EUCAST reading guide for broth microdilution

Version 2.0
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Changes from previous version (1.0)

<table>
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<td>• Specific reading instructions for Gram-negative organisms with tigecycline and eravacycline added.</td>
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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](http://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 $5 \times 10^5$ 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 12-16.
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 combinations require specific reading instructions:
  - Gram-positive cocci with bacteriostatic antimicrobial agents
    - For example chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline
  - Gram-negative organisms with tigecycline and eravacycline
  - All organisms with trimethoprim and trimethoprim-sulfamethoxazole
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.
Gram-negative organisms with tigecycline and eravacycline

- 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.
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).