

## Evaluation of Etest for Testing Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Isolates with Different Growth Media

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Received 21 May 1993/Returned for modification 5 July 1993/Accepted 11 August 1993

**The MICs for 101 isolates of *Neisseria gonorrhoeae* obtained by Etest (AB-Biodisk, Solna, Sweden) and the agar dilution method on GC medium base supplemented with 1% Kellogg's defined supplement (GCMB) were compared. The overall percent agreement (within 1 log<sub>2</sub> dilution) between methods was greater than 97.9. The Pearson's correlation coefficients for penicillin, tetracycline, erythromycin, and ceftriaxone for the two methods were 0.98, 0.97, 0.93, and 0.93 ( $P = 0.001$ ), respectively, for comparisons on GCMB. The overall percent agreement was lower when hemoglobin-supplemented GCMB was used. Etest is an attractive alternative to the agar dilution method for gonococcal antimicrobial susceptibility testing and should be further analyzed in multicenter studies.**

Etest (AB Biodisk, Solna, Sweden) is a new technique for quantitative (MIC) antimicrobial susceptibility testing which allows for the direct determination of the inhibitory concentrations (1-3). Etest has been compared with different recommended "gold standard" reference methods for antimicrobial susceptibility determinations with panels of gram-positive and gram-negative microorganisms (1, 4), anaerobes (3), and fastidious microorganisms such as *Haemophilus influenzae* and *Streptococcus pneumoniae* (7). Those studies reported excellent agreement (generally, >90%) with the recommended reference methods. Some reports indicated problems in agreement because of differences in inoculum or medium or the use of certain antimicrobial agents (7).

Few studies have evaluated Etest in comparison with the agar dilution method for comparing the antimicrobial susceptibilities of *Neisseria gonorrhoeae* isolates (8, 10, 11, 12, 14). The method recommended for determining the antimicrobial susceptibilities of isolates of *N. gonorrhoeae* is the agar dilution method (9, 13). Because of the difficulty of the technique and because many laboratories receive a small number of gonococcal isolates for testing each month, the laborious agar dilution method is beyond the practical capability of many except reference laboratories. Thus, a simpler reproducible method such as Etest would enable a large number of laboratories to determine the MICs for gonococcal isolates.

In the present study, we compared the agar dilution and Etest methods for measuring the MICs of penicillin, tetracycline, erythromycin, and ceftriaxone for 101 isolates of *N. gonorrhoeae*.

**Comparison of MICs determined by Etest and agar dilution methods.** The 101 confirmed (13) *N. gonorrhoeae* isolates, including reference strains GC 1-20 (WHO III), GC 1-21 (WHO V), GC 1-22 (WHO VII), and GC 6770 (ATCC 49226), comprising 49 auxotype/serovar classes with various antibiotic resistance patterns and resistance mechanisms (35 susceptible isolates, 29 *N. gonorrhoeae* isolates with chromosomally mediated resistance [CMRNG], 22 penicillinase-

producing *N. gonorrhoeae* isolates [PPNG], 8 penicillinase-producing, plasmid-mediated tetracycline-resistant [TR] *N. gonorrhoeae* isolates [PP-TRNG], and 7 TR *N. gonorrhoeae* isolates [TRNG]) were selected from the culture collection of the National Laboratory for Sexually Transmitted Diseases, Laboratory Centre for Disease Control, for the present study.

Etest (AB Biodisk) and agar dilution antibiotic susceptibility tests to penicillin (Ayerst Laboratories, Montreal, Canada), tetracycline (Pfizer Canada Inc., Point-Claire-Dorval, Canada), erythromycin (Eli Lilly Canada Inc., Scarborough, Canada), and ceftriaxone (Hoffman-LaRoche, Mississauga, Canada) were determined by using GC medium base (GCMB) agar (Difco, Detroit, Mich.) with 1% Kellogg's defined supplement (4) with or without 1% hemoglobin (5). Hemoglobin-containing medium was included in the study since this medium has been recommended by the World Health Organization (13) for use in gonococcal antimicrobial susceptibility testing and has been used in a previous Etest evaluation of gonococcal isolates (10). Both Etest and the agar dilution method were completed simultaneously with the same inoculum, which was prepared by suspending an overnight culture in diluent (pH 7.2) (6) and adjusting the opacity to equal that of a 0.5 McFarland optical standard. For Etest, four different antibiotic strips were placed in an equidistant radial fashion on the surface of the inoculated plates. The plates were then incubated at 35°C in 5% CO<sub>2</sub> for 18 to 20 h. The MICs obtained by Etest were interpreted by directly reading the intercept of the antibiotic gradient strip and the zone of inhibition. MIC determination by the agar dilution method was completed as described previously (9). The same cell suspension prepared for Etest was diluted 10-fold prior to inoculation onto the antibiotic-supplemented agar plates with a Steers replicator (5). Plates were incubated at 35°C in 5% CO<sub>2</sub>, and the MIC was determined as recommended by the National Committee for Clinical Laboratory Standards (9).

