

Evaluation of Sensititre Automated Reading and Incubation System for Automated Reading of Sensititre Broth Microdilution Susceptibility Plates

Kimberle C. Chapin^{1*} and Michael C. Musgnug²

Department of Pathology, Lifespan Academic Medical Centers, Rhode Island and Miriam Hospitals, Providence, Rhode Island,¹ and Binax, Portland, Maine²

Received 11 July 2003/Returned for modification 10 September 2003/Accepted 13 November 2003

The Sensititre Automated Reading and Incubation System (ARIS) performs automated reading of Sensititre identification and susceptibility plates. ARIS susceptibility results were compared to manually read results. A total of 212 isolates (3,352 organism-antimicrobial agent combinations) yielded 99.4% essential agreement and categorical error rates of 0.2% minor, 0% major, and 1.0% very major. ARIS yields accurate susceptibility results compared to plates read manually.

Microdilution susceptibility tests are manually interpreted by visual inspection or using an automated instrument. The Sensititre Automated Reading and Incubation System (ARIS) (TREK Diagnostic Systems, Inc., Cleveland, Ohio) is an automated system that detects fluorescence liberated by bacterial enzymes that cleave fluorogenic substrates in the broth. A computer algorithm converts this fluorescent signal to a MIC. Two different instruments are available to perform automated reading of the fluorescent plates: the semiautomated Autoreader, which requires off-line incubation of plates and reads one plate at a time, and the fully automated, robotics-driven ARIS, which incubates and reads plates with minimal user intervention.

Sensititre susceptibility plates have been available for more than 20 years. Plates produced in the early 1980s were interpreted by nonfluorescent methods and interpreted manually by visualizing macroscopic growth with a light box. Early studies yielded results equivalent to the National Committee for Clinical Laboratory Standards (NCCLS) frozen reference microdilution method (2–4). Subsequently, susceptibility plates that used a fluorogenic substrate strip and allowed automated interpretation by the Autoreader, a semiautomated system, as well as manual interpretation were developed. Studies in the 1980s showed that the plates read by the Autoreader yielded results that were equivalent to the results from plates that were visually inspected using a light box (8, 9).

At this time, there are two Sensititre plate modifications. While all Sensititre susceptibility plates can still be read manually, the two modifications use fluorogenic substrates to allow for automated interpretation. Both modifications of Sensititre plates can be used in conjunction with both the semiautomated Autoreader and the fully automated ARIS instruments. One plate format has dried fluorogenic substrate in the wells. This substrate in well (SIW) modification simplifies plate setup by eliminating the addition of a separate substrate strip. Recently,

ARIS compared favorably to a popular commercial automated method, MicroScan WalkAway (Dade Behring, West Sacramento, Calif.) (1) using Sensititre SIW plates. SIW plates are preferred for testing sites that have custom-formatted plates and/or have higher testing volume. The second plate format requires the addition of a fluorogenic substrate strip to the broth prior to inoculation. Plates requiring a substrate strip may be preferable for laboratories with smaller testing volume where manual or semiautomated interpretation is appropriate. Both plate formats, those requiring a substrate strip and SIW plates, produce the same results (Roger Grist [TREK Diagnostic], personal communication).

The objective of this investigation was to evaluate the performance of the ARIS for automated reading of Sensititre plates requiring the addition of a substrate strip compared to visual inspection of the same plates. In addition, the background of increasing resistance to antimicrobial agents should provide a greater challenge for this system compared to earlier studies.

A total of 212 clinical isolates were evaluated (Table 1). Appropriate quality control strains recommended by NCCLS (6, 7) were tested each day of testing. Isolated colonies that had been incubated overnight on solid medium were added to demineralized water to prepare a 0.5 McFarland suspension as verified by a Sensititre nephelometer. A fluorogenic substrate strip was added to a 10-ml sample of Sensititre cation-adjusted Mueller-Hinton broth. Thirty minutes later, a 10- μ l aliquot of the organism suspension was transferred to the broth containing the substrate strip. Sensititre MG and MH susceptibility plates (for gram-positive and gram-negative organisms, respectively) were inoculated with this suspension. A Sensititre Auto-Inoculator, a robotics-driven dosing platform, delivered 50 μ l of this suspension to each well of the susceptibility plates. Adhesive plate seals were added, and plates were placed in the ARIS at 35°C for 18 to 24 h. Automated plate reading was performed by the ARIS, and manual plate reading was done using the SensiTouch computer-driven light box.

