



Limitations of gradient tests

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The Standards (1)

ISO 20776-1 (2006)

*Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 1: **Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.***

- Broth dilution is the reference method for determining MIC
- BMD = broth micro dilution



The Standards (2)



- EUCAST disk diffusion method is calibrated to broth dilution
- EUCAST MIC breakpoints are valid for broth dilution methods

If other MIC method is used:

- the method should be calibrated to broth dilution in order to use EUCAST breakpoints → responsibility of the manufacturer
- the method should be used according to the instructions by the manufacturer → responsibility of the user

Responsibility of the manufacturer

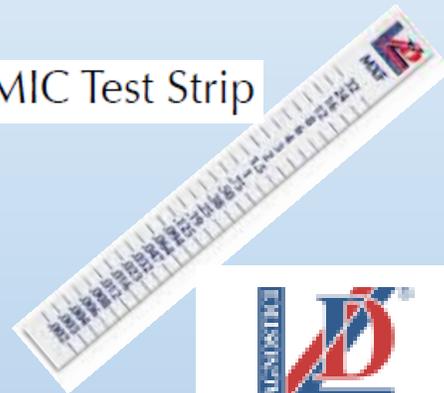
To produce gradient strips where antibiotic concentration is exactly precise on every single spot along the test strip.



Etest[®]



MIC Test Strip



M.I.C. Evaluator Strips (M.I.C.E.™)

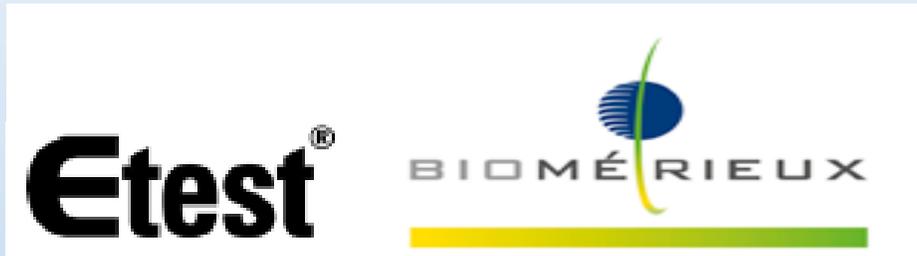
Thermo
SCIENTIFIC

Oxoid Microbiology Products

On every single spot
on every single test strip
on every produced LOT.

Responsibility of the user

To use the gradient test according to the instructions of the manufacturer



- Instructions available on website
- Registration required: www.mybiomerieux.com
- Technical Library
 - Etest Application Guide:
 - Information on the requirements for the plate, suspension medium, suspension turbidity, incubation time and atmosphere
 - Summary of Etest Performance, Interpretive Criteria and Quality Control Ranges

Responsibility of the user

To use the gradient test according to the instructions of the manufacturer



MIC Test Strip
for antimicrobial susceptibility testing



- Instruction available on website, registration not required
- www.liofilchem.net
 - Support center
 - Open areas: MIC Test Strip

http://www.liofilchem.net/pdf/mic/tabella_interpretazione.pdf

http://www.liofilchem.net/pdf/mic/MIC_APPLICATION_GUIDE.pdf

Technical Sheets

Responsibility of the user

To use the gradient test according to the instructions of the manufacturer



M.I.C.Evaluator Strips (M.I.C.E.™)

General basic level documents available free on the website

- Instructions for handling strips and reading results

<https://tools.thermofisher.com/content/sfs/manuals/MICE-Instruction-Guide-EN.pdf>

<https://tools.thermofisher.com/content/sfs/manuals/MICE-Interpretation-Guide-EN.pdf>

Detailed information available after registration.

Examples of limitations – 1

Not all gradient test products are compatible with EUCAST

Amoxicillin-clavulanic acid, fast concentration of clavulanic acid 2 µg/ml:

- Liofilchem (MIC Test Strip) and Thermo Scientific (M.I.C.Evaluator): available
- bioMérieux: **no Etest with fast concentration of clavulanic acid available**
(only constant ratio)

www.eucast.org → AST of bacteria → Compliance of manufacturers

Examples of limitations - 2

Streptococcus pneumoniae and erythromycin



Etest Application Sheet EAS 010:

- incubation +35°C / 5% CO₂ / 20-24h
- CO₂ lowers agar pH value which may have influence on the activity of erythromycin
 - Etest QC-range and SIR-interpretations are adapted to incubation at CO₂

Erythromycin	Etest [®]	EUCAST QC	Etest [®]	EUCAST SIR
CO ₂	0.064-0.25		$S \leq 1, R \geq 4$	
Ambient		0.032-0.125		$S \leq 0.25, R \geq 1$

Examples of limitations - 2

Streptococcus pneumoniae and erythromycin

EUCAST MIC breakpoints are validated for BMD method, including incubation in ambient air

Because CO₂ influences the activity of erythromycin, the EUCAST MIC breakpoints can not be used for the gradient tests that are incubated at CO₂ atmosphere according to the instructions by the manufacturer



The easiest way to avoid this problem: use EUCAST disk diffusion method.