The overall percent agreement, within the acceptable 1 log<sub>2</sub> dilution difference (1), between MICs obtained by Etest and those obtained by the agar dilution method with GCMB for both comparisons was 97.9 (Table 1). The agreement

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TABLE 1. Comparison of the MICs for 101 *N. gonorrhoeae* isolates measured by Etest (with GCMB) and the agar dilution method (GCMB without and with hemoglobin)

Antimicrobial agent	% Etest MICs within the following dilution ( $\log_2$ ) of agar dilution MICs on GCMB without (with) hemoglobin:						% Agreement within $\pm 1 \log_2$ dilution without (with) hemoglobin	No. of results outside range of Etest strips <sup>a</sup>
	<-2	-2	-1	Same	+1	+2		
Penicillin		1.3 (1.3)	31.2 (16.9)	57.1 (55.8)	7.8 (20.8)	2.6 (5.2)	96.1 (93.5)	24
Tetracycline	(1.0)	2.0 (35.6)	63.4 (57.4)	32.7 (5.9)	2.0		98.0 (63.4)	0
Erythromycin	(1.0)	3.0 (1.0)	8.9 (9.9)	74.3 (71.3)	13.9 (15.8)	(1.0)	97.0 (97.0)	0
Ceftriaxone			17.6 (5.9)	64.7 (34.1)	17.6 (54.1)	(5.9)	100.0 (94.1)	16
All agents	(0.5)	1.6 (10.4)	30.8 (23.6)	56.9 (41.2)	10.2 (21.4)	0.5 (2.7)	97.9 (88.9)	

<sup>a</sup> The penicillin MICs for some PPNG isolates were higher than the range on the Etest strips and were not included in the statistical analysis; some ceftriaxone MICs were lower than the range of the Etest strips.

between the MICs of penicillin was 96.1% (74 of 77 isolates; the remaining 24 isolates were PPNG or PP-TRNG). The MICs for 24 PPNG and PP-TRNG isolates exceeded the upper range for the Etest strip; all would have been correctly interpreted as being resistant to penicillin. Conversely, the MICs of ceftriaxone for 16 isolates were lower than the range indicated on the ceftriaxone Etest strip, and the isolates would have been correctly interpreted as being susceptible. The MICs for reference isolates were within the indicated concentration ranges by both Etest and the agar dilution method. Penicillin and tetracycline MICs obtained by Etest tended to be lower than the MICs obtained by the agar dilution method; 32.5% of the penicillin MICs and 65.4% of the tetracycline MICs were  $\geq 1 \log_2$  dilution lower by Etest. With erythromycin, 11.9% of the MICs obtained by Etest were lower than those obtained by the agar dilution method and 16.8% were higher, while an equal percentage (17.6%) of the MICs of ceftriaxone obtained by Etest were either lower or higher than those obtained by the agar dilution method.

When Etest results obtained with GCMB were compared with agar dilution results obtained on hemoglobin-supplemented GCMB, the overall percent agreement was 88.9 (Table 1). The agreement for tetracycline MICs was particularly affected, with only 63.4% being within 1  $\log_2$  dilution; 94.1% of the MICs obtained by Etest were less than the MICs obtained by the agar dilution method. With the other antibiotics, the percent agreements between Etest (GCMB) and the agar dilution method (GCMB with hemoglobin) were 93.5, 97, and 94.1 for penicillin, erythromycin, and ceftriaxone, respectively (Table 1).

