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Automated Reading of MIC Microdilution Trays Containing Fluorogenic Enzyme Substrates with the Sensititre Autoreader

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The Sensititre Autoreader is a microcomputer-driven instrument capable of automatically reading antimicrobial susceptibility microdilution trays. The instrument measures the fluorescence liberated by bacterial enzymatic activity on fluorogenic substrates as an indicator of growth in each well. A mathematical algorithm converts the fluorescent signals from an antimicrobial dilution series to an MIC endpoint. A three-center study evaluated the performance of the Autoreader in comparison with MIC determined visually in a duplicate set of control plates lacking fluorogenic substrate. Among 828 isolates of gram-negative bacilli tested against 17 antimicrobial agents, Autoreader 18-h MIC were within ±1 twofold dilution of control MIC values (agreement) in 95.3% of instances. In 3.5% of the instances, Autoreader values occurred ±2 half-step dilutions from control values (minor discrepancy), and in only 1.2% of instances did Autoreader values deviate from control values by greater than ±2 dilution steps (major discrepancy). Agreement, minor discrepancies, and major discrepancies were noted among 148 gram-positive cocci tested against 11 antimicrobial agents in 93.5, 4.8, and 1.7% of the instances, respectively. Over half of the major discrepancies noted with gram-negative bacilli occurred with *Proteus mirabilis*–beta-lactam combinations, a problem that was resolved when a lower initial inoculum was used. Inter- and intralaboratory reproducibility was excellent. Standard Sensititre susceptibility trays may be instrument read at 18 h reproducibly and accurately with only slight modification of conventional procedures to include fluorogenic enzyme substrates in the incubation broth.

Traditionally, the endpoint of antimicrobial susceptibility determinations has been the direct visual or instrument recognition of microbial growth in either a broth or agar matrix. Sensititre (GIBCO Laboratories, Life Technologies, Inc., Chagrin Falls, Ohio) is a commercially packaged system for quantitative antimicrobial susceptibility testing in broth microdilution trays. MICs of antimicrobial agents against bacteria are determined by visual inspection of inhibitory endpoints in a drug dilution series. The Sensititre system yielded results that were in close agreement with those from a reference microdilution procedure (2, 3).

Recently, the Sensititre system has been modified by the manufacturer to permit reading of the microdilution trays with a new instrument, the Autoreader. In this procedure, microbial growth is monitored by the measurement of fluorescence produced by bacterial enzymatic action on fluorogenic substrates. These fluorescence signals are interpreted by the computer-controlled instrument and converted to MICs.

The object of this collaborative investigation was to evaluate the performance of the new automated procedure in three different hospital laboratories. Results of the Autoreader procedure were compared with those of the conventional, visually read Sensititre trays. In addition, reproducibility studies were carried out on selected clinical and quality control bacterial isolates.

MATERIALS AND METHODS

Organisms. Strains of 828 gram-negative and 148 gram-positive bacteria, recently isolated from clinical specimens, were used in the following study centers: University Hospital, Cincinnati, Ohio; Indiana University Hospital, Indianapolis, Ind.; and John Dempsey Hospital, Farmington, Conn. Representatives of 25 bacterial species consisting of both gram-negative bacilli and gram-positive cocci were selected in numbers roughly proportional to their expected isolation frequency in a clinical laboratory. These included 99 isolates of *Staphylococcus aureus*, 24 *Staphylococcus epidermidis*, 17 group D streptococci, 8 group B beta-hemolytic streptococci, 232 *Enterobacter-Klebsiella-Serratia*, 172 *Proteus-Morganella-Providentia*, 148 *Escherichia coli*, 114 *Pseudomonas aeruginosa* and other *Pseudomonas* species, 83 *Salmonella-Shigella-Yersenia*, 34 *Citrobacter-Hafnia*, 26 *Acinetobacter* spp., and 19 *Aeromonas* spp. One or more of the following quality control organisms (recommended by the manufacturer for use with Sensititre trays) were run daily to monitor the control MIC microdilution procedure: *Escherichia coli* ATCC 25922, *Streptococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 25619, and *Flavobacterium meningosepticum* ATCC 13254.

