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Evaluation of the AutoSCAN-3, a Device for Reading Microdilution Trays

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The AutoSCAN-3 (American MicroScan, Mahwah, N.J.) is an instrument capable of automated reading of commercially available microdilution trays for identification and quantitative susceptibility testing of rapidly growing bacteria. This study compared the results of visual and automated reading of microdilution trays for determination and interpretation of minimum inhibitory concentrations of 471 selected gram-negative and gram-positive clinical bacterial isolates. Visual and automated readings were performed in a double-blind fashion, and all discrepancies were examined by a referee. A quantitative comparison of minimum inhibitory concentrations was performed for 201 organisms, yielding 2,472 drug-organism combinations. After exclusion of off-scale values, complete quantitative agreement was obtained in 94% of 959 on-scale combinations, and agreement within ±1 well was obtained in 99.3%. Considering the minimum inhibitory concentration interpretations routinely furnished by the instrument, a qualitative comparison was performed for all 471 organisms. Complete agreement in interpretation was obtained in 97.6% of 5,843 drugs-organism combinations, with very major discrepancies accounting for only 0.1% and major discrepancies accounting for 0.2% of all combinations tested.

The broth microdilution technique for quantitative susceptibility testing has enjoyed increasing popularity in clinical microbiology laboratories. Commercially prepared microdilution trays are now widely available with predetermined dilutions of antibiotics stored in either frozen or dried form. The performance of many of these products has been evaluated and found to correlate satisfactorily with reference methods (2, 4, 5, 6; B. Flejzor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C326, p. 325; C. Mohla, M. J. Chang, and G. Controni, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C338, p. 368). Recently, automated readers have been introduced for use with microdilution trays which, in conjunction with a computer and a printer, offer a moderate degree of automation while preserving the option of visual reading in case of instrument failure. Both identification and quantitative susceptibility testing of rapidly growing bacteria can be performed. Preliminary evaluations of these instruments have been generally favorable (C. N. Baker, L. O. Helsel, D. L. Rhoden, and C. Thornsberry, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C329, p. 366; T. L. Gavan, C. L. Corlett, and M. J. Telenson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C262, p. 315).

The AutoSCAN-3 (American MicroScan, Mahwah, N.J.) is one such instrument which, in addition to automated reading of commercially prepared microdilution trays, also offers computerized interpretation of minimum inhibitory concentrations (MICs) based on achievable antibiotic blood levels (8).

This study evaluated the performance of AutoSCAN-3 as compared with the results obtained by visual reading of microdilution trays for quantitative susceptibility testing of gram-negative and gram-positive bacteria.

(This study was presented in part previously [P. C. DeGirolami, K. A. Eichelberger, L. C. Salfity, and M. F. Rizzo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C263, p. 315].)

MATERIALS AND METHODS

The bacteria tested were 471 fresh clinical isolates consisting of 154 gram-positive and 317 gram-negative organisms selected so as to provide adequate representation of the species recovered in clinical microbiology laboratories. Gram-negative bacteria included 81 *Escherichia coli*, 66 *Klebsiella* spp., 30 *Enterobacter* spp. and *Hafnia* spp., 12 *Serratia* spp., 15 *Citrobacter* spp., 49 *Proteus* spp. (also including *Morganella* spp. and *Providencia rettgeri*), 2 *Salmonella* spp., 53 *Pseudomonas aeruginosa*, and 9 other nonfermenters (including *Pseudomonas maltophilia*, *Acine-*

tobacter spp., and *Moraxella* spp.). Gram-positive bacteria included 69 *Staphylococcus aureus*, 21 *Staphylococcus* spp., 61 enterococci, and 3 viridans streptococci.

Microdilution trays used included MicroScan gram-positive MIC, gram-negative combo, and gram-negative urine combo panels. All trays were stored frozen until use and were inoculated according to the instructions of the manufacturer by the stationary-phase technique to obtain a final concentration of 10^5 CFU/ml (1, 3).

