PERFORMANCE EVALUATION OF THE LIFESCALE AST RAPID AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY SYSTEM

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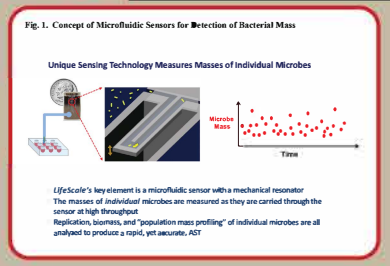
ABSTRACT

Background: Reducing the time to reporting antimicrobial susceptibility test (AST) results directly from positive blood cultures is essential for managing patients with suspected sepsis/bacteremia. The aim of this study was to evaluate the LifeScale AST system using a custom LifeScale 96-well broth microdilution panel. The system employs a microfluidic sensor that detects and measures the mass of individual organisms at high throughput with MIC results available within 4 – 6 hours. **Methods:** Fresh blood cultures detected as positive by the BACTECFX Continuous Monitoring blood culture system and confirmed to be positive by Gram stain for Gram-negative rods were enrolled in the study. If a mixed Gram stain was observed, the sample was ruled as ineligible for the study. All testing was performed within 12 hours of the blood culture being flagged as positive. LifeScale performance was evaluated by comparing MIC and interpretative results to our current standard of practice (SOC, MicroScan Walkaway 96 broth microdilution). The following metrics were assessed: Essential Agreement (EA), Category Agreement (CA; CLSI), Very Major (VMJ), Major Discrepancy (MAJ)), and Minor Discrepancies (MIN). **Results:** A total of 100 samples were tested that met criteria for LifeScale (intended) claimed species: E. coli (N:42), Klebsiella pneumoniae (N:25), Pseudomonas aeruginosa (N:16), Klebsiella oxytoca (N:7) Klebsiella aerogenes (N:2), and Acinetobacter spp. (N:8); 2 of 44 E. coli, 2 of 27 K. pneumoniae, and 3 of 19 P. aeruginosa were not evaluable due to insufficient growth, user or amplitude error. The average time to LifeScale results was 4 hr 30 min versus 36-40 hours for SOC generated results. Following resolution of AST results, there was 95.8% final agreement between LifeScale and Microscan WalkAway; 97.4% categorical agreement, no very major discrepancies, and 0.29% major discrepancies. **Conclusion:** The LifeScale system provided reliable results for AST for Gram negative organisms directly from positive blood cultures with minimal hands-on time (approximately < 8 minutes), allowing for more rapid antimicrobial management compared to standard methods. This is the first AST system that detects and measures the mass of each organism compared to traditional growth-based AST methods.

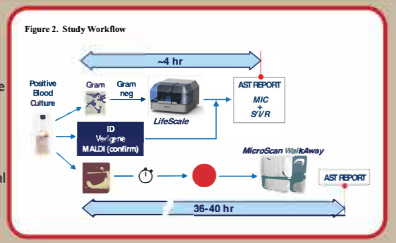
INTRODUCTION

Blood cultures are the gold standard for the detection and recovery of bacteria responsible for bacteremia and sepsis. Rapid antimicrobial susceptibility testing (AST) plays a significant role in the optimal management of patients, especially in the selection of appropriate antibiotics. Compounding this problem is the emergence of antimicrobial resistance (AMR) which has surpassed the discovery and availability of new antimicrobial agents. The average turnaround time to detect, recover, identify, and generate AST results is 48 – 96 hours. Of the emerging rapid phenotypic AST systems, only one, PhenoTest BC® (Accelerate Diagnostics, Tucson, AZ) has US FDA approval. The aim of this study was to evaluate the accuracy, time to results, ease-of-use, and potential impact on clinical outcomes of the LifeScale (Affinity Biosensors, Santa Barbara, CA) rapid AST system, whose U.S. clinical trial results are currently under review by the FDA. LifeScale employs a microfluidic sensor that detects and measures the mass of individual organisms at high throughput (Fig.1). Antimicrobial Susceptibility is determined by assessing growth in liquid cultures (proprietary cation adjusted Mueller-Hinton broth) after an incubation period. The technology is based on microchannel resonators, also known as individual microcantilevers, and assesses antibiotic activity via changes in mass of individual cells following passage through the microfluidics channels (Fig. 1). The system generates minimal inhibitory concentration (MIC) results for each antibiotic tested and generates interpretive (S/I/R) results based on current CLSI or FDA-defined breakpoints following the entry of final organism identification. The current platform is restricted to the testing of Gram-negative bacilli isolates identified to be included in the LifeScale database and blood cultures confirmed to be positive by Gram stain. Testing can be performed direct from a positive blood culture, which is the focus of this study. Results are available within 4 – 6 hours after inoculation of the LifeScale AST susceptibility plate.