Don't confirm the result with gradient test.

Examples of limitations - 3

Colistin

- EUCAST-CLSI – working group for colistin:
 - BMD is the only validated method to determine colistin susceptibility
 - Disk diffusion method does not work (reason: large molecule)

EUCAST Development Laboratory:

ECCMID 2017: poster P 161

- Colistin gradient tests from bioMérieux and Liofilchem
- Müller-Hinton –plates from BBL, Oxoid and bioMérieux
- 75 isolates (E.coli, K.pneumoniae, P.aeruginosa, Acinetobacter sp.)



Both gradient tests underestimated MIC values by one or more twofold dilutions, especially for concentrations on or above the breakpoint of 2 mg/L, **leading to false susceptible results (VME).**

Examples of limitations - 3

Colistin

Actions to take:

- **Don't use gradient tests or disk diffusion for colistin**
- **Use broth microdilution: it works fine**
- **QC: mcr-1 positive *E. coli* NCTC 13846 (colistin MIC target value 4 mg/L)**

Examples of limitations - 4

Piperacillin-tazobactam

EUCAST Development Laboratory, January 2016:

- Several batches of tests from both bioMérieux and Liofilchem
- Isolates: Enterobacteriaceae and *Pseudomonas aeruginosa*



- Gradient tests from both manufacturers gave variable and unreliable results
- The difference between gradient tests and BMD was not systematic, some values were two dilutions too high, others too low.
- There was also considerable variation between batches from the same manufacturer.



EUCAST disk diffusion test for pip-tazo works fine.

Don't confirm the disk test result with gradient test!

**Eucast breakpoint tables:
MIC required**

- Enterobacteriaceae: colistin (BMD)
- Pseudomonas sp: colistin (BMD)
- Pseudomonas aeruginosa: ceftolozane-tazobactam
- Acinetobacter sp: colistin (BMD)
- Staphylococcus: glycopeptides (BMD), tedizolid, daptomycin, fosfomicin
- Streptococcus A,B,C,G: dalbavancin, oritavancin, tedizolid, daptomycin
- Streptococcus agalactiae: trimethoprim
- **Streptococcus pneumoniae: betalactams if oxacillin zone <20 mm, meropenem if meningitis**
- Streptococcus viridans group: cefazolin, carbapenems if penicillin R, HLAR test
- Streptococcus anginosus group: dalbavancin, oritavancin, tedizolid
- Haemophilus influenzae: ceftaroline, meropenem if meningitis
- **Neisseria gonorrhoeae, Neisseria meningitidis**
- **Anaerobes**
- Helicobacter pylori
- Non-species related
- Candida sp., Aspergillus sp.

Evaluation of β -lactam gradient tests (Etest, M.I.C.E. and MIC Test Strip) for *Streptococcus pneumoniae*

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Conclusions

“Gradient tests (Etest, M.I.C.E. and MTS) generally **underestimated MIC values for β -lactam antibiotics for *S. pneumoniae*, potentially giving false susceptible results.** This effect was seen in all brands, but most pronounced for Etest. Quality control with *S. pneumoniae* ATCC 49619 was not sufficient to disclose these differences.

These results emphasize **the need for manufacturers to improve gradient test performance.**”

**Kvalitetskontroll gradienttester (svenska)/
Gradienttitestien laaduntarkkailu (suomeksi)**

1. Every new LOT: test with a susceptible QC strain (strains listed)
2. In addition, perform regular QC
 - frequency depending on antibiotic, package and the frequency of use
3. The QC result is compared to the EUCAST QC table:
 - if out of QC range, repeat the test excluding possible sources of error
 - if still out of range, test another strip from another package if possible
 - if still out of range, contact the manufacturer

Take home message: Don't drive the car eyes closed

MIC value is not automatically better or more reliable result than the disk diffusion test result.

Only broth (micro)dilution is a reference method.

Gradient tests:

- read through the instructions by the manufacturer
- piperacillin-tazobactam and colistin: major risk to false result
→ use disk diffusion (pip-tazo) or BMD (colistin)
- perform quality control: erroneous LOTs and methodological problems can be detected