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

EUCAST reading guide for broth microdilution

Version 5.0
January 2024

Changes from previous version (4.0)

Slide	Change
17	Clarification on disregarding haze when reading MIC endpoints for cefiderocol

Broth microdilution

- Broth microdilution is the reference method for antimicrobial susceptibility testing of rapidly growing aerobic bacteria, except for mecillinam and fosfomycin, where agar dilution is the reference method.

- EUCAST recommends testing according to the International Standard ISO 20776-1, but with the use of MH-F broth (Mueller-Hinton broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD, see instructions for preparation at www.eucast.org) for fastidious organisms.

- Results are recorded as the lowest concentration of antimicrobial agent that inhibits visible growth of a microorganism, the Minimum Inhibitory Concentration (MIC), expressed in mg/L or µg/mL.

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.

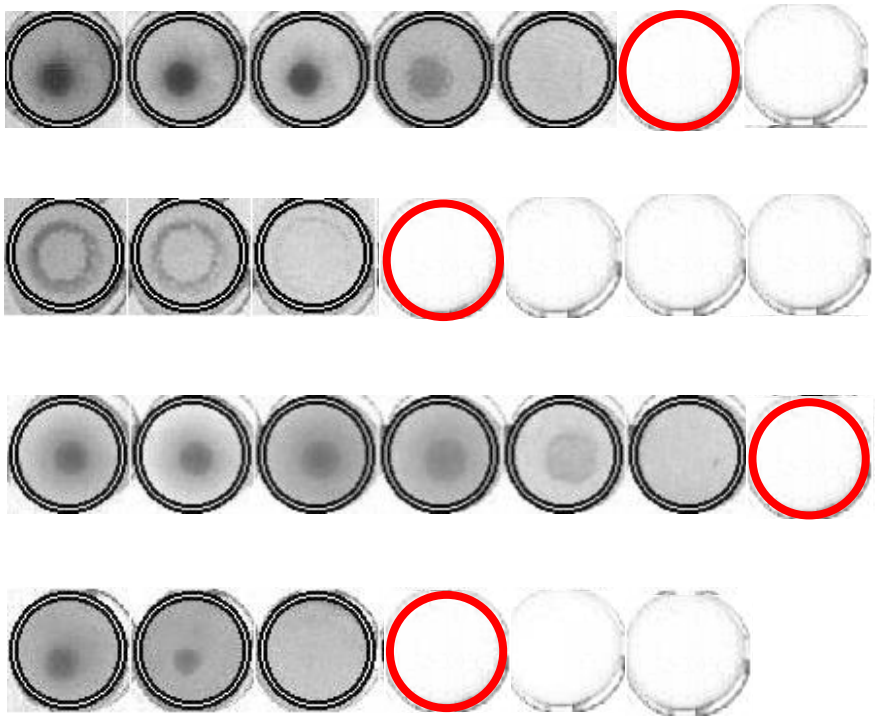
- Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.
  For exceptions, see slides 12-18.

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 (for exceptions, see slides 12-16).

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.

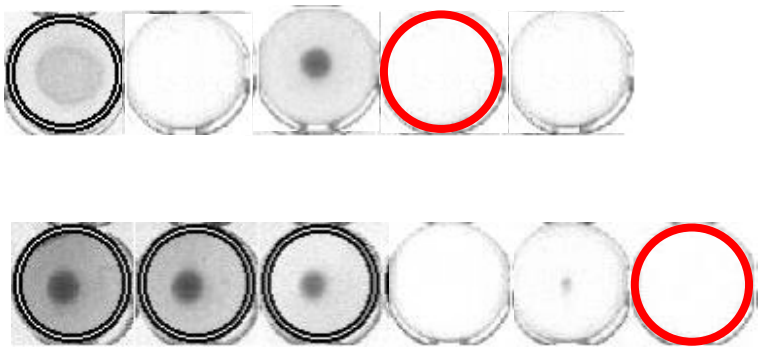
Skipped wells

- 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.