Inoculum preparation. Two or more identical colonies were picked from primary enriched or selective agar plates after overnight incubation and suspended in saline to match a McFarland 0.5 turbidity standard, using an API IR inoculum reader (Analytab Products, Plainview, N.Y.). Appropriate dilution was made in Mueller-Hinton broth so that the final inoculum density in both control and test plates contained ca. 10^5 CFU/ml. Bacterial isolates that were frozen at −70°C before testing were subcultured twice on agar before inoculum preparation.

Control microdilution MIC procedure. MICs, determined with commercially prepared dehydrated antibiotic microdilution trays (Sensititre), were used as control MICs for each bacterial isolate. The assay broth consisted of Mueller-Hinton broth supplemented with 50 μg of calcium

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and 25 μg of magnesium per ml. Three plate formats were utilized, each containing a variety of dehydrated antimicrobial agents arranged in twofold dilution steps. Gram-positive isolates were tested on AP01 panels with the following antimicrobial agents and concentration ranges (micrograms per milliliter): penicillin, 0.06 to 8; methicillin, 0.12 to 16; ampicillin, 0.12 to 16; cephalothin, 0.5 to 64; gentamicin, 0.12 to 16; kanamycin, 0.5 to 64; erythromycin, 0.25 to 32; chloramphenicol, 0.25 to 32; clindamycin, 0.12 to 16; tetracycline, 0.12 to 16; and vancomycin, 0.25 to 32.

AP02A panels were used for gram-negative bacilli isolated from specimens other than urine and contained (in micrograms per milliliter): ampicillin, 0.25 to 32; carbenicillin, 4 to 512; piperacillin, 2 to 256; cephalothin, 1 to 128; cefoxitin, 1 to 128; cefamandole, 1 to 128; cefotaxime, 0.25 to 32; moxalactam, 1 to 128; amikacin, 0.25 to 32; gentamicin, 0.25 to 16; chloramphenicol, 1 to 64; and tobramycin, 0.25 to 16. Gram-negative urinary tract isolates were tested on AP03 panels containing (in micrograms per milliliter): trimethoprim-sulfa-methoxazole, 0.25-4.75 to 32-608; nalidixic acid, 1 to 128; nitrofurantoin, 2 to 256; ampicillin, 1 to 128; carbenicillin, 4 to 512; cephalothin, 1 to 128; cefoxitin, 1 to 128; cefamandole, 1 to 128; gentamicin, 0.5 to 64; kanamycin, 2 to 256; tetracycline, 0.5 to 64.

Trays were inoculated with a Sensititre Auto-inoculator, which delivered 50 μl of inoculated assay broth to each well of the microdilution panel. After inoculation, panels were covered with an adhesive plastic seal and incubated at 35°C under ambient atmosphere for 18 h. The MIC was the lowest dilution of drug that completely inhibited macroscopic growth. The control plates were visually read with a Sensititre computer-assisted reader with a mirrored view box equipped with indicator lights that matched an on-screen graphic display of the microdilution panel. Keyboard entry of inhibitory endpoints into a dual disk drive microcomputer allowed for data storage, interpretation categorization, and report printing.

Test MIC procedure. Adapation of the standard Sensititre panels for instrument reading required the use of a modified cation-supplemented, buffered, Mueller-Hinton broth formulation (Mueller-Hinton TES) as assay broth to which predried fluorogenic enzyme substrates were delivered by means of a paper strip. Alcohol-flamed tweezers were used to aspectically transfer each strip from a foil packing envelope to the broth tube. After a 15-min incubation at room temperature of the broth-substrate mixture, the bacterial inoculum was added to approximately 10^5 CFU/ml. Panels were then inoculated and incubated at 35°C for 18 h in a fashion identical to that for the control plates.

