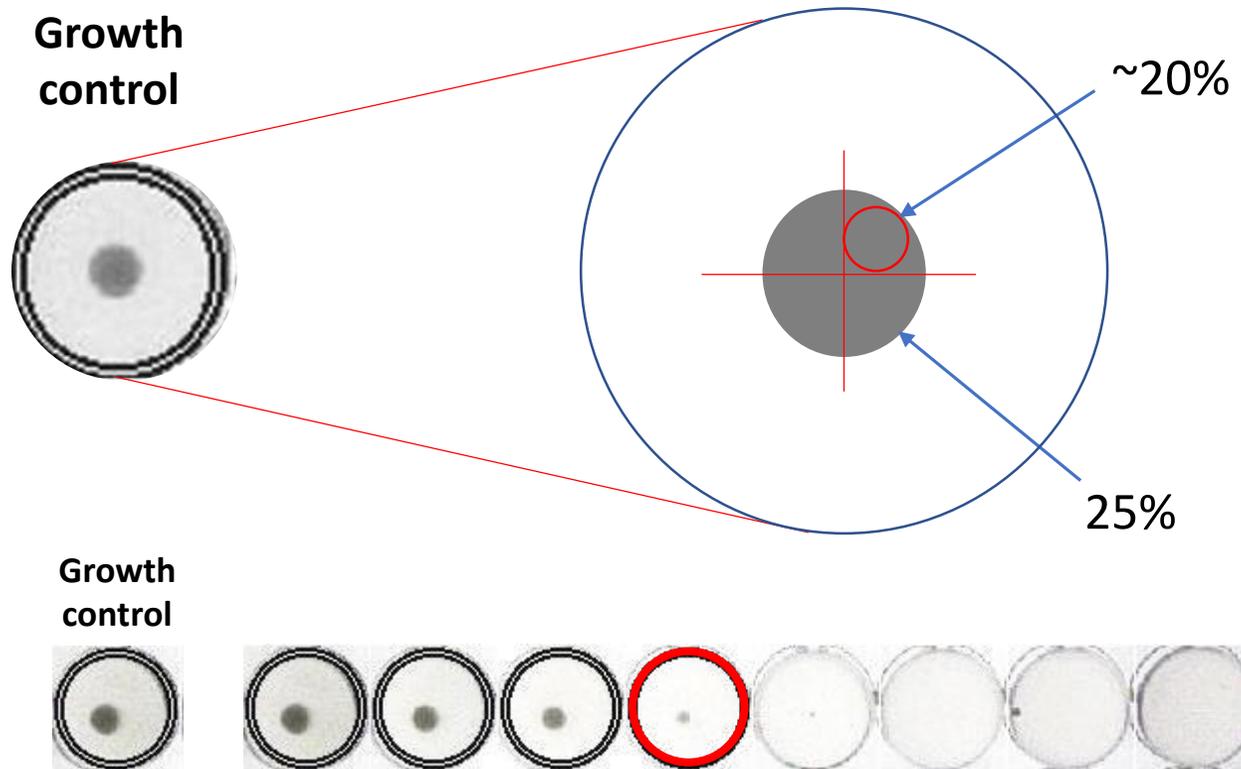
Read the MIC at the lowest concentration that inhibits  $\geq 80\%$  of growth as compared to the growth control.