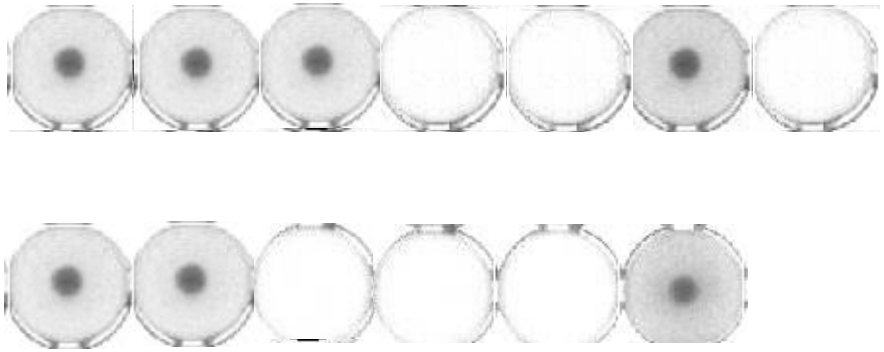
- When a single skipped well occurs, retest the isolate or read the highest MIC value to avoid reporting isolates as false susceptible.

- 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

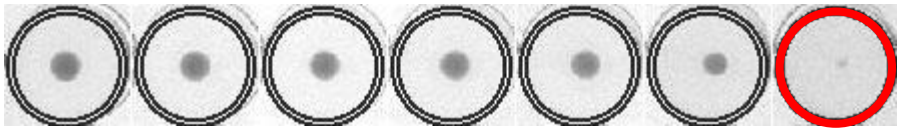
- The following antimicrobial agents require specific reading instructions:
  - Bacteriostatic antimicrobial agents, both with Gram-positive and Gram-negative organisms
  - Trimethoprim and trimethoprim-sulfamethoxazole
  - Cefiderocol

Gram-positive cocci with bacteriostatic antimicrobial agents

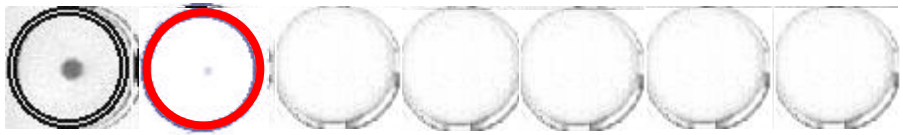
- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

Doxycyline

Fusidic acid



Tetracycline

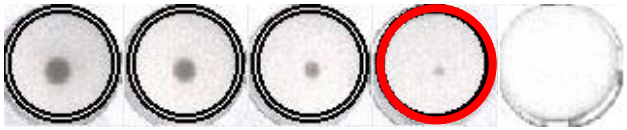


Doxycyline

Gram-positive cocci with bacteriostatic antimicrobial agents

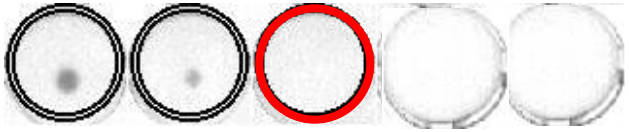
- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

Linezolid

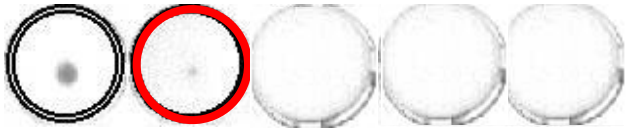


Linezolid

Tedizolid



Tedizolid

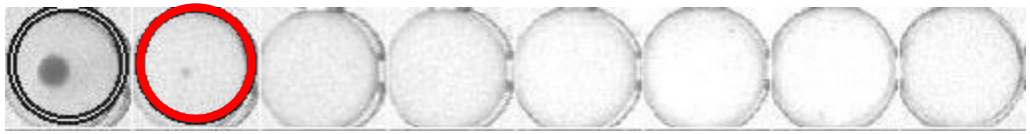


Gram-negative organisms with bacteriostatic antimicrobial agents

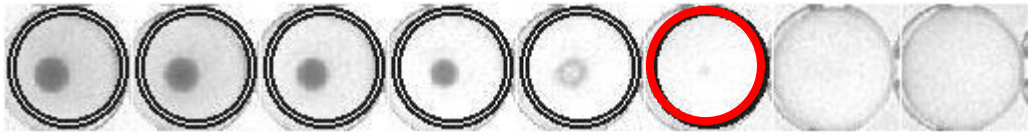
- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