Autoreader instrument. Instrument reading of the fluorogenic substrate containing plates was accomplished with a computer (Apple II+)-driven reader (Sensititre Autoreader). Upon keyboard command, the plate carriage of the instrument transports the microdilution panel over an optical path that pulses the excitation wavelength of light and records at the emission wavelength the intensity of light as fluorescence resulting from bacterial enzymatic activity upon the fluorogenic substrates in each well of the plate. Raw fluorescence signals from the antibiotic-free control wells are compared with signals generated in the antibiotic-containing test wells. An algorithm is used by the microcomputer to automatically interpret the signals from the dilution series of each drug. An MIC is then displayed, printed, and stored together with any patient demographic data entered for each isolate.

Study protocol. Each test organism was inoculated into one control plate and one fluorogenic substrate-containing test plate. After overnight incubation, MIC values were visually determined from the control plate and entered via keyboard into the microcomputer. Test MIC values were then determined by the Autoreader from the fluorogenic substrate-containing plate and automatically stored in the computer. In addition, a visual determination of MIC values was made from the substrate-containing plate and entered by keystroke into the computer.

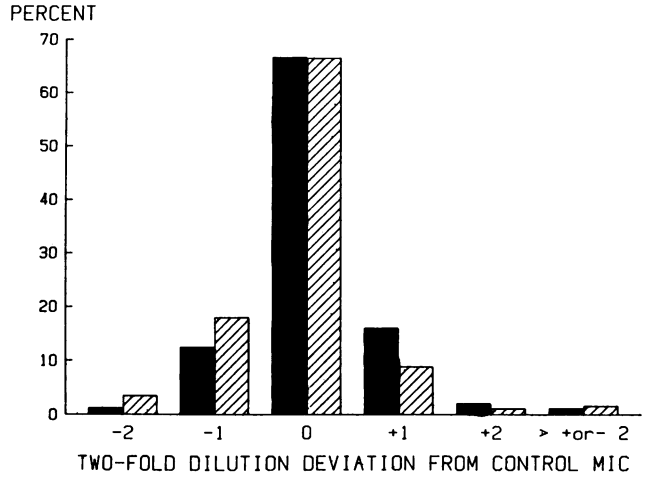

FIG. 1. Frequency distribution by half-step dilution wells of Autoreader test MIC values relative to control MIC values expressed as percentage of total gram-negative (■; n = 9,619) and total gram-positive (▨; n = 1,628) data pairs, respectively.

Data expression. Visually read control MICs and those MICs determined in the test plate by the Autoreader were stored on floppy data disks and analyzed with a compilation microcomputer program at the University Hospital in Cincinnati. This program summarized the distribution of MIC test values relative to those values obtained by the visually read control plates. Each comparison between the control and the Autoreader MIC for an individual drug-organism combination was referred to as a "data pair." A data pair was considered in agreement when the Autoreader MIC fell within ±1 well (or one twofold dilution) of that of the control MIC value. A deviation of the test MIC ±2 wells from the control MIC was considered a minor discrepancy, and a major discrepancy occurred when the test MIC was more than ±2 wells from the control MIC.

RESULTS

Figure 1 summarizes the distribution of test MIC values generated by the Autoreader relative to the control MIC values. The MIC data are shown as percentages of all gram-negative and gram-positive data pairs. Only 1.2% of the 9,619 gram-negative data pairs and 1.7% of the 1,628 gram-positive data pairs studied showed major discrepancies. Minor discrepancies were noted in 3.5 and 4.8% of the gram-negative and gram-positive data pairs, respectively.

