

Validation of the Sensititre® Automated Reading and Incubation System (ARIS®) for Antimicrobial Susceptibility Testing in a High Volume Clinical Microbiology Laboratory

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ABSTRACT

The Sensititre ARIS (TREK™ Diagnostic Systems, Westlake, OH) is a feasible alternative for automated susceptibility testing in a clinical laboratory. Custom gram positive (GP) and gram negative (GN) Sensititre plates (test system) were tested in parallel with MicroScan (Dade Behring, West Sacramento, CA) PC10 GP and NUMIC10 GN plates as part of an in-house validation. Plates were inoculated according to manufacturer's instructions. Sensititre inocula were adjusted to a 0.5 McFarland suspension while the Prompt Inoculation System was used for MicroScan. Viable counts were obtained for QC organisms daily. The ARIS and Walkaway-96 performed automated reading of Sensititre and MicroScan plates respectively. Results were analyzed for essential (+/- one well) and categorical (sensitive, intermediate and resistant) agreement. Antimicrobials that resulted in major (false resistance) or very major (false susceptibility) errors when compared to in-house results were read manually to assure that instrument reading was correct. Isolates with errors not resolved by manual reading were tested again in parallel. Errors not resolved via repeat testing in-house were arbitrated by blinded testing with NCCLS M-7 standard frozen reference microdilution plates (TREK Diagnostic Systems). A total of 350 isolates (4105 antimicrobial-organism combinations) were evaluated. The following error rates for 246 GN organisms were observed: 1.3% minor, 0.1% major and 0.8% very major. GN essential agreement was 96.0%. Error rates for the 104 GP organisms were 0.9% minor, no major, and no very major. Essential agreement for GP organisms was 95.0%. Overall, the systems performed similarly, however the Sensititre system offers a number of added benefits. The inoculum is standardized and the plates can be manually read if necessary. Custom plates are available in lot sizes that are appropriate for both high and low volume laboratories and may be changed frequently. This, coupled with the fact that Sensititre offers more FDA-approved antibiotics than MicroScan or Vitek allows susceptibility testing of a hospital's actual antibiotic formulary.

INTRODUCTION

This in-house validation of Sensititre for automated antimicrobial susceptibility testing (AST) was conducted to demonstrate that performance was equivalent to the existing automated AST system (MicroScan). No previous validation has compared the Sensititre ARIS to another automated AST system.

MATERIALS & METHODS

- Clinical and stock isolates were tested.
- Reproducibility of both systems was checked by an initial test of 10 isolates in triplicate for three days.
- Appropriate QC organisms as per NCCLS were run each day of testing.
- Colony counts were performed on QC isolates to ensure that the final inoculum concentration was approximately 5 X 10⁸ CFU/ml.
- Microscan plates:**
 - Prompt™ Inoculation System used to make inoculum.
 - Plates were dosed with the RENOK inoculator.
 - Plates were read at 18-24 hours via the Walkaway™-96.
- Sensititre plates:**
 - Bacterial suspensions were adjusted using a nephelometer to be equivalent to a 0.5 McFarland (1.5 X 10⁸ CFU/ml).
 - 10 µl of this was transferred to 10 ml Sensititre cation-adjusted Mueller Hinton broth.
 - Plates were dosed with the Sensititre AutoInoculator™.
 - Plates were read at 18-24 hours via the ARIS.
- Data analysis:**
 - MicroScan was considered the reference method.
 - Essential agreement (+/- one well)
 - Categorical agreement (S, I, R)
 - Minor error: one method = I; one method = S or R.
 - Major error: MicroScan S; Sensititre R
 - Very major error: MicroScan R; Sensititre S

MATERIALS & METHODS con't

- Discrepant analysis:**
 - NCCLS M7 standards were used as the reference method.
 - For major or very major errors, both plates were read manually and results compared again.
 - If major or very major errors still existed, both plates were set up a second time and read manually if necessary.
 - Any isolates with persistent major or very major errors were sent out for blinded discrepant testing via frozen reference microdilution plates (TREK Diagnostic Systems Lab Services), according to NCCLS M7 standards.