Growth control

# Trimethoprim and trimethoprim-sulfamethoxazole

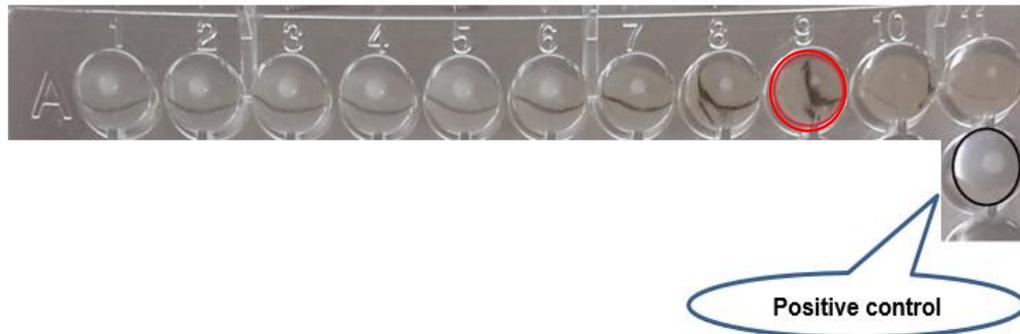
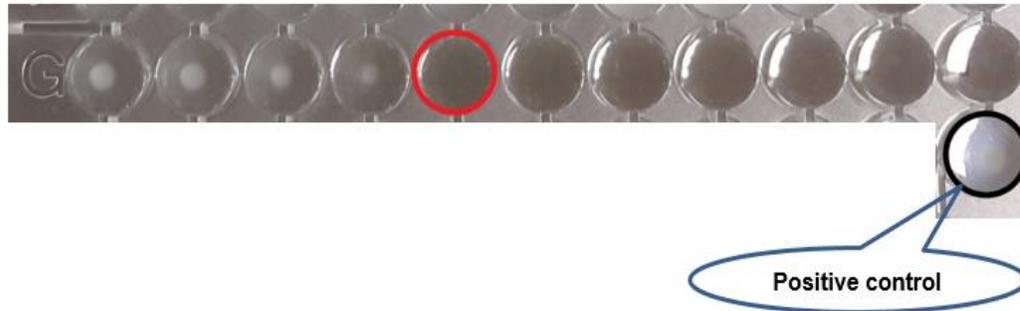
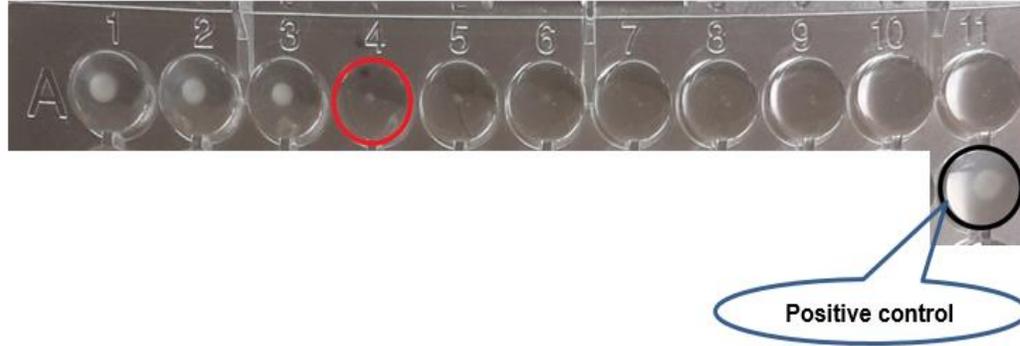
Reading the MIC at 80 % inhibition means that pellets with a size <20 % as compared to the growth control should be disregarded!



# Cefiderocol

- Broth microdilution MIC determination must be performed in iron-depleted Mueller-Hinton broth and specific reading instructions must be followed. For testing conditions, see <https://www.eucast.org/eucastguidancedocuments>.
- The MIC is read as the first well in which the reduction of growth corresponds to a button of <1 mm or is replaced by the presence of light haze/faint turbidity ( $\geq 80\%$  reduction in haze as compared to the growth control).
- The positive control should show strong growth in the form of a button of >2 mm or heavy turbidity.
- See next slide for pictures with reading examples.

# Cefiderocol



# **Difficult reading situations**

# Reading difficulties

- Trailing endpoints
- Haze/turbidity without pellet
- Scattered growth
- Haemolysis
- Skipped wells
- Very tiny pellets
- Bubbles
- Condensation

# 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 2-3 wells.
- Unless otherwise stated, endpoints should be read at complete inhibition of growth.