Tigecycline

Tigecycline



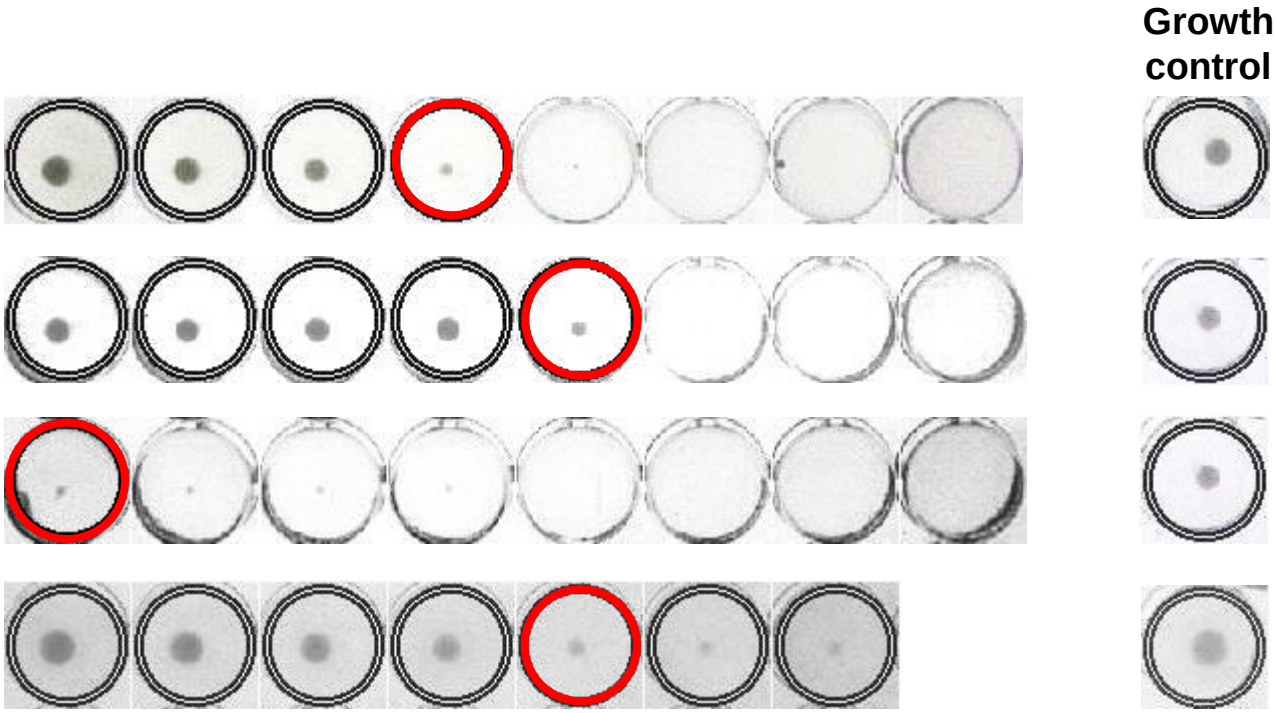
Eravacycline



Eravacycline

Trimethoprim and trimethoprim-sulfamethoxazole

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



Cefiderocol

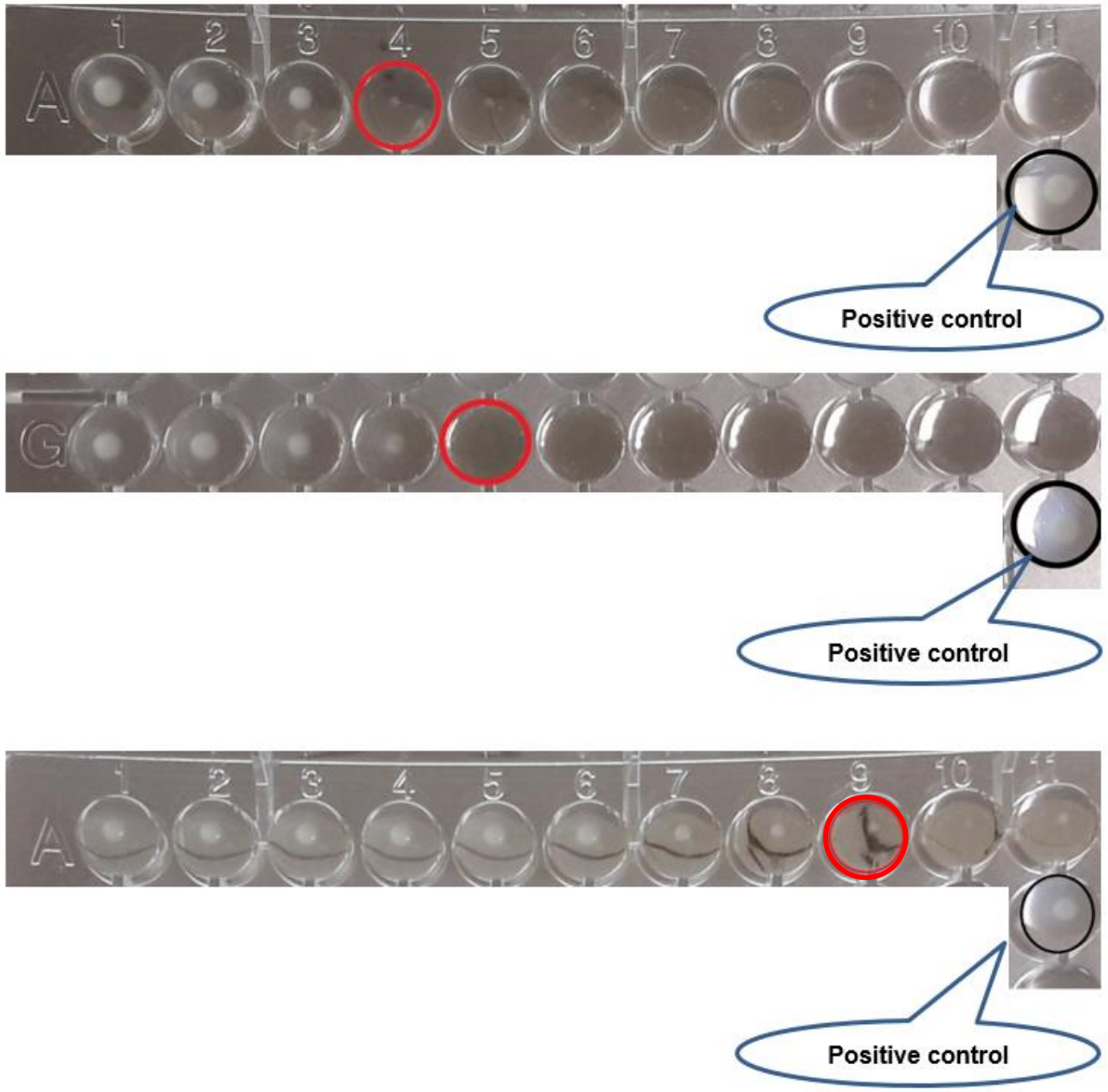
- Broth microdilution MIC determination must be performed in iron-depleted Mueller-Hinton broth and specific reading instructions must be followed. For testing conditions, see http://www.eucast.org/guidance_documents/.

- 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 (≥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



Interpretation of results

- Make sure that MIC values for relevant Quality Control strains are within acceptable ranges before reporting results for clinical isolates.
  - See quality control criteria in EUCAST QC Tables (www.eucast.org).

- Interpret MIC values into susceptibility categories (S, I and R) according to the current EUCAST Breakpoint Tables (www.eucast.org).

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