The reference method for MIC determination is broth microdilution (BMD) according to ISO standard 20776-1 [1]. For some agents and some species, BMD is not suitable. In these cases, agar dilution may be appropriate.

Many clinical microbiology laboratories have not implemented BMD and gradient tests are commonly used for MIC determination. There are no EUCAST recommendations for gradient tests and users are recommended to strictly follow the manufacturers' instructions for storage, handling and testing. There are two different brands of gradient tests on the market: Etest (bioMérieux) and MIC Test Strip (Liofilchem).

The performance of a test is a shared responsibility between manufacturer and user. Manufacturers are responsible for calibrating the product against reference methodology and issuing directives for the use of the product:

- which media were validated?
- which atmospheres were validated?
- which species were validated?
- was the full concentration gradient, lower and upper end of the concentration scale, validated for relevant species?
- if the gradient test was not validated using broth microdilution as reference, what other method was used as reference?

The user is responsible for the everyday use and performance of the gradient test (as for any other commercial antimicrobial susceptibility testing device). Strict adherence to the manufacturers' instructions concerning storing and handling of reagents is of great importance. Also, gradient tests should only be used for the species and with the variables (e.g. inoculum, media, incubation time, incubation temperature and atmosphere) that have been validated by the manufacturer.

Manufacturers sometimes list quality control (QC) criteria in package inserts and mostly these will agree with EUCAST criteria. If not, follow the EUCAST criteria (targets and ranges). EUCAST QC criteria and clinical breakpoints are connected through reference methodology. Only product or methods calibrated to reference methodology can be used with EUCAST breakpoints.

There are several studies showing quality problems and poor correlation with BMD for gradient tests and EUCAST has issued warnings for some products (see the EUCAST warnings page). However, EUCAST is not mandated or financially equipped to systematically check all antimicrobial susceptibility testing products. The lack of a warning is no guarantee that a product is without problems.

NordicAST recommendations for QC of gradient tests:

- Each new lot of gradient tests should be tested with a standard QC strain.
    - See Table 1 in EUCAST QC tables [2] for information on recommended strains for quality control. The recommendations (i.e. principal QC) are based on using a strain of the same (or a similar) species as the organism to be tested, but sometimes other QC strains have to be added to cover all agents.
    - Beta-lactam beta-lactamase inhibitor combinations must also be tested with a beta-lactamase producing strain to control the inhibitor component (see Table 2 in EUCAST QC tables [2]).
    - For agents routinely used on both un-supplemented Mueller-Hinton agar and MH-F agar (Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD), it is recommended to quality control the test on both media using relevant control strains.
- The frequency of quality control of gradient tests depends on the antimicrobial agent, storage conditions, type of package and how often the test is used.
    - Some antimicrobial agents are more sensitive to degradation during handling and storage than others and it is advisable to control these tests more often. Examples of these are beta-lactam agents, particularly amoxicillin-clavulanic acid, ertapenem, imipenem and meropenem.
    - Gradient tests in single packs should be preferred over packages with several tests. Large packages tend to be moved back and forth between room temperature and cold storage, which may result in loss of activity.
    - Rarely used antimicrobial agents/tests should be checked in conjunction with each clinical test.
- The result should be evaluated against EUCAST QC tables [2] and not against the criteria listed by the manufacturer in the product insert, except when EUCAST criteria have not been determined.
    - If the results are out of range: Repeat the test after having excluded obvious errors related to the shelf life of the test, media, incubators or testing procedure.
    - If results are still out of range: Repeat the test using a gradient test from an unopened package or a new lot and include one or two other strains (QC strain or other well-defined strain) with known MIC values. If possible, include a media from a different batch or a different manufacturer.
    - If the results continue to be out of range, list all data and report to the manufacturer.

Standard QC strains are mostly pan-susceptible and for most agents belong to the wild-type population. The performance of QC with such strains, checks the lower end of the scale but leaves the upper (non-wild type) end open to errors. In situations where it is important to ascertain the whole scale of concentrations for an agent, the use of a battery of strains, such as that offered by EUCAST for benzylpenicillin and *Streptococcus pneumoniae* (https://www.eucast.org/ast_of_bacteria/strains_with_defined_susceptibility/), is appropriate.

References

1. ISO. *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*. ISO 20776-1:2019. Geneva, Switzerland: International Standards Organization; 2019.
2. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 11.0; 2021. http://www.eucast.org/ast_of_bacteria/qc_tables/.

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Changes

Date	Change
2021-05-11	Document translated into English. Major revisions. Responsible committee members changed.
2017-03-07	Rekommenderad QC-stam för minocyklin ändrad till S. aureus ATCC 29213.
2016-09-05	Dokumentet uppdaterat med finsk översättning. QC-intervall för S. aureus ATCC 29213 och telitromycin tillagt.
2016-03-30	Ny utgåva. Omfattande revideringar i hela dokumentet. Nya antibiotika: Ceftolozan-tazobaktam, dalbavancin, oritavancin, telavancin och tedizolid. Dokumentansvariga uppdaterade.
2015-03-20	Lagt till ceftobiprol. Ändrat dokumentansvarig.
2014-02-05	Lagt till ceftarolin
2012-10-15	Nytt dokument