Isolates Tested			
Organism	No. tested	Organism	No. tested
Gram-negative:		Gram-positive:	
<i>Klebsiella</i> spp.	42	<i>Enterococcus faecalis</i>	28
<i>Escherichia coli</i>	40	<i>Staphylococcus aureus</i> (MRSA)	24
<i>Proteus mirabilis</i>	30	<i>Staphylococcus aureus</i> (MSSA)	12
<i>Pseudomonas aeruginosa</i>	27	<i>Enterococcus faecium</i> (vancomycin S)	10
<i>Enterobacter</i> spp.	27	<i>Enterococcus faecium</i> (vancomycin R)	10
<i>Citrobacter</i> spp.	16	Coagulase-negative <i>Staphylococcus</i>	11
<i>Serratia marsecens</i>	12	Total Gram-positive	95
Misc fermenters	23		
Misc nonfermenters	14		
Total Gram-negative	231		

Total 326

Antimicrobials Common to both Sensititre and MicroScan Plates

Gram Negative ^a	Gram Positive ^b
Amikacin	Ampicillin
Ampicillin	Cefazolin
Ampicillin/Sulbactam	Cлиндamycin
Aztreonam	Erythromycin
Cefazolin	Gentamicin
Cefepime	Levofloxacin
Ceftazidime	Nitrofurantoin
Ceftriaxone	Oxacillin
Gentamicin	Tetracycline
Levofloxacin	Vancomycin
Nitrofurantoin	
Piperacillin	
Tetracycline	
Tobramycin	

^a Sensititre: custom plate; MicroScan: NUMIC10
^b Sensititre: custom plate; MicroScan: PC10

RESULTS

Performance of Sensititre Compared to MicroScan after Discrepant Testing^a

Organism	No. tested	Error Rates			
		Essential agreement ^b	Minor ^c	Major ^d	Very Major ^e
Gram-negative	231	96.0%	1.3%	0.1%	0.8%
Gram-positive	95	95.0%	0.8%	0.0%	0.0%
Overall	326	95.8%	1.2%	0.1%	0.7%

^a NCCLS M7 standard
^b Results within one (1) two-fold dilution
^c One method: intermediate; One method: sensitive or resistant
^d Sensititre: R; MicroScan: S
^e Sensititre: S; MicroScan: R

Discrepant Analysis^a using NCCLS M7 Standard Reference Panels

Antimicrobial	Organism	Sensititre	MicroScan	Reference ^b	Resolution ^c
Ampicillin	<i>Providencia</i> sp.	8	>16	4	Sensititre
Ampicillin/Sulbactam	<i>Klebsiella oxytoca</i>	8	>16	16	Dual agreement ^d
Cefepime	<i>Stenotrophomonas maltophilia</i>	≤1	>16	1	Sensititre
Cefazolin	<i>Staphylococcus aureus</i>	>16	≤2	32	Sensititre
	Coagulase-negative <i>Staph</i>	≤2	>8	2	Sensititre
	<i>Klebsiella oxytoca</i>	8	>16	16	Dual agreement
	<i>Providencia</i> sp.	1	>16	8	Non-agreement ^e
	<i>Enterobacter aerogenes</i>	4	>16	4	Sensititre
Erythromycin	<i>Staphylococcus aureus</i>	>4	≤0.25	>16	Sensititre
Nitrofurantoin	<i>Providencia</i> sp.	≤32	>64	32	Sensititre
Oxacillin	Coagulase-negative <i>Staph</i>	2	>4	>8	MicroScan
Piperacillin	<i>Escherichia coli</i>	≤8	>64	64	MicroScan
	<i>Flavobacterium</i> sp.	16	>64	16	Sensititre
	<i>Flavobacterium</i> sp.	16	>64	16	Sensititre
	<i>Klebsiella oxytoca</i>	≤8	64	8	Sensititre
	<i>Klebsiella pneumoniae</i>	≤8	>64	8	Sensititre
	<i>Klebsiella pneumoniae</i>	16	>64	64	MicroScan
	<i>Klebsiella pneumoniae</i>	≤8	32	16	Dual agreement
	<i>Stenotrophomonas maltophilia</i>	>64	16	64	Sensititre
Tetracycline	<i>Enterococcus faecalis</i>	>8	≤2	≤1	MicroScan
	<i>Enterococcus faecium</i>	>8	≤2	≤1	MicroScan
	<i>Enterococcus</i> spp.	>8	≤2	≤1	MicroScan