Table 1 shows the percentage agreement and discrepancies between data pairs arranged by antibiotic. The overall agreement of data pairs for the gram-negative organisms was 95.3% and ranged from 91.9% for ampicillin to 98.4% for kanamycin. Further analysis of the gram-negative data pairs showing major discrepancies revealed that ca. 60% of these

TABLE 1. Agreement of 18-h Sensititre Autoreader MICs with control for 828 gram-negative bacilli

Drug	% of MICs showing the following relative to control:			No. of data pairs
	Agreement[a]	Minor discrepancy[b]	Major discrepancy[c]	
Amikacin	96.5	3.3	0.2	511
Gentamicin	97.9	1.7	0.4	828
Kanamycin	98.4	1.0	0.6	317
Tobramycin	97.1	2.7	0.2	511
Ampicillin	91.9	6.5	1.6	828
Carbenicillin	96.5	2.7	0.8	828
Piperacillin	96.7	2.3	1.0	511
Cefamandole	92.0	5.7	2.3	828
Cefotaxime	97.7	1.0	1.3	511
Cefoxitin	92.3	4.3	3.4	828
Cephalothin	92.4	5.6	2.0	828
Moxalactam	96.5	2.5	1.0	511
Chloramphenicol	96.9	2.9	0.2	511
Cotrimoxazole	96.5	1.9	1.6	317
Nalidixic acid	97.2	2.2	0.6	317
Nitrofurantoin	95.6	4.4	0	317
Tetracycline	95.6	3.1	1.3	317
All drugs	95.3	3.5	1.2	

[a] Autoreader MIC within ±1 well of control MIC.
[b] Autoreader MIC ±2 wells from control MIC.
[c] Autoreader MIC greater than ±2 wells from control MIC.

instances occurred with *Proteus* spp., in particular *Proteus mirabilis*. In addition, all *Proteus* discrepant data pairs involved beta-lactam drugs, most notably cefoxitin, cefamandole, cephalothin, and ampicillin. All major discrepancies in this group exhibited test MICs greater than two doubling dilutions higher than the control values. It should be noted that these major discrepancies among the Autoreader values were not observed when MICs were visually determined from the *Proteus* trays containing fluorogenic substrates. Other than those data pair discrepancies involving *Proteus* spp., the distribution of major discrepancies appeared random with respect to organisms, drug type, and direction of deviation from the control MIC.

It was of interest to determine whether the fluorogenic substrate used in the test panel had any effect on the visual determination of MIC endpoints. Therefore, MICs were determined by visual examination of the test plate and compared with control plate MIC values. Greater than 97% agreement was noted among the 9,619 gram-negative data pairs, with less than 0.6% of the data pairs occurring in the major discrepancy category. Therefore, a visual determination of MIC endpoints can be made in the presence of fluorogenic substrates with confidence should an automatic reading not be done or be questioned. Also, when the gram-negative test plate MICs determined by the Autoreader were compared with the MIC values determined by visual examination of endpoints on those same test plates, agreement approached 98%. This same plate agreement between visually and instrument-determined MICs was slightly higher than the comparison agreement between Autoreader test MICs and control values that were determined on parallel but separate plates. Therefore, MIC determinations made by the Autoreader in a majority of cases accurately reflected growth patterns in substrate-containing test plates.

Table 2 shows the distribution of data for 148 isolates of gram-positive cocci that were tested against 11 antimicrobial agents. Agreement was noted in 93.5% of the 1,628 data pairs examined. Minor discrepancies were seen in 4.8% of the data pairs, and only 1.7% of the data pairs were considered major discrepancies. Agreements ranged from 86% among ampicillin and chloramphenicol data pairs to 98% for gentamicin, kanamycin, and vancomycin data pairs. Examination of major discrepancies showed that penicillin had the highest rate (4.7%), whereas tetracycline and vancomycin had no major discrepant pairs. Among the 148 gram-positive isolates tested, the 99 isolates of *Staphylococcus aureus* showed the greatest agreement between Autoreader and control MICs, with 96, 3, and 1% of the data pairs in the agreement, minor discrepancy, and major discrepancy categories, respectively. It should be noted that approximately half of the *Staphylococcus epidermidis* isolates tested either failed to grow in both the control and test plates or failed to generate sufficient fluorescent signal to allow Autoreader MIC determinations. Of the 24 *Staphylococcus epidermidis* isolates which gave sufficient fluorescence for Autoreader MIC reports, only 2% of data pairs showed major discrepancies. Other than *Staphylococcus epidermidis*, rarely was an organism encountered, gram-positive or gram-negative, which failed to generate an interpretable fluorescence signal after 18 h of incubation.

