

# A Comparative Study Of Antibiotic Gradient Devices For The Determination Of Daptomycin MIC

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## Overview

**Purpose:** To compare the performance of Thermo Scientific™ Oxoid™ daptomycin 256 M.I.C.Evaluator™ (M.I.C.E.™) strips (Thermo Fisher Scientific) to the CLSI broth dilution method and daptomycin 256 Etest® strip (bioMérieux) method according to CLSI guidelines.

**Methods:** One hundred and forty six isolates comprising staphylococci, streptococci and enterococci were used to evaluate the performance of daptomycin 256 M.I.C.E. strips and compare performance to daptomycin 256 Etest strips and the CLSI broth dilution method.

**Results:** Daptomycin 256 M.I.C.E. strips achieved at least 90.0% essential agreement (EA) with the CLSI broth dilution method across all groups of organisms tested, outperforming daptomycin 256 Etest strips.

## Introduction

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism and are used by diagnostic laboratories mainly to confirm resistance<sup>1</sup>.

Thermo Scientific™ Oxoid™ M.I.C.E.™ strips provide a gradient of stabilised antimicrobial, covering fifteen doubling dilutions on a convenient polymer strip format. Upon application, the antimicrobial is released from the strip, forming a defined concentration gradient in the surrounding agar. After appropriate incubation, the MIC value is easily read off where the interface between the zone and growth of the organism touches the strip<sup>2</sup>. Each M.I.C.E. strip is also individually packaged and can be obtained in stackable boxes of 10 or 50 strips (see Figure 1.).

The current M.I.C.E. range has been extended to include daptomycin, an antibiotic used in the treatment of infections caused by aerobic Gram-positive bacteria<sup>3</sup>.

FIGURE 1. Thermo Scientific Oxoid M.I.C.E. strips and packaging.



## Methods

In total, one hundred and forty six isolates comprising staphylococci, streptococci and enterococci (see Table 1. below for further information) were used to evaluate the performance of daptomycin 256 M.I.C.E. strips and compare performance to daptomycin 256 Etest strips and the CLSI broth dilution method<sup>4</sup>.

TABLE 1. Organisms tested during the study.

Organism Group	Sub-group / Number of isolates tested
Staphylococci (n=90)	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) / 47
	Methicillin-sensitive <i>Staphylococcus aureus</i> (MSSA) / 19
	Coagulase-negative staphylococci (CNS) / 24
Streptococci (n=20)	<i>Streptococcus agalactiae</i> / 5
	<i>Streptococcus pyogenes</i> / 5
	<i>Streptococcus pneumoniae</i> / 5
	<i>Streptococcus viridans</i> / 5
Enterococci (n=36)	<i>Enterococcus faecium</i> / 19
	<i>Enterococcus faecalis</i> / 8
	<i>Enterococcus gallinarum</i> / 4
	<i>Enterococcus casseliflavus</i> / 2
	<i>Enterococcus durans</i> / 1
	<i>Enterococcus avium</i> / 1
	Other <i>Enterococcus</i> spp. / 1

For each of the methods tested, a 0.5 McFarland standard was prepared and either swabbed onto the media outlined in Table 2. for strip testing or inoculated into the required broth (after adjusting the inoculum level for the volume in the microtitre wells) for the CLSI broth dilution method. The MIC of each of the isolates tested was interpreted using daptomycin M.I.C.E. and daptomycin Etest strips tested according to CLSI guidelines, as well as the CLSI broth dilution method.