After 15 to 18 h of incubation at 35°C, MICs were determined by visual examination and by AutoSCAN-3 in a double-blind fashion. Visual readings were performed by two experienced technologists. For all discrepancies, a second visual reading was performed by a referee to exclude or correct technical or clerical errors. Before being inserted into the instrument, all trays were examined visually for flaws (scratches or imperfections of the tray plastic) and for peculiar growth patterns (side growth resulting in dishomogeneous turbidity) which could interfere with the optics of the instrument. A total of 10 quality control bacterial strains were tested each day and interpreted both visually and by AutoSCAN-3. All results were within the established ranges.

RESULTS AND DISCUSSION

A quantitative comparison was performed for 201 organisms (Table 1). Of the resulting 2,472 drug-organism combinations tested, 959 (38.8%) were on scale. After exclusion of off-scale data, complete quantitative agreement (i.e., the same MIC) was obtained in 94% of the cases. Excluding the species represented by fewer than 10 isolates, the highest rate of complete quantitative agreement was obtained for *Proteus* spp. (96.4%) and the lowest for *Enterobacter* spp. (89.2%). Overall quantitative agreement within ±1 well was obtained in 99.3% of the cases. These data were derived from all wells tested, without exclusion of those known to contain flaws or side growth. Of all trays tested, 8% had scratches or other plastic imperfections and 6.5% contained at least one well with side growth. To determine the usefulness of visually checking the trays before insertion in the instrument, the agreement was recalculated after exclusion of all wells with flaws or side growth. Surprisingly, complete agreement rose only by 0.5% (94 to 94.5%). Similarly, agreement within ±1 well rose only by 0.2% (99.3% to 99.5%).

Since interpretations of MICs based on achievable antibiotic blood levels are provided routinely by the instrument, we undertook to analyze the effect of location of the discrepancies in relation to the MIC breakpoints upon the interpretations. The instrument uses a four-point qualitative interpretation system, indicating high susceptibility, moderate susceptibility, slight susceptibility, and resistance. We defined very major, major, and minor discrepancies as previously established by Thornsberry et al. (7) and added a fourth category of very minor discrepancies to indicate a change from moderately to highly susceptible or vice versa. Qualitative agreement (agreement in interpretation) was determined for 471 organisms. Complete agreement in interpretation was obtained in 97.6% of 5,843 drug-organism combinations. Most discrepancies were minor or very minor, with very major discrepancies accounting for only 0.1%

TABLE 1. Quantitative agreement *by taxon* for all antibiotics

Organism	Strains tested	Total no. (%[a]) of on-scale drug-organism combinations	Agreement (%)	
			Complete	± 1 dilution
E. coli	38	214 (44.2)	93	98.1
Klebsiella spp.	31	167 (43.2)	94.6	100
Enterobacter spp.[b]	14	37 (21.5)	89.2	94.6
Serratia spp.	2	4 (16.6)	100	100
Citrobacter spp.	5	23 (27.3)	95.7	100
Proteus spp.[c]	30	137 (36.2)	96.4	100
Salmonella spp.	2	5 (20.9)	100	100
P. aeruginosa	23	79 (26.8)	94.9	100
Other nonfermenters[d]	5	17 (27.4)	100	100
S. aureus	19	78 (34.2)	93.6	100
Staphylococcus spp.	9	32 (29.6)	93.8	96.9
Enterococcus spp.	23	171 (62)	93	100
Total	201	959 (38.8)	94	99.3

[a] Percentages (in parentheses) indicate the number of on-scale drug-organism combinations/total number of drug-organism combinations.

[b] Includes *Enterobacter* spp. and *Hafnia* spp.

[c] Includes *Proteus* spp., *Morganella* spp., and *Providencia rettgeri*.

[d] Includes *Pseudomonas maltophilia*, *Acinetobacter* spp., and *Moraxella* spp.

TABLE 2. Qualitative discrepancies and interpretation agreement for 471 organisms yielding 5,843 drug-organism combinations

Type of discrepancy	Including flaws		Excluding flaws	
	Discrepancies (%)	Agreement (%)	Discrepancies (%)	Agreement (%)
Very major	0.1		0.1	
Major	0.2		0.1	
Minor	1.1	99.7[a]	0.8	99.8[a]
Very minor	1.0	98.6[b]	0.2	99.0[b]
Total	2.4	97.6[c]	1.2	98.8[c]

[a] Agreement considering only major and very major discrepancies.
[b] Agreement considering only major, very major, and minor discrepancies.
[c] Agreement considering all discrepancies.

and major ones for 0.2% of all drug-organism combinations. When wells with flaws or side growth were excluded, agreement rose to 98.8%. If minor and very minor discrepancies were disregarded, agreement was 99.7% before and 99.8% after exclusion of wells with flaws or side growth (Table 2). Discrepancies were rather evenly distributed, with the automated reading giving more resistant results in 42% and more susceptible results in 58% of cases.