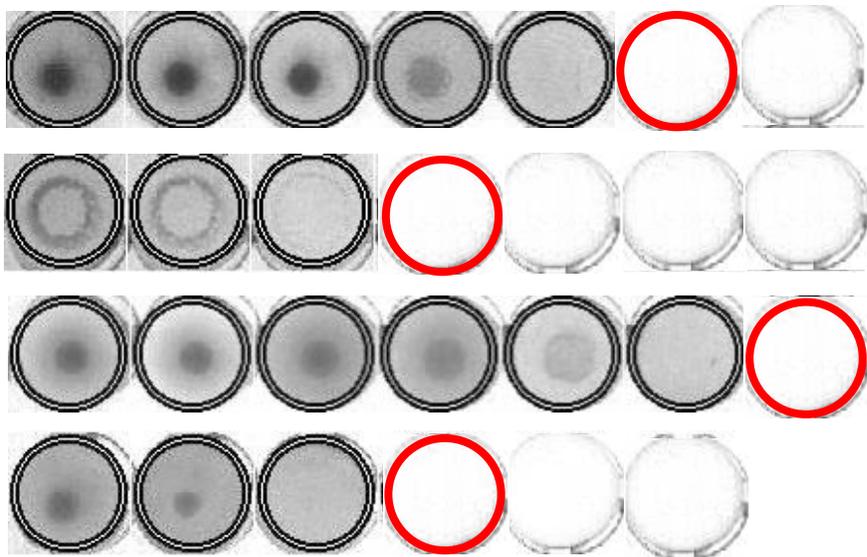
Example *P. aeruginosa* and colistin (breakpoint  $S \leq 4$ ,  $R > 4$  mg/L)



0.25   0.5   1   2   4   8   16   mg/L

# Haze or turbidity without pellet

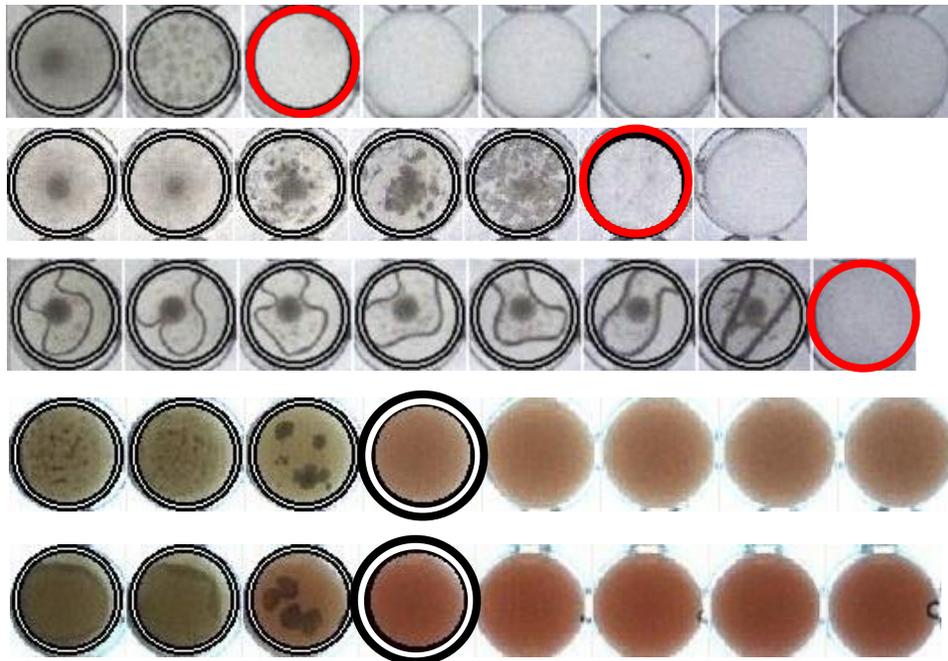
- Haze or turbidity without a pellet is often seen for *Pseudomonas* and *Acinetobacter* spp. This should be regarded as growth and the endpoint read at the first well with complete inhibition (clear broth).



# Scattered growth

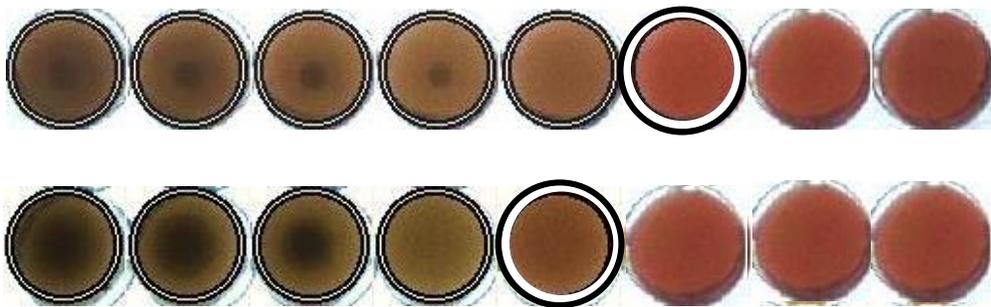
- Growth may not always appear as a clear pellet centered in the middle of the well, but can also be scattered.
  - Check for purity if you suspect a contamination.
  - If the culture is pure, take scattered growth into account.

