



Comparison of Etest and Agar Dilution for the Susceptibility Testing of 933 Anaerobic Isolates

T. Stevens¹, D. Hoban¹, C. Gaylord¹, M. Hackel¹, M. McCarthy¹, M Person¹, D. Hecht², J. Osmolski²

¹Laboratories International for Microbiology Studies, Schaumburg, IL, USA

²Loyola University Medical Center, Maywood, IL, USA

Presented at the 103rd ASM General Meeting, Washington, DC May 18-22, 2003

Revised Abstract

Background Agar dilution (Wadsworth) and broth microdilution are the two susceptibility testing methods recognized by the NCCLS for determining MIC endpoints against anaerobic bacteria. Agar dilution remains the reference standard for anaerobic susceptibility testing while broth microdilution is limited to *Bacteroides fragilis* group organisms and selected antibiotics. Many microbiology laboratories do not have the resources for performing agar dilution and must rely on simpler methods such as a concentration gradient agar diffusion method (Etest®). If Etest are used for testing, NCCLS requires laboratories to perform validation studies demonstrating that their results are comparable to the reference method. **Methods** MIC's to amoxicillin-clavulanic acid, ampicillin-sulbactam, clindamycin, metronidazole, and piperacillin-tazobactam were initially determined on 933 recent anaerobic isolates using Etest strips and interpreted following manufacturer's instructions and NCCLS guidelines. Isolates were retested using the agar dilution reference method following NCCLS guidelines. **Results** Overall agreement comparing Etest to reference NCCLS specified agar dilution method is shown below.

Antimicrobial Agent	≤1 dilution difference (%)	≤2 dilution difference (%)	≤3 dilution difference (%)	Major Errors S -> R (%)
Amox-clav	77.3	93.4	6.6	0.1
Amp-sulb	65.7	87.8	12.2	0.2
Clindamycin	76.5	90.2	9.8	1.8
Metronidazole	63.2	80.2	19.8	5.9
Pip-tazo	75.7	88.8	11.2	1.1

Conclusion The data shows the concentration gradient agar diffusion method (Etest) highly correlates to the agar dilution method within a two dilution difference. With the exception of metronidazole, interpretive results of Etest has a >98% correlation rate to the agar dilution test method. The Etest strips provide an important tool for anaerobic susceptibility testing in the clinical microbiology laboratory.

Introduction

Many Microbiology Laboratories do not have the resources nor staffing to perform anaerobic sensitivity testing by the agar dilution method recognized by NCCLS. Since microbroth dilution is significantly limited for anaerobic sensitivity testing, laboratories are forced to use alternate methods not approved by NCCLS. The Etest is simple to perform and is generally reliable method that is optimally read after 48 h of incubation. It should be an acceptable alternative to the agar dilution standard, although results with certain organism-antimicrobial combinations should be read very conservatively because of the frequency of major errors (MEs).² Over 900 clinical strains of anaerobes were tested against 5 antibiotics using the concentration

gradient agar diffusion method (Etest) and agar dilution method.

Materials and Methods

- ◆ Clinical isolates were collected in 2001-2002 from over 300 laboratories.
- ◆ Identifications were performed by using the RapID ANA II System (Remel Inc. Lenexa, KS) and additional PRAS biochemicals (Anaerobe Systems, Morgan Hill, CA.) as needed.
- ◆ 933 isolates of anaerobes consisting of 86 species were tested.

Antimicrobial Susceptibility Testing

- ◆ Antibiotics tested were amoxicillin-clavulanic acid, ampicillin-sulbactam, clindamycin, metronidazole and piperacillin-tazobactam.

Etest susceptibility testing:

- ◆ MIC's were determined by using the concentration gradient agar diffusion method (Etest, AB Biodisk, Sweden). Testing was performed according to NCCLS guidelines¹ and manufacturers instructions.
- ◆ Organism suspension was inoculated into Brain Heart Infusion broth equivalent to an 1 McFarland standard.
- ◆ PRAS brucella agar w/Vitamin K and Hemin (Anaerobe Systems, CA) was used as growth medium for testing.
- ◆ The determination of endpoints was determined according to Manufacturers guidelines.³
- ◆ Quality Control of antibiotic Etest strips and media was performed using *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotamicrons* ATCC 29741, *Eubacterium lentum* ATCC 43055.

Agar Dilution susceptibility testing:

- ◆ NCCLS recommended reference agar dilution method for anaerobes (M11-A5) was used for susceptibility testing.¹
- ◆ Brucella agar supplemented w/Vitamin K and Hemin was the test medium.
- ◆ Each inoculum was prepared and standardized by using a Vitek colorimeter to deliver approximately 10⁶ CFU/spot. All antibiotic plates were prepared and tested on the same day.
- ◆ The determination of endpoints was determined according to NCCLS guideline M11-A5.¹
- ◆ Quality Control of antibiotics and media was performed using *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotamicrons* ATCC 29741, *Eubacterium lentum* ATCC 43055.