MATERIALS & METHODS

Fresh blood cultures identified as positive by the BACTEXFX Continuous Monitoring System (Becton Dickinson, Sparks, Maryland) and confirmed positive by Gram stain for only Gram-negative rods, were eligible for the study. If the Gram stain revealed mixed morphotypes (polymicrobial), the sample was not included in the study. Per standard of care (SOC) in our laboratory, positive blood cultures were screened on the Luminex Verigene (Austin, TX), subcultured followed by confirmatory identification per standard of care (SOC) (MALDI-TOF, Bruker Daltonics; Billericia, MA) and antimicrobial susceptibility testing performed on the MicroScan Walkaway 96 (Beckman Coulter, Sacramento, CA), which served as the "gold standard" for comparing LifeScale. AST results (Fig 2). Repeat blood cultures from the same patient and contaminates were excluded from the study. We compared each individual bacteria AST combination to determine agreement to SOC and categorial agreement based on current CLSI 2022 M100 guidelines to produce categorical results (S/I/R) results. Errors in agreement were classified as very major errors (VME) or major errors (ME). Quality control was performed according to the LifeScale user manual and tested on the LifeScale AST platform each week of testing. QC organisms were measured on a rotating basis of the following strains: E. coli ATCC 25922, E. coli ATCC 35218, K. pneumoniae ATCC 700603, P. aeruginosa ATCC 27853, S. aureus ATCC 29213, and E. faecalis ATCC 29212.



RESULTS

1. 2 of 44 E. coli were not evaluable due to user error or amplitude error, 2 of 27 tested K. pneumoniae were not evaluable due to failure to grow or amplitude error, and 3 of 19 tested P. aeruginosa were not evaluable due to amplitude error **(Table 1)**.
2. Agreement was 90% or higher for 12 of 14 antibiotics tested, with categorical agreement exceeding 95% for all but one antibiotic (Piperacillin/Tazobactam), **(Table 2)**. When comparing LifeScale to the MicroScan Walkaway96, a total of 5 VMEs and 7 MEs occurred in 8 patient samples. Following resolution of these discordant results by the reference broth microdilution method, of 1,049 total susceptible strain-antimicrobial evaluations, 3 (0.29%) MEs were observed with LifeScale versus 5 MEs (0.48%) for the MicroScan Walkaway96, Of the 159 total resistant combinations, no VMEs were reported by LifeScale versus 2 VMEs (1.3%) for the MicroScan Walkaway96 **(Table 2)**.
3. Overall agreement and categorical agreement were >95% for 5 of 6 species and >90% for P. aeruginosa following resolution by broth microdilution **(Table 3)**.
4. Average time to results for the LifeScale AST system for positive blood culture specimens was 4 hours and 30 minutes **(Table 4)** versus 36 hours – 40 hours (time from detection of a positive blood culture, Gram stain, subculture, identification and AST test result generation with the MicroScan Walkaway96.
5. Organisms that were detected in positive blood cultures but are not in the LifeScale Database were: Brevundimonsas diminuta, Moraxella osloensis, Fusobacterium mortiferum, Bacteroides fragilis, Haemophilus parainfluenzae, Hungatella effluvii; one E. coli and one Klebsiella pneumoniae did not initially grow but grew on the second attempt.
6. A total of 16 polymicrobial cultures were encountered as determined by confirmatory MALDI results. Of these, LifeScale produced results for 11 polymicrobial cultures for which the Verigene identified a single organism reportable by LifeScale. Further work will assess agreement between these LifeScale results and the SOC ASTs for the individual organisms.

