

Thermo Scientific Oxoid M.I.C.Evaluator Strips For The Testing Of Haemophilus Isolates Following CLSI And ISO/EUCAST Guidelines

Milena Oleksiuk¹, Anne Butler², Kate Powell²

¹Thermo Fisher Scientific, Basingstoke, UK, ²Thermo Fisher Scientific, East Grinstead, UK

Overview

Purpose: This study was conducted to evaluate the performance of the penicillin range of Thermo Scientific™ Oxoid™ M.I.C.Evaluator™ (M.I.C.E.™) strips (Thermo Fisher Scientific) for Haemophilus species minimum inhibitory concentration (MIC) testing.

Methods: Haemophilus isolates were tested according to M.I.C.E. strips method and CLSI agar dilution and ISO/EUCAST broth dilution methods for antimicrobial susceptibility testing. Organism MICs were compared and used to determine essential agreement (EA).

Results: Haemophilus spp. demonstrated greater than 90% EA across all antimicrobials tested.

Introduction

Oxoid M.I.C.Evaluator strips, shown in Figure 1, are a quantitative assay combining the accuracy of traditional broth dilution with the ease of use of antibiotic discs. Each polymer strip contains an antibiotic gradient, which when placed onto the inoculated agar plate, diffuses into the agar. Following appropriate inoculation and incubation, the minimum inhibitory concentration of an organism can be visually determined without the need for any measurements or calculations.

This study compared the performance of amoxycillin 256, amoxycillin/clavulanic acid 256, ampicillin 256, oxacillin 256, penicillin 256 and penicillin 32 M.I.C.E. strips to the CLSI agar dilution method and ISO/EUCAST broth dilution method for determining the MIC of Haemophilus spp.

Methods

A total of 20 Haemophilus spp. clinical isolates were grown on Thermo Scientific™ Columbia Agar with Chocolate Blood overnight in appropriate conditions, according to CLSI¹ and ISO/EUCAST^{2,3} guidelines.

ISO/EUCAST Broth Dilution Method

Microtitre plates containing cation adjusted Thermo Scientific™ Mueller-Hinton Broth supplemented with 5% Lysed Horse Blood, 20 mg/L β-NAD and appropriate concentrations of each antimicrobial agent (amoxycillin, oxacillin, amoxycillin/clavulanic acid, ampicillin and penicillin), were inoculated with a 0.5 McFarland inoculum suspension of each Haemophilus isolate (to achieve an inoculum level of 7.5x10⁵ cfu/mL) using a multi-channel pipette, as shown in Figure 2. All microtitre plates were incubated in aerobic conditions at 35±2C° according to ISO/EUCAST guidelines.

CLSI Agar Dilution Method

Thermo Scientific™ Mueller-Hinton Agar supplemented with 5% Defibrinated Horse Blood, 20 mg/L β-NAD containing appropriate concentrations of each antimicrobial agent listed in Table 1, were inoculated with the same inoculum suspension using a multi-point inoculator. Plates were incubated in 5% CO₂ at 35±2C°, according to CLSI methodology.

FIGURE 1. Oxoid M.I.C.Evaluator strips.



M.I.C.Evaluator Strip Method

The same inoculum suspension of each Haemophilus isolate was used to create a bacterial lawn on Mueller-Hinton Agar supplemented with 5% Defibrinated Horse Blood and 20 mg/L β-NAD. All tested M.I.C.E. strips listed in Table 1. were applied onto each agar plate using sterile forceps. Following the CLSI and ISO/EUCAST guidelines, plates were incubated in 5% CO₂ at 35±2C°.

MIC results were then read and used to determine EA (the percentage of the M.I.C.E. strips giving an MIC within +1.0 and -1.5 doubling dilution difference between to the CLSI agar dilution and ISO/EUCAST broth dilution results.

Results

Haemophilus isolates demonstrated greater than 90% EA across all compounds tested, as shown in Table 1. Penicillin 256 M.I.C.E. strips, oxacillin 256 M.I.C.E. strips and ampicillin 256 M.I.C.E. strips achieved 100% EA for both the CLSI agar dilution and ISO/EUCAST broth dilution methods. Amoxycillin 256 M.I.C.E. strips and amoxycillin/clavulanic acid 256 M.I.C.E. strips achieved 100% EA with the CLSI agar dilution and 90% with the ISO/EUCAST broth dilution methods. Penicillin 32 M.I.C.E. strips achieved 100% EA and 95% EA with the CLSI and ISO/EUCAST dilution methods respectively.

FIGURE 2. Microtitre plate for broth dilution method.

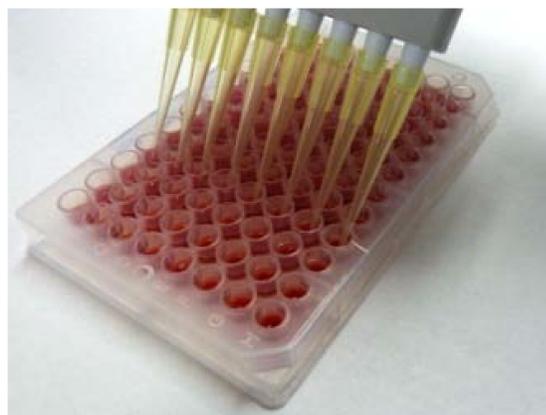


TABLE 1. The essential agreement results calculated for each antimicrobial for the CLSI and ISO/EUCAST dilution methods.

M.I.C.E. Strips	Method	EA (%)
Amoxycillin 256	ISO/EUCAST	90
	CLSI	100
Amoxycillin/Clavulanic acid 256	ISO/EUCAST	90
	CLSI	100
Ampicillin 256	ISO/EUCAST	100
	CLSI	100
Oxacillin 256	ISO/EUCAST	100
	CLSI	100
Penicillin 32	ISO/EUCAST	100
	CLSI	95
Penicillin 256	ISO/EUCAST	100
	CLSI	100

Conclusion

The presented data show that the Oxoid M.I.C.Evaluator strips are an accurate and reliable method for measuring Haemophilus spp. susceptibility to amoxycillin, amoxycillin/clavulanic acid, ampicillin, oxacillin and penicillin when following the CLSI agar dilution and ISO/EUCAST broth dilution methods.

References

1. CLSI Standards for antimicrobial susceptibility testing; Twenty-second informational supplement M100 S22 January 2012.
1. ISO guidelines; Clinical laboratory testing and *in vitro* diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – ISO 20776-1:2006 (E).
2. EUCAST guidelines: European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0 01/01/2012.

© 2013 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Folio no. LT 2091A/September/2013.