Results

Results are depicted in the following tables.

Table 1. In Vitro susceptibility of 933 anaerobic bacteria determined by Etest and Agar Dilution *

Antimicrobial Agent	Etest (mcg/mL)		Agar Dilution (mcg/mL)	
	MIC50	MIC90	MIC50	MIC90
Amox/Clav	0.25	2	0.5	2
Amp/Sulb	0.25	2	0.5	4
Clinda	0.5	>256	0.5	>16
Metro	0.5	64	1	4
Pip/Tazo	0.25	8	0.25	8

* 86 distinct species represented

Table 2. Percentage (%) agreement between Etest and agar dilution

Antimicrobial Agent	≤2 Dilutions (Agreement)	>=3 Dilutions (Disagreement)	% of Major Errors	% of Very Major Errors
Amox/Clav	93.4	6.6	0.1	0.6
Amp/sulb	87.8	12.2	0.2	0.5
Clinda	90.2	9.8	1.8	1.7
Metro	80.2	19.8	5.9	0.9
Pip/tazo	88.8	11.2	1.1	0.2
Total	88.1	11.9	1.8	0.8

Discussion

Etest determination of MICs has been a very useful tool for microbiologist and has been shown to be both reliable and convenient.⁴⁻⁵ The NCCLS does not recognize Etest as a reference method of susceptibility testing primarily due to the longstanding policy of not endorsing commercial products and therefore does not publish any guidelines for its use. However, the Etest can be a useful tool especially for laboratories that are short staffed and because of the particular limitations and difficulties involved in testing anaerobic bacteria by the NCCLS agar dilution methodology.

Generally, the Etest MICs were one log₂ dilution lower than the agar dilution method with the notable exception for metronidazole. Metronidazole showed significantly reduced susceptibility with Etest as has been shown by others.⁶ Etest may not be reliable for the determination of metronidazole MICs against anaerobic bacteria.

The in vitro activity of Etest and agar dilution are presented in Table 1. The MIC₅₀s of amoxicillin-clavulanic acid, ampicillin-sulbactam and metronidazole were one log₂ dilution lower than those seen with agar dilution. The MIC₅₀s of clindamycin and piperacillin-tazobactam were identical by both methodologies. The MIC₉₀s of amoxicillin-clavulanic acid, piperacillin-tazobactam and clindamycin (MIC₉₀s for clindamycin exceeded the limits of both testing systems) were the same for both Etest and agar dilution and one log₂ dilution lower for ampicillin-sulbactam and clindamycin. Only metronidazole had a significantly higher MIC₉₀ with Etest.

Generally, Etest MICs for all drugs, including metronidazole, were within one log₂ dilution of agar dilution against all isolates with 88.1% of all MICs within two log₂ dilutions. Hsueh et al had similar finding in a previous anaerobic study but limited to a single species.⁷ Excluding metronidazole, over 90% of all drugs are within two log₂ dilutions. More importantly, there was very high agreement between Etest and agar dilution with respect to categorical interpretive changes. Table 2 shows that there was >98% agreement overall for the antimicrobial agents tested with < 2% major errors and < 1% very major errors.

Conclusions

- ◆ 90% of Etest MICs were within ± 2 log₂ dilutions of agar dilution MICs
- ◆ Etest vs agar dilution resulted in >98% categorical agreement.
- ◆ Etest is not recommended for the evaluation of metronidazole in vitro susceptibilities against anaerobes.
- ◆ Etest is an acceptable alternative to agar dilution for the determination of in vitro susceptibilities in anaerobic bacterial species.

References

- 1 National Committee for Clinical Laboratory Standards. 2001. Approved Standard M11-A5. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- 2 Rosenblatt, J.E, Gustafson, D.R. (1995) Evaluation of the Etest for Susceptibility Testing of Anaerobic Bacteria. Diagn Microbiol Infect Dis 1995;22:279-284.
- 3 Etest Technical Manual, Edition 2000. Susceptibility Testing of Anaerobes. AB Biodisk, Solna, Sweden.
- 4 Brown, D. F., and L. Brown. 1991. Evaluation of the E test, a novel method of quantifying antimicrobial activity. J Antimicrob Chemother 27:185-90.
- 5 Baker, C. N., S. A. Stocker, D. H. Culver, and C. Thornsberry. 1991. Comparison of the E Test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. J Clin Microbiol 29:533-8.
- 6 Pierard, D., A. De Meyer, P. Rosseel, and S. Lauwers. 1996. Use of the E-test for determining antimicrobial susceptibility of anaerobic bacteria. Pathol Biol (Paris) 44:358-62.
- 7 Hsueh, P. R., J. C. Chang, L. J. Teng, P. C. Yang, S. W. Ho, W. C. Hsieh, and K. T. Luh. 1997. Comparison of Etest and agar dilution method for antimicrobial susceptibility testing of *Flavobacterium* isolates. J Clin Microbiol 35:1021-3.