Five quality control isolates were repeatedly tested on different days in the three study centers. The percentage of repetitive readings identical to the median MIC for each repetitive antibiotic series was similar for the Autoreader and the control values, and ranged from 65 to 86% for the Autoreader and from 75 to 86% for the control series (Table 3). Also, the percentage of repetitive readings that fell within one doubling dilution of the median MIC was 98 to 100% for both the Autoreader and the control values, with the exception of *Streptococcus faecalis* Autoreader values (93%) and *Staphylococcus aureus* control values (95%). Distribution of repetitive values around the median MIC did not appear to vary between study centers. Lastly, comparison of the average of the geometric means for Autoreader and control values for each organism expressed as the log to the base 2 + 9 (5) indicates no difference except for *Pseudomonas aeruginosa* (the Autoreader average geometric mean was only 0.6 higher than the control mean). Therefore, both the Autoreader and the control tray values proved to be highly

TABLE 2. Agreement of Sensititre Autoreader MICs with control values for 148 gram-positive cocci

Drug	% of MICs showing the following relative to control:			No. of data pairs
	Agreement[a]	Minor discrepancy[b]	Major discrepancy[c]	
Ampicillin	85.8	11.5	2.7	148
Cephalothin	95.3	4.0	0.7	148
Chloramphenicol	85.8	10.8	3.4	148
Clindamycin	95.3	3.4	1.3	148
Erythromycin	94.6	2.0	3.4	148
Gentamicin	98.0	1.4	0.6	148
Kanamycin	98.0	1.4	0.6	148
Methicillin	93.9	5.4	0.7	148
Penicillin	90.6	4.7	4.7	148
Tetracycline	93.9	6.1	0	148
Vancomycin	98.0	2.0	0	148
All drugs	93.5	4.8	1.7	

[a] Autoreader MIC within ±1 well (doubling dilution) of control MIC.
[b] Autoreader MIC ±2 wells from control MIC.
[c] Autoreader MIC greater than ±2 wells from control MIC.

TABLE 3. Sensititre Autoreader and control plate reproducibility with quality control organisms

QC organism	No. of repeat assays	No. of antibiotics tested per Assay	% of readings identical to median MIC		% of readings + or − 1 doubling dilution from median MIC		Differences between Autoreader and control geometric means for all antibiotics[a]
			Autoreader	Control	Autoreadei	Control	
E. coli ATCC 25922	25	11	84	80	100	100	0.03
Streptococcus faecalis ATCC 29212	8	11	65	78	93	100	0.12
Staphylococcus aureus ATCC 25923	13	11	77	76	98	95	0.10
Pseudomonas aeruginosa ATCC 25619	22	12	74	75	99	99	0.60
F. meningosepticum ATCC 13254	20	12	84	86	99	99	0.08

[a] Geometric means were calculated after conversion of antibiotic concentrations to $\log_2 + 9$. Differences represent average geometric mean Autoreader MICs minus average geometric mean control MICs for all antibiotics tested.

reproducible among the three laboratories and on a day-to-day basis within each laboratory.