Examples for *Pseudomonas*, *Acinetobacter* and *Streptococcus* spp.



# Haemolysis

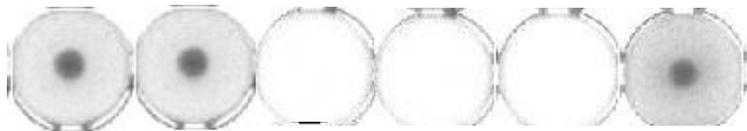
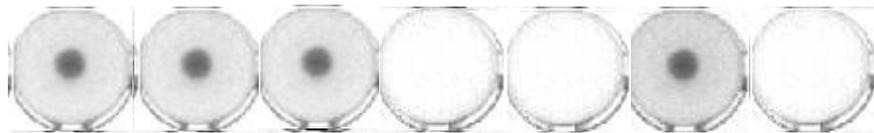
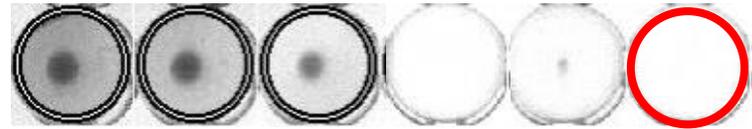
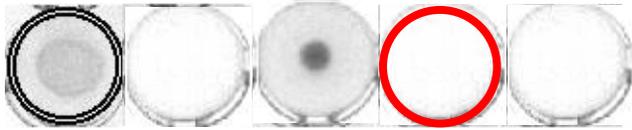
- Haemolysis of the blood may occur for fastidious organisms tested in MH-F broth. Hemolysis is often accompanied by turbidity or a deposit of growth (pellet).
- Haemolysis with turbidity or pellet should be regarded as growth when determining endpoints.



# Skipped wells

- Occasionally a skip may be seen, *i.e.* a well showing no growth bordered by wells showing growth.
- Possible explanations for skipped wells:
  - Contaminations
  - Incorrect inoculation
  - Heterogenous resistance
- **Single skipped well:** Retest the isolate or report the highest MIC value to avoid reporting isolates as false susceptible.
- **Multiple skipped wells:** Do not report results for an agent with more than one skipped well.

# Examples skipped wells



Retest or read the highest MIC value!

Results invalid!

# Advice for skipped wells

- Check for purity by subculturing from growth in the higher concentration or perform MALDI-TOF directly from the pellet.
  - If a contaminant is detected in the higher concentration, disregard growth and read the lower MIC.
- Check for purity by looking at disk diffusion agar plates if performed from the same inoculum suspension.
- Retest the isolate.
  - Note that in the case of heteroresistance, retesting may give a skipped well in another concentration.
- Refrain from reporting results for the agents with skipped wells.

# Very tiny pellets

- **Bacteriostatic agents:** disregard tiny pellets (pinpoint growth).
- **Bactericidal agents:** very tiny pellets that are clearly visible in the last well should be taken into account.
- When tiny pellets are present in all wells of the panel it may be caused by precipitations.
  - If uncertain, subculture from the tiny pellets in higher concentrations.

*Identity Specifications*  
**Difco™ Mueller Hinton Broth**  
Dehydrated Appearance: Light beige, free-flowing, homogeneous with a few dark specks.  
Solution: 2.1% solution, soluble in purified water upon boiling. Solution is very light amber, clear, may have a slight precipitate.  
Prepared Appearance: Very light amber, clear, may have a slight precipitate.

- For MH-F broth, incomplete lysing and centrifugation of the horse blood can lead to precipitations in all wells.