CONCLUSIONS

1. The results indicate that the LifeScale AST system, based on changes in microbial mass, provides rapid, reliable, curate AST results in less than five hours directly from positive blood cultures containing Gram negative organisms contained in the current data base; minimal hands-on time for processing specimens for LifeScale AST testing was less than 8 minutes.
2. This the first rapid AST system that detects and measures the mass of each organism compared to traditional growth-based AST methods.
3. There is a need to expand the LifeScale test menu to include more organisms and antibiotics.
4. Further work is needed to assess and address the challenges posed by polymicrobial blood cultures.
5. The LifeScale rapid AST system has the potential benefit of influencing and improving patient outcomes and promoting antimicrobial stewardship due to the rapid provision of AST results.

A specific and most important benefit would be limiting patient exposure to unnecessarily broad-spectrum antibiotics.

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ACKNOWLEDGMENTS

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Table 1. Evaluated Samples

Genus	Species	No. of Samples Evaluated
Acinetobacter	*species*	8
Escherichia	*coli*	42
Klebsiella	*aerogenes*	2
Klebsiella	*pneumoniae*	25
Klebsiella	*oxytoca*	7
Pseudomonas	*aeruginosa*	16
Total		100

Table 2. Performance of LifeScale compared to the MicroScan Walkaway 96 for antibiotics evaluated byboth systems

Antibiotic	Number Evaluated	% Agreement to SOC	% Categorical Agreement to SOC[a]	VMEs[b]	MEs[b]
All	1227	95.84%	97.39%	0.00% (0/159)	0.29% (3/1049)
Amikacin	100	100.00%	100.00%	0.00% (0/0)	0.00% (0/100)
Ampicillin	78	96.05%	100.00%	0.00% (0/55)	0.00% (0/21)
Aztreonam	92	94.57%	95.65%	0.00% (0/13)	1.33% (1/75)
Cefazolin	79	88.10%	96.05%	0.00% (0/21)	0.00% (0/50)
Cefepime	100	87.00%	98.00%	0.00% (0/13)	0.00% (0/85)
Ceftazidime	100	96.00%	97.00%	0.00% (0/13)	1.18% (1/85)
Ceftazidime/Avibactam	92	98.91%	98.91%		0.00% (0/92)
Ertapenem	83	100.00%	100.00%	0.00% (0/1)	0.00% (0/82)
Gentamicin	100	94.00%	100.00%	0.00% (0/4)	0.00% (0/90)
Levofloxacin	100	88.00%	88.00%	0.00% (0/13)	0.00% (0/86)
Meropenem	100	99.00%	99.00%		0.00% (0/100)
Mero/Vaborbactam	24	100.00%	100.00%		0.00% (0/24)
Piperacillin/Tazobactam	99	87.88%	87.88%	0.00% (0/1)	0.00% (0/95)
Trimethoprim/Sulfamethoxazole	83	94.05%	95.24%	0.00% (0/20)	1.56% (1/64)

[a] Adjusted error rates: categorical discrepancies not included with MICs are in agreement

[b] Very major errors (VMEs) and major errors (MEs) after resolution by reference broth microdilution

Table 3. Performance of LifeScale AST versus MicroScan Walkaway96 for genera and species evaluated by both systems.

Genus	Species	% Agreement[a]	% Categorical Agreement[a]
Acinetobacter	*species*	98.59% (70/71)	98.59% (70/71)
Escherichia	*coli*	97.45% (534/548)	98.36% (539/548)
Klebsiella	*aerogenes*	96.15% (25/26)	100.00% (26/26)
Klebsiella	*pneumoniae*	96.48% (329/341)	97.95% (334/341)
Klebsiella	*oxytoca*	97.94% (95/97)	98.97% (96/97)
Pseudomonas	*aeruginosa*	90.97% (131/144)	95.83% (138/144)
Total		96.50%	98.04%

[a] Agreement and categorical agreement rates reflect results after resolution by reference broth microdilution for strain-antimicrobial combinations resulting in VMEs and MEs

Table 4. Time to results for the *LifeScale* System

Genus	Species	Time to Result (h:mm)	No. Samples
Acinetobacter	*species*	4:38	8
Escherichia	*coli*	4:33	42
Klebsiella	*aerogenes*	4:27	2
Klebsiella	*oxytoca*	4:29	7
Klebsiella	*pneumoniae*	4:24	25
Pseudomonas	*aeruginosa*	4:27	16
Average (h:mm)		4:30	