Only those antimicrobial-organism combinations suggested by NCCLS as appropriate for routine use and reporting were evaluated (7). The ranges of antimicrobial concentrations

* Corresponding author. Mailing address: Department of Pathology, Lifespan Academic Medical Centers, Rhode Island and Miriam Hospitals, 593 Eddy St., Providence, RI 02903. Phone: (401) 444-2526. Fax: (401) 444-8514. E-mail: kchapin@lifespan.org.

TABLE 1. Clinical isolates tested in this study

Organism	No. tested
Gram-negative bacteria	
<i>Escherichia coli</i>	31
<i>Pseudomonas aeruginosa</i>	20
<i>Klebsiella pneumoniae</i>	23
<i>Enterobacter cloacae</i>	15
<i>Klebsiella oxytoca</i>	10
<i>Acinetobacter</i> spp.	7
<i>Enterobacter aerogenes</i>	6
<i>Citrobacter</i> spp.	4
<i>Proteus</i> spp.	3
Other gram-negative organisms	3
Subtotal.....	122
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	42
<i>Enterococcus faecalis</i>	24
<i>Enterococcus faecium</i>	19
Coagulase-negative staphylococcus.....	5
Subtotal.....	90
Total.....	212

tested are shown in Table 2. Results from the automated read and manual read were compared for essential agreement (results within 1 twofold dilution). Results were analyzed for the following categorical errors: minor (intermediate versus sus-

TABLE 2. Sensititre antimicrobial agents and concentrations tested

Antimicrobial agent(s)	Concns tested (µg/ml)	
	MH ^a	MG ^b
Amikacin	1-32	
Amoxicillin-clavulanate	0.5/0.25-16/8	
Ampicillin	0.5-16	0.12-8
Ampicillin-sulbactam	8/4-16/8	8/4-16/8
Cefazolin		2-16
Cefepime	0.25-16	
Cefotaxime	4-32	
Ceftazidime	1-16	
Cefuroxime	1-16	
Cephalothin	1-16	2-16
Chloramphenicol		4-16
Ciprofloxacin	0.06-2	0.06-2
Clarithromycin		0.12-4
Clindamycin		0.25-2
Erythromycin		0.12-4
Gentamicin	0.25-8	0.25-8
Imipenem	0.25-8	
Lomefloxacin		0.5-4
Mezlocillin	4-64	
Nitrofurantoin		32-64
Norfloxacin		4-8
Ofloxacin		0.5-4
Oxacillin		0.25-2
Penicillin		0.03-8
Piperacillin	4-64	
Piperacillin-tazobactam	2/4-64/4	
Rifampin		0.5-2
Tetracycline		0.25-8
Ticarcillin-clavulanate	4/2-64/2	
Tobramycin	1-8	
Trimethoprim-sulfamethoxazole	0.5/9.5-2/38	0.5/9.5-2/38
Vancomycin		0.5-16

^a For gram-negative organisms.
^b For gram-positive organisms.

TABLE 3. Accuracy of ARIS for reading Sensititre susceptibility plates

Organism	No. of isolates tested	Essential agreement ^a (%)	No. (%) of categorical errors		
			Minor ^b	Major ^c	Very major ^d
Gram-negative	122	99.5	3 (0.1)	0 (0)	5 (1.0)
Gram-positive	90	99.4	2 (0.2)	0 (0)	4 (1.1)

^a Within 1 twofold dilution.
^b Intermediate versus sensitive or resistant.
^c Falsely resistant.
^d Falsely susceptible.

ceptible or resistant result), major (ARIS, resistant result; manual, susceptible result [falsely resistant]), and very major (ARIS, susceptible result; manual, resistant result [falsely susceptible]). Any minor error that was in essential agreement was not tallied as an error. For example, a result of 8 µg/ml (susceptible) compared to 16 µg/ml (intermediate) would be in essential agreement and therefore not be tallied as a minor error.