^a Performed on major and very major errors after retesting
^b Reference panel as per NCCLS M7 standards
^c For 22 discrepancies, the reference method agreed with Sensititre 12 times, MicroScan 6 times, both systems 3 times, and neither system once.
^d Both systems yielded results within one well of the NCCLS reference method
^e Non-agreement is >1 well difference from reference result

Sensititre Errors After Comparison with Reference Testing Results

Antimicrobial	Organism	No. (%) Major Errors	No. (%) VM ^a Errors
Cefazolin	<i>Providencia</i> sp.	-	1 (13%)
Oxacillin	Coagulase-negative <i>Staph</i>	-	1 (9%)
Piperacillin	<i>Escherichia coli</i>	-	1 (3%)
	<i>Klebsiella pneumoniae</i>	-	1 (3%)
Tetracycline	<i>Enterococcus</i> spp.	3 (6%)	-

^a Very Major

RESULTS con't

MicroScan Errors After Comparison with Reference Testing Results

Antimicrobial	Organism	No. (%) Major Errors	No. (%) VM ^a Errors
Ampicillin	<i>Providencia</i> sp.	1 (13%)	-
Cefepime	<i>Stenotrophomonas maltophilia</i>	1 (17%)	-
Cefazolin	<i>Staphylococcus aureus</i>	-	1 (3%)
	Coagulase-negative <i>Staph</i>	1 (9%)	-
	<i>Providencia</i> sp.	1 (13%)	-
	<i>Enterobacter aerogenes</i>	1 (11%)	-
Erythromycin	<i>Staphylococcus aureus</i>	-	1 (3%)
Nitrofurantoin	<i>Providencia</i> sp.	1 (13%)	-
Piperacillin	<i>Flavobacterium</i> sp.	2 (67%)	-
	<i>Klebsiella oxytoca</i>	2 (25%)	-
	<i>Stenotrophomonas maltophilia</i>	-	1 (17%)

^a Very Major

Standard Susceptibility Plate Pricing

Manufacturer	List Price ^a
Vitek ^b	\$ 5.69
Vitek2 ^b	\$ 7.85
MicroScan	\$ 9.55
Sensititre	\$ 6.40

Custom Susceptibility Plate^a

Manufacturer	Min. Order
Sensititre	500
Vitek ^b	5,000
MicroScan	NA ^c

^a Per manufacturers on 4/24/2002
^b Instrumentation required (no manual read option)

^a Pricing varies according to plate layout and volume
^b Instrumentation required (no manual read option)
^c Not available for automated system

CONCLUSIONS

- Performance was equivalent between Sensititre custom formatted susceptibility plates and Microscan standard susceptibility plates.
- Most discrepant testing results favored Sensititre when compared to the NCCLS frozen microdilution plates.
- Resolution of discrepant testing may have favored Sensititre more frequently due the standardized inoculum verified by a nephelometer.
- Sensititre offers more antimicrobial agents for its plates than MicroScan or Vitek.
- Custom-formatted plates allow for greater antibiotic flexibility and testing actual agents on hospital formulary.
- TREK offers custom formatted plates in lots as small as 500 plates, while Vitek requires a minimum of 5,000 and MicroScan does not offer custom-formatted plates for use with the automated system.

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