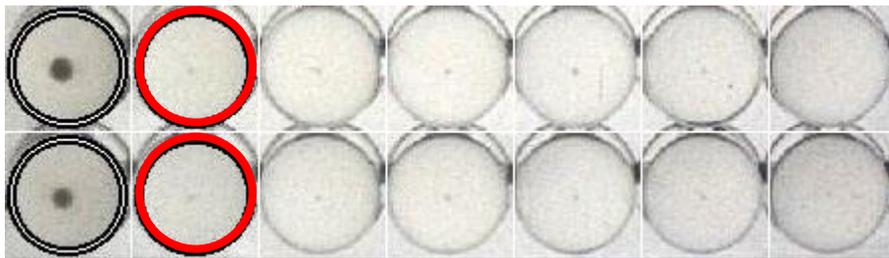
# Very tiny pellets

- If precipitations are suspected, subculture from the tiny pellets in higher concentrations or compare results with disk diffusion results for the agent in question.

Examples for *S. aureus* and ceftobiprole

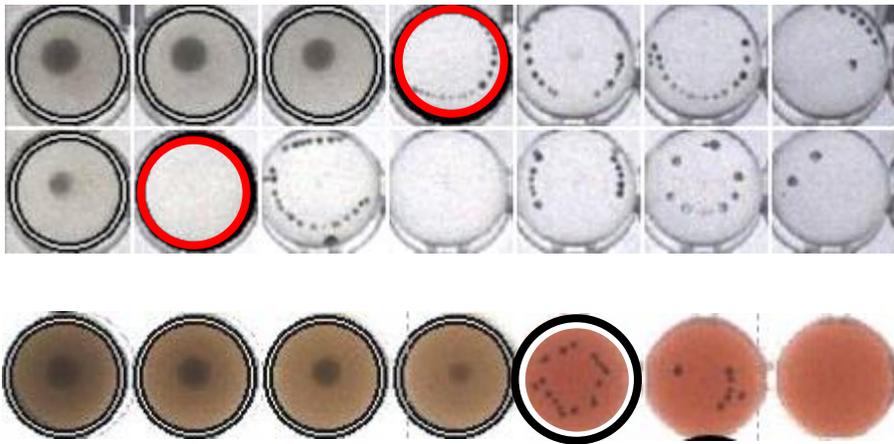


Examples of enterococci and fluoroquinolones



# Bubbles

- Bubbles can occur but usually do not interfere with reading as they are typically not centered in the middle of the well.
- Ignore bubbles when reading.



# Condensation

- Condensation on the plastic film may interfere with reading of MICs.
- To reduce problems with condensation, place an empty panel at the top of each stack
- Be very careful if removing the plastic film!



# **Reading exercises for BMD**

# Reading exercises in the lab

- Perform reading exercises for staff involved in BMD testing.
- Use EUCAST BMD Reading Guide to present general and specific reading instructions.

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

## EUCAST reading guide for broth microdilution

### Reading MIC endpoints

... should be read manually. The use of a mirror facilitate reading.

... automated reader or camera system is used, it ... be calibrated to manual reading.

... the MIC as the lowest concentration of ... robial agent that completely inhibits growth of the ... sm as detected by the unaided eye. ... ceptions, see slides 12-18.

### Gram-positive cocci with bacteriostatic antimicrobial agents

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

oxycycline	
usidic acid	
tetracycline	
oxycycline	

## AST of bacteria

Organization

Public consultations

EUCAST News

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**

IVDR

Media preparation

**MIC determination**

Disk diffusion methodology

Disk diffusion implementation

Breakpoint tables

Quality Control

Strains with defined susceptibility



## 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** - see separate guidance (agar and broth for non-fastidious and fastidious organisms)

**Broth microdilution** - [EUCAST reading guide v 5.0](#) (1 January 2024)

Guidance on cefiderocol MIC testing - see guidance document.

MIC determination

# Selection of isolates and agents

- Practise on reading the same BMD panels.
- Include QC strains plus clinical isolates representing challenging examples and agents with specific reading instructions:
  - Haemolysis
  - Trailing endpoints
  - Bacteriostatic agents
  - Trimethoprim-sulfamethoxazole
- Calibrate your reading against ranges and target values for the QC strains.

# Frequency of reading exercises

- Always when first implementing the broth microdilution method.
- Always when introducing:
  - A new BMD panel
  - An agent that is difficult to read
- If possible, repeat once a year to keep staff updated.