Essential agreement was 99.5% for 122 gram-negative isolates (2,092 antimicrobial-organism combinations). Categorical errors were as follows: 3 (0.1%) minor, 0 (0%) major, and 5 (1.0%) very major errors. Ninety gram-positive isolates (1,260 antimicrobial-organism combinations) yielded 99.4% essential agreement and the following categorical errors: 2 (0.2%) minor, 0 (0%) major, and 4 (1.1%) very major errors (Table 3).

Very major errors occurred with *Pseudomonas aeruginosa* and ciprofloxacin and trimethoprim-sulfamethoxazole and with *Acinetobacter* spp. and ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. *Enterococcus faecalis* and rifampin yielded three very major errors, while *Enterococcus faecium* and rifampin yielded one very major error (Table 4).

Although ciprofloxacin and trimethoprim-sulfamethoxazole each yielded two very major errors, this represents only 2% of gram-negative isolates. It is unclear why 12% of *Enterococcus* isolates yielded errors with rifampin.

The ARIS performs well for automated reading of Sensititre susceptibility plates requiring the addition of a substrate strip compared to results determined manually by visual inspection. Performance exceeds 90% essential agreement, which is generally accepted as satisfactory (5). Categorical error rates are also well within accepted limits. The Sensititre ARIS/Auto-reader automated system appears to work well in this era of new antimicrobials and increasing resistance and proves to be

TABLE 4. Details of very major errors

Organism	Antimicrobial	No. of VM ^a errors
<i>Pseudomonas aeruginosa</i>	Ciprofloxacin	1
<i>Pseudomonas aeruginosa</i>	Trimeth-sulfa ^b	1
<i>Acinetobacter baumannii</i>	Ciprofloxacin	1
<i>Acinetobacter baumannii</i>	Gentamicin	1
<i>Acinetobacter baumannii</i>	Trimeth-sulfa	1
<i>Enterococcus faecalis</i>	Rifampin	3
<i>Enterococcus faecium</i>	Rifampin	1

^a VM, very major (falsely susceptible).
^b Trimeth-sulfa, trimethoprim-sulfamethoxazole.

a viable alternative in automated antimicrobial susceptibility testing.

This study was funded by TREK Diagnostic Systems and the Robert E. Wise, MD, Research and Education Institute, Lahey Clinic.

REFERENCES

1. **Chapin, K. C., and M. C. Musgnug.** 2003. Validation of the Automated Reading and Incubation System with Sensititre plates for antimicrobial susceptibility testing. *J. Clin. Microbiol.* **41**:1951–1956.
2. **Gavan, T. L., R. N. Jones, and A. L. Barry.** 1980. Evaluation of the Sensititre system for quantitative antimicrobial drug susceptibility testing: a collaborative study. *Antimicrob. Agents Chemother.* **17**:464–469.
3. **Hansen, S. L., and P. K. Freedy.** 1983. Concurrent comparability of automated systems and commercially prepared microdilution trays for susceptibility testing. *J. Clin. Microbiol.* **17**:878–886.
4. **Jones, R. N., T. L. Gavan, and A. L. Barry.** 1980. Evaluation of the Sensititre microdilution antibiotic susceptibility system against recent clinical isolates: three-laboratory collaborative study. *J. Clin. Microbiol.* **11**:426–429.
5. **Jorgensen, J. H.** 1993. Selection criteria for an antimicrobial susceptibility testing system. *J. Clin. Microbiol.* **31**:2841–2844.
6. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
7. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing. 12th informational supplement. M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Nolte, F. S., K. K. Krisher, L. A. Beltran, N. P. Christianson, and G. E. Sheridan.** 1988. Rapid and overnight microdilution antibiotic susceptibility testing with the Sensititre breakpoint Autoreader system. *J. Clin. Microbiol.* **26**:1079–1084.
9. **Staneck, J. L., S. D. Allen, E. E. Harris, and R. C. Tilton.** 1985. Automated reading of MIC microdilution trays containing fluorogenic enzyme substrates with the Sensititre Autoreader. *J. Clin. Microbiol.* **22**:187–191.