TABLE 2. Media and incubation conditions for each of the methods tested

Organism group	Medium for strip testing	Incubation conditions for strip testing	Medium for broth microdilution	Incubation conditions for broth microdilution
Staphylococci	Thermo Scientific™ Mueller-Hinton Agar	35-37°C for 16-18 hrs.	Cation-adjusted Thermo Scientific™ Mueller-Hinton Broth	35-37°C for 16-20 hrs. in ambient air
Streptococci	95% Thermo Scientific™ Mueller-Hinton Agar with 5% sheep blood	35-37°C for 20-24 hrs. 16-18 hrs. for β-haemolytic streptococci. 5% CO <sub>2</sub> for <i>S. pneumoniae</i> and <i>S. viridans</i> , otherwise aerobic conditions.	Cation-adjusted Thermo Scientific™ Mueller-Hinton Broth supplemented with 2.5-5% Lysed Horse Blood	35-37°C for 20-24 hrs. in ambient air
Enterococci	Thermo Scientific™ Mueller-Hinton Agar	35-37°C for 16-18 hrs.	Cation-adjusted Thermo Scientific™ Mueller-Hinton Broth	35-37°C for 16-20 hrs. in ambient air

## Results

EA (the percentage of strips giving an MIC within +1.0 and -1.5 doubling dilution difference to the gold standard result) was calculated for each of the 3 organism groups tested (streptococci, staphylococci and enterococci) as well as sub-groups e.g. MRSA and species e.g. *S. agalactiae*.

Overall, as demonstrated by the results in Table 3., daptomycin 256 M.I.C.E. strips performed considerably better than daptomycin Etest strips, achieving 95.9% EA compared to 58.9%. Of the 10 sub-groups tested, 9 achieved at least 90.0% EA between M.I.C.E. strips and the CLSI broth dilution method compared to just 2 for the daptomycin Etest. Additionally, M.I.C.E. strips achieved 100.0% EA with the CLSI broth dilution method for 8 of the 10 sub-groups tested.

### Streptococci

Daptomycin 256 M.I.C.E. strips performed excellently when tested with streptococci, achieving 95.0% EA with the CLSI broth dilution method with 3 of the 4 species tested (*S. agalactiae*, *S. pyogenes* and *S. pneumoniae*) achieving 100.0% EA.

In comparison, daptomycin Etest strips achieved 70.0% EA when tested with streptococci, with 3 of the 4 species tested failing to achieve 90.0% EA. Furthermore, *S. pneumoniae* achieved 20.0% EA with the CLSI broth dilution method.

### Staphylococci

When tested with staphylococci, M.I.C.E. strips again performed excellently, achieving 94.4% EA. Additionally, M.I.C.E. strips achieved at least 90.0% EA for all sub-groups tested, achieving 91.5% EA for MRSA and 100.0% EA for MSSA and CNS. Performance of the daptomycin Etest was again lower, achieving 47.8% EA for staphylococci. Additionally, CNS and MSSA sub-groups achieved 27.7% and 57.9% EA respectively.

### Enterococci

As shown in Table 3, M.I.C.E. strips performed excellently when tested with enterococci, achieving 100.0% EA for all species tested. In comparison, daptomycin Etest strips achieved 80.6% EA for enterococci with each of the sub-groups tested achieving less than 90.0% EA.

TABLE 3. Daptomycin M.I.C.E. and daptomycin Etest EA values with the CLSI broth dilution method

	EA (%)	
	M.I.C.E.	Etest
<b>Overall (n=146)</b>	95.9	58.9
<b>Streptococci</b>	95.0	70.0
<i>S. agalactiae</i>	100.0	80.0
<i>S. pyogenes</i>	100.0	100.0
<i>S. viridans</i>	80.0	80.0
<i>S. pneumoniae</i>	100.0	20.0
<b>Staphylococci</b>	94.4	47.8
MRSA	91.5	27.7
MSSA	100.0	57.9
CNS	100.0	100.0
<b>Enterococci</b>	100.0	80.6
<i>E. faecalis</i>	100.0	75.0
<i>E. faecium</i>	100.0	84.2
Enterococcus spp.	100.0	77.8

## Conclusion

Daptomycin 256 M.I.C.E. strips performed considerably better than daptomycin 256 Etest strips, achieving excellent EA with the CLSI broth dilution method. With their individually packaged sachets (see Figure 2.), daptomycin M.I.C.E. strips offer an accurate and convenient alternative to the traditional broth dilution method.

FIGURE 2. An individually packaged M.I.C.E. strip



## References

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