**Classification of resistant isolates.** Statistical analysis by the chi-square test (McNemar test) for paired comparisons indicated that there was no significant difference between the results of Etest and the agar dilution method using GCMB agar in classifying gonococcal isolates into resistant, moderately susceptible, and susceptible categories for penicillin, erythromycin, and ceftriaxone (data not shown). With tetracycline MICs, however, statistically significant differences were noted. Overall, eight isolates classified as resistant and seven isolates classified as susceptible to tetracycline by the agar dilution method were classified as moderately susceptible ( $\chi^2 = 6.12$ ;  $P < 0.025$ ) and susceptible ( $\chi^2 = 5.14$ ;  $P < 0.025$ ), respectively, by Etest. The comparison with hemoglobin-supplemented GCMB indicated that nine isolates classified as resistant and eight isolates classified as moderately susceptible by the agar dilution method were classified as moderately susceptible ( $\chi^2 = 7.11$ ;  $P < 0.01$ ; data not shown) and susceptible ( $\chi^2 = 6.12$ ;  $P < 0.025$ ) by Etest, respectively. These discrepant results for tetracycline susceptibilities were noted with isolates which were suscepti-

ble, CMRNG and PPNG isolates, but not with PP-TRNG or TRNG isolates (Table 2).

**Regression analysis between Etest and agar dilution MICs.** The MICs measured by Etest and obtained by the agar dilution method showed a linear relationship (data not shown). For all antibiotics tested, the fitted regression lines had slopes of close to 1 (range, 0.89 for ceftriaxone to 1.1 for penicillin) and small intercepts (range, 0.01 for erythromycin to 0.86 for tetracycline); that is, the MICs obtained by Etest were generally within the acceptable 1  $\log_2$  dilution. The significant relationship was also reflected in Pearson's correlation coefficients, which were 0.98, 0.97, 0.93, and 0.93 ( $P = 0.001$ ), respectively, for penicillin, tetracycline, erythromycin, and ceftriaxone for comparisons on GCMB between the two methods. High Pearson's correlation coefficients (range, 0.92 to 0.98) were also obtained with hemoglobin-supplemented GCMB medium.

Few studies have evaluated Etest for determining the antimicrobial susceptibilities of gonococcal isolates. Bolmström et al. (2) first indicated that the method might be used with fastidious microorganisms, such as *N. gonorrhoeae*. Initially, the medium used was unsupplemented PDM antibiotic sensitivity medium (AB Biodisk); however, a subse-

TABLE 2. Comparison of tetracycline susceptibility classifications of 101 gonococcal isolates, including CMRNG and PPNG, by the agar dilution method and Etest on GCMB agar

Isolate	Method	No. of gonococcal isolates with the following tetracycline susceptibility classifications <sup>a</sup> :		
		Resistant ( $\geq 2$ mg/liter)	Moderately susceptible (0.5–1.0 mg/liter)	Susceptible ( $\leq 0.25$ mg/liter)
All	Agar dilution	51	31	19
	Etest	43	32	26
Susceptible <sup>b</sup>	Agar dilution	0	16	19
	Etest	0	12	23
CMRNG <sup>c</sup>	Agar dilution	23	6	0
	Etest	18	11	0
PPNG	Agar dilution	13	9	0
	Etest	10	9	3

<sup>a</sup> Susceptibility classifications of the National Committee for Clinical Laboratory Standards (9).

<sup>b</sup> Susceptible, gonococcal isolate which is susceptible to all antibiotics tested.

<sup>c</sup> CMRNG, gonococcal isolate which is chromosomally resistant to one or more antibiotics.

quent study showed that 30% of gonococcal isolates tested did not grow on this medium (14). Subsequent studies as well as the present one evaluating Etest for use in gonococcal susceptibility testing have used GCMB (11) and hemoglobin-supplemented GCMB (10). In the present study, a 97.9% overall agreement was achieved between Etest and the agar dilution method with GCMB in comparison with a 95.3% overall agreement on hemoglobin-supplemented GCMB. Sanchez et al. (11), who used GCMB, reported an overall agreement of 87%, while another study reported >90% agreement (with hemoglobin-supplemented GCMB) for tetracycline, ciprofloxacin, and cefuroxime MICs and 85% agreement for ampicillin and penicillin MICs (10). This is the first study to compare Etest results on GCMB with and without hemoglobin. Our results showed that hemoglobin does not enhance the performance of Etest. Sanchez et al. (11) reported minor interpretive discrepancies for CMRNG isolates for penicillin, tetracycline, and cefoxitin but no false-resistant or false-susceptible isolates. However, in the present study, no statistically significant differences were noted in the classification of isolates resistant or susceptible to penicillin, erythromycin, or ceftriaxone. Significant differences were noted with tetracycline MICs for PPNG and CMRNG isolates. In the present study, we noted that the penicillin and tetracycline MICs obtained by Etest were lower, irrespective of the medium used, than the MICs obtained by the agar dilution method, while erythromycin MICs obtained by Etest may be slightly higher than those obtained by the agar dilution method, an observation that has also been made by others (8, 10, 11). In addition, the present study also confirmed previous observations (5) that tetracycline MICs obtained by the agar dilution method were higher on hemoglobin-supplemented medium.