Fifteen randomly selected clinical isolates (3 gram-positive and 12 gram-negative) were tested against 11 or 12 antibiotics on three separate days in all three laboratories. Analysis of data for deviation from the median MIC for each repetitive series indicated a high degree of reproducibility similar to that observed with the QC isolates for both the Autoreader and the control values, with one notable exception. Autoreader repetitive values for ampicillin, carbenicillin, cephalothin, cefoxitin, cefamandole, and moxalactam showed poor inter-laboratory reproducibility with one isolate of *Proteus mirabilis*. Autoreader values at the University of Cincinnati and the University of Connecticut were several dilutions greater than those generated at Indiana University; the latter Autoreader values were in agreement with the visually read controls. Review of Autoreader major discrepancies among gram-negative bacilli in the study indicated that Indiana experienced only half the number of major discrepancies than either the Cincinnati or Connecticut group. Furthermore, there was not a single *Proteus*–beta-lactam major discrepancy among the Indiana data, which accounted for that center's low number of major discrepant data pairs. The *Proteus mirabilis* reproducibility isolate and six *Proteus mirabilis* isolates originally found to have discrepant Autoreader values in the study were retested with a reduced initial inoculum size of 10^4 CFU/ml for the fluorogenic substrate-containing test plates. The lower inoculum produced Autoreader MICs that in all cases were in agreement with control MICs.

DISCUSSION

This study tested the ability of a system to automatically read and interpret three formats of microdilution trays for determining the susceptibility of bacterial clinical isolates to 23 different antimicrobial agents. The Autoreader MIC data and the visually determined MICs in the parallel series of control plates were very similar. Agreement, defined as ±1 half-step (twofold) dilution between test and control values, was obtained in over 95% of data pairs involving gram-negative organisms. Deviation of test values greater than two dilutions from the control values was present in only 1.2% of the 9,619 gram-negative data pairs analyzed. Interestingly, ca. 60% of the major discrepancies occurred with *Proteus mirabilis*. This was the only trend noted among the major discrepancies and was seen most commonly with the penicillins and cephalosporins. It has since been learned by the manufacturer that *Proteus mirabilis* exhibits high enzymatic activity against one or more of the fluorogenic substrates utilized in the system. A *Proteus* sp. isolate may be capable of producing sufficient enzymatic cleavage of the substrates to be interpreted falsely by the Autoreader as growth during the short growth phase necessary for beta-lactam antibiotics to act on cell wall synthesis. That is, certain inhibitory concentrations of beta-lactam compounds may have a reduced signal-to-noise ratio with *Proteus* spp. because of an increased fluorescent background. This background prompts a failure of the analysis algorithm to recognize an inhibitory endpoint. The relative fluorescence units produced by *Proteus mirabilis* in wells containing inhibitory concentrations of beta-lactam antibiotics are higher than those values recorded in inhibitory concentrations of aminoglycosides at which less background occurs, presumably because there is little organism growth in the aminoglycoside wells before inhibition. Also in support of this hypothesis is the observation that all of the *Proteus*–beta-lactam discrepancies had Autoreader values higher than the control values. The finding that a reduction in the *Proteus* sp. inoculum from 10^5 to 10^4 CFU/ml produces accurate Autoreader values is consistent with the above hypothesis since background enzymatic activity is less with a decreased initial inoculum density. It is not clear whether the success of the Indiana group with *Proteus* was due to slight, but not detected, differences in initial inoculum size compared with those used in the other laboratories, or to the ability of the Indianapolis instrument to better discriminate between fluorescent signal and noise. We suspect that slight differences in the initial calibration of the Autoreader instruments may have been responsible for these observations. It has since been learned from the manufacturer that the second generation of Autoreader instruments, calibrated according to more narrow tolerance specifications, better distinguishes between background noise and true signal. For the present, the problem appears to be easily resolved by the use of a lowered inoculum for *Proteus mirabilis*, an organism that fortunately has a very characteristic colonial morphology and thus may be easily channeled to a low inoculum protocol in most cases. Elimination of the *Proteus*-associated discrepancies would lower the major discrepancy rate between the Autoreader and control MICs to ca. 0.6% for gram-negative

bacilli. In addition, many of the minor discrepancies noted in the study would have been eliminated.