When individual drug-organism combinations were examined, only the wells with physical imperfections were excluded; any wells with anomalous growth patterns were included. Side growth occurred in 0.97% of all drug-gram-positive-organism combinations but only in 0.3% of all drug-gram-negative-organism combinations.

For *Enterobacteriaceae*, agreement in interpretation was obtained in over 96% of the cases with all antibiotics except tetracycline (Table 3). The lower qualitative agreement for tetracycline (91.3%) was due to minor discrepancies clustering around the breakpoints (1, 2, and 4 μg/ml). For nonfermenting gram-negative organisms, interpretation agreement was obtained in 98.3% of cases with carbenicillin and tobramycin and in 100% of the cases with all other antibiotics tested (Table 3). For staphylococci, qualitative agreement was obtained in over 97% of cases except with chloramphenicol (Table 3). The lower agreement with chloramphenicol (95.6%) was due to four very major discrepancies clustered around the highest concentration tested (8 μg/ml). In all four instances, the instrument read the MIC at 8 μg/ml, interpreted as moderately susceptible, whereas visual reading gave results greater than 8 μg/ml, necessarily but perhaps incorrectly interpreted as resistant. This problem may have occurred because of the lack of higher chloramphenicol concentrations (16 μg/ml) in the microdilution tray, representing intermediate levels of susceptibility.

For streptococci, qualitative agreement was over 98% except with cephalothin (91.9%) and clindamycin (93.8%) (Table 3). There were 5 minor discrepancies for enterococci and cephalothin owing to the failure of the instrument to detect side growth at 16 μg/ml, and there were four minor discrepancies for enterococci and

TABLE 3. Qualitative agreement for each antibiotic

Antibiotic	Agreement (%)			
	Enterobacteriaceae	Nonfermenters	*Staphylococci*	Streptococci
Amikacin	100	100	NT[a]	NT
Ampicillin	98.8	100	100	100
Carbenicillin	99.2	98.3	NT	NT
Cefamandole	99.6	100	NT	NT
Cefoxitin	99.6	100	NT	NT
Cephalothin	98.4	100	100	91.9
Chloramphenicol	99.6	100	95.6	100
Clindamycin	NT	NT	100	93.8
Erythromycin	NT	NT	98.9	100
Gentamicin	NT	NT	98.9	100
Kanamycin	99.6	100	NT	NT
Methicillin	NT	NT	97.8	100
Nalidixic acid	98	100	NT	NT
Nitrofurantoin	99.2	100	100	100
Penicillin G	NT	NT	100	98.4
Sulfisoxazole	100	100	NT	NT
Tetracycline	91.3	100	97.8	100
Tobramycin	96.6	98.3	NT	NT
Trimethoprim-sulfamethoxazole	99.6	100	100	100
Vancomycin	NT	NT	100	100

[a] NT, Not tested.

clindamycin similarly produced and located at the levels of 4 and 8 μg/ml. It should be noted that neither cephalothin nor clindamycin are useful drugs in the treatment of enterococcal infections.

In conclusion, AutoSCAN-3 performed with a high degree of accuracy in the automated reading of microdilution trays for the quantitative susceptibility testing of gram-negative and gram-positive bacteria. It should be noted that in this study the performance of the instrument was compared with visual readings obtained by two experienced technologists whose results, if discrepant with those of the instrument, were rechecked by a referee. The high degree of agreement obtained under these ideal conditions suggests that the instrument would probably compare equally or favorably with visual readings performed by a larger number of technologists engaged in the daily routine activities of a clinical microbiology laboratory.

Although a quick visual scan before inserting each tray in the instrument might detect physical imperfections and anomalous growth patterns which could interfere with automated reading, the effect of this precaution on the overall accuracy of the instrument was minimal.

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