Etest is an attractive alternative to the agar dilution method for testing the susceptibilities of gonococcal isolates to antimicrobial agents. The significantly high correlation coefficients between Etest and the agar dilution method on GCMB agar indicate that Etest MICs can be used to estimate MICs that would be obtained by the agar dilution method. Etest offers the advantage that laboratories that process small numbers of specimens could determine the susceptibilities of gonococcal isolates reasonably accurately. Certain methodological difficulties, such as the number of Etest strips that should be applied to each plate and the concentration range of certain antimicrobial agents (e.g., ceftriaxone), could be resolved specifically for gonococcal susceptibility testing. Further evaluation of Etest for gonococcal susceptibility determinations seems warranted.

We thank Marielle Pauzé, Stephanie Hickey, and Michele Lemieux for technical assistance and Walter Tostowaryk (Division of Biometrics, Laboratory Centre for Disease Control) for statistical

advice. We especially thank Anne Bolmström (AB Biodisk) for supplying Etest strips and for critical review of the manuscript.

#### REFERENCES

1. Baher, 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**:533-538.
2. Bolmström, A., S. Arvidson, M. Ericsson, and A. Karlsson. 1988. A novel technique for direct quantification of antimicrobial susceptibility of microorganisms. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1209.
3. Citron, D. M., M. I. Ostovari, A. Karlsson, and E. J. C. Goldstein. 1991. Evaluation of the E test for susceptibility testing of anaerobic bacteria. *J. Clin. Microbiol.* **29**:2197-2203.
4. Derek, F., J. Brown, and L. Brown. 1991. Evaluation of the E-test, a novel method of quantifying antimicrobial activity. *J. Antimicrob. Chemother.* **27**:185-190.
5. Dillon, J. R., W. Tostowaryk, and M. Pauze. 1987. Effects of different media and methods of inoculum preparation on results of antimicrobial susceptibility testing of *Neisseria gonorrhoeae* by agar dilution. *Antimicrob. Agents Chemother.* **31**:1744-1749.
6. Hendry, A. T. 1983. Growth responses of *Neisseria gonorrhoeae* auxotypes to required amino acids and bases in liquid medium. *Can. J. Microbiol.* **29**:1309-1313.
7. Jorgensen, J. H., A. W. Howell, and L. A. Maher. 1991. Quantitative antimicrobial susceptibility testing of *Haemophilus influenzae* and *Streptococcus pneumoniae* by using the E-test. *J. Clin. Microbiol.* **29**:109-114.
8. Lind, I., L. Berthelsen, and M. W. Bentzon. 1991. Performance of the E-test (AB Biodisk) as to antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. Abstr. 7th Int. Pathog. Neisseria Conf., p. 155.
9. National Committee for Clinical Laboratory Standards. 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. Olsson-Liljequist, B., B. M. Hoffman, M. Ramberg, and Y. Skoglund. 1991. The E test compared with agar dilution for susceptibility test of *Neisseria gonorrhoeae* (NG). Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 176.
11. Sanchez, M. L., M. S. Barrett, and R. N. Jones. 1992. The E test applied to susceptibility test of gonococci, multiple-resistant enterococci, and *Enterobacteriaceae* producing potent  $\beta$ -lactamases. *Diagn. Microbiol. Infect. Dis.* **15**:459-463.
12. Sanchez, M. L., and R. N. Jones. 1993. E test, an antimicrobial susceptibility testing method with broad clinical and epidemiological application. *Antimicrob. Newsl.* **8**:1-7.
13. Van Dyck, E., P. Piot, and A. Meheus. 1989. Bench-level laboratory manual for sexually transmitted diseases. WHO/VDT/89.443. World Health Organization, Geneva.
14. Yeung, K.-H., L.-K. Ng, and J. R. Dillon. 1991. Evaluation of the E-test and PDM ASM agar in antimicrobial susceptibility testing of *Neisseria gonorrhoeae* isolates. Abstr. Int. Soc. STD Res. 9th Int. Meet.