Among the gram-positive cocci, greater than 93% of the data pairs were in agreement. Only 1.7% of the data pairs were major discrepancies. Sixteen of the 27 major discrepancies occurred with either *Streptococcus faecalis* (an organism for which routine susceptibility testing has little value) or *Staphylococcus epidermidis*, an organism that is generally troublesome in conventional microdilution systems because of poor growth. Of the 148 gram-positive isolates tested, 99 were *Staphylococcus aureus*. For these isolates, 96, 3, and 1% of the data pairs fell within the agreement, minor discrepancy, and major discrepancy categories, respectively. The study included only six isolates of methicillin-resistant *Staphylococcus aureus*, all of which gave Autoreader values in agreement with control values, with the exception of two cephalothin data pairs in the minor discrepancy category.

The intralaboratory and interlaboratory data accumulated for both the QC isolates and the 15 selected clinical isolates that were concurrently tested in each of the study centers showed excellent reproducibility. Likewise, reproducibility testing data generated by the Autoreader compared with control reproducibility data were in close agreement. The only notable deviations were seen with *Proteus*–beta-lactam combinations. This problem was resolved by lowering the inoculum size.

Preparation of trays for instrument reading requires only that the fluorogenic substrate strip be added to a modified Mueller-Hinton assay broth before inoculation. All other materials and procedures are identical to those used in the conventional Sensititre antimicrobial susceptibility test system. Inoculation of plates via the mechanized Sensititre inoculator has been described elsewhere (4), and it may be added that recent technical upgrading of these instruments has greatly increased reliability, a problem encountered with earlier models. The Autoreader component itself was trouble-free and dependable in each laboratory throughout the 18 months of development and validation studies. Since program alterations or updates are accomplished merely by changing floppy disks, great flexibility of the system with respect to demographic, printing, and custom-plate formatting is possible.

One limitation of the Autoreader system is that the present configuration is more "mechanized" than "automated." Use of the system requires an operator to load each plate and to command via keystroke the reading sequence. Although a laboratory could theoretically realize a cost saving by shifting plate reading responsibilities from medical technologists and microbiologists to less skilled and presumably lower paid personnel, significant labor savings will be accomplished only when the system permits truly automated, walk-way operation.

Two other instruments have been successfully used to read MIC microdilution trays automatically, the AutoSCAN 3 (American Micro Scan, Mahwah, N.J.) (1) and the Dynatech MR580 Microelisa Autoreader (Dynatech Laboratories Ltd., Billingshurst, England) (6). Both systems function through turbidometric measurements of the microdilution wells and utilize microcomputers for data interpretation, storage, and handling. Although these two systems share similarities with the Sensititre Autoreader with regard to reading of trays after 18 h of incubation, various Autoreader system parameters such as inoculum size, fluorogenic substrate type and content, and the interpretation algorithm can be altered to allow for same-day MIC determinations. Data from a partially completed study of 5-h MIC determinations with the Sensititre Autoreader have indicated very good correlation with control MIC values for *Enterobacteriaceae* (J. L. Staneck, S. D. Allen, and R. C. Tilton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C89, p. 314). Use of the Autoreader for same-day reading of the recently released Sensititre Breakpoint Plate for qualitative susceptibility testing would be an attractive development for those laboratories interested in routine, rapid, nonquantitative susceptibility testing.

The development of an instrument capable of reading and interpreting quantitative antimicrobial susceptibility tests in microdilution trays by the use of fluorogenic enzyme substrates for the monitoring of microbial growth has been highly successful. Sensititre dehydrated antimicrobial susceptibility dilution trays containing fluorogenic substrate in a modified Mueller-Hinton broth can, after 18 h of incubation, be instrument read both accurately and reproducibly. This innovative fluorogenic substrate technology should find application in other areas of clinical microbiology in which monitoring of the growth parameters is integral to the testing modality.

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