RESOLUTION OF ANTIMICROBIAL SUSCEPTIBILITY RESULTS GENERATED WITH A RAPID FIVE HOUR METHOD INVOLVING POLYMICROBIAL BLOOD CULTURES

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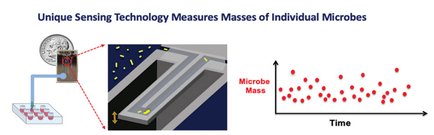
ABSTRACT

Background: We previously completed an evaluation of the LifeScale AST platform for generating antimicrobial susceptibility results within 4-6 hours directly from blood cultures containing Gram-negative microorganisms. Since most rapid AST systems are validated for monomicrobial samples, it is important to assess the impact that polymicrobial samples have on rapid AST results. The objective of this study was to compare LifeScale AST results to those of our SOC and reference microbroth dilution for polymicrobial samples. Methods: Fresh blood cultures detected as positive by the BACTEC FX system and confirmed by Gram stain to contain Gram-negative bacteria were tested with LifeScale with a panel of 14 antibiotics. Samples showing polymicrobial morphology by Gram stain were not tested. Species-level organism identification was performed directly from blood cultures with Verigene. Isolates were produced for each sample tested on LifeScale and mixed cultures were confirmed on subculture. Each isolate recovered on subculture was identified by Bruker MALDI-TOF and AST was performed on the MicroScan Walkaway. Categorical results (S/I/R) from LifeScale were compared to those generated by MicroScan. Very major and major discrepancies were resolved by reference microbroth dilution. Results: Sixteen polymicrobial samples not detected by Gram stain were tested on LifeScale and recovered in subculture. Verigene results for 4 samples revealed organisms not contained in the LifeScale database and AST results were not reported. Of the remaining 12 samples, 127 organism/drug combinations were available for comparison. Seven samples produced at least one VME or ME when LifeScale results were compared to SOC for the isolates identified by Verigene; four VMEs from three samples were resolved by reference BMD and none of these isolates were resistant, indicating agreement with the LifeScale results. Of the total 13 MEs from 4 samples, all were resolved when a resistant result was obtained by SOC data was unavailable for the remaining all were resolved when a resistant result was obtained by SOC or the reference BMD for at least one of the isolates from a polymicrobial culture. Conclusion: For all organism/antibiotic combinations, LifeScale showed a resistant result when either mixed isolate was resistant. If these results prove robust on further testing, this may justify the reporting of rapid LifeScale results without the need for separate confirmation.

INTRODUCTION

Rapid phenotypic antimicrobial susceptibility tests (AST) have, and continue to emerge, in efforts to provide rapid AST results directly from positive blood cultures. Our laboratory recently completed an evaluation of the LifeScale AST system (Affinity Biosensors, Santa Barbara, CA) by testing 100 patient samples. LifeScale employs a unique microfluidic sensor (Fig. 1) that measures the masses and number of individual bacteria at high throughput to determine susceptibility. The technology is based on a microchannel resonator comprising microcantilevers with an embedded microfluidic channel and assesses antibiotic activity via changes in the number and mass of individual cells following passage through microfluidics channels (Fig. 1). While testing directly from positive blood culture enables rapid results, it opens the possibility that the AST may unknowingly be performed on polymicrobial/mixed samples. Companion diagnostics (e.g., Gram stain) can screen most polymicrobial samples with the aim of isolating the constituents so they can be tested using the conventional standard of care (SOC). However, some polymicrobial samples may escape such a screen and the rapid AST assesses the mixed culture and reports results before the polymicrobial nature of the sample can be detected. This was the case for a few samples in our previous study (ID Week 2022; Abstract#1262539). In the current study, we analyzed these samples by comparing detailed analysis of the associated isolates. The results suggest how the rapid AST results for polymicrobial samples might impact patient care and whether mitigations are indicated.

Fig. 1. Concept of Microfluidic Sensors for Detection of Bacterial Mass

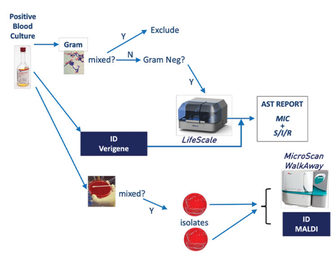


- *LifeScale's* key element is a microfluidic sensor with a mechanical resonator
- The masses of *individual* microbes are measured as they are carried through the sensor at high throughput
- Replication, biomass, and "population mass profiling" of individual microbes are all analyzed to produce a rapid, yet accurate, AST

MATERIALS & METHODS

Fresh blood cultures detected as positive by the BACTEC FX system (Becton Dickinson, Sparks, Md) and confirmed positive by Gram stain for only Gram-negative rods were tested with LifeScale using a panel of 14 antibiotics (Table 1). Workflow for detection and analysis of polymicrobial samples in shown in Figure 2. Samples showing polymicrobial morphology by Gram stain were not tested on the LifeScale. Species-level organism identification was performed directly from blood cultures with Verigene (Luminex Corp., Austin, TX) with the BC-GN assay. LifeScale reported MICs and interpretative results based on CLSI breakpoints, using the Verigene species-level identification as input. Isolates were produced for each sample run on LifeScale. Mixed cultures were detected by subculture. Each isolate recovered on subculture was identified by Bruker MALDI-TOF (Bruker Daltonics, Billericia, MA) and AST performed on the MicroScan Walkaway96 (Beckman Coulter, Sacramento, CA), as the vSOC. Categorical results (S/I/R) from LifeScale were compared to those generated by the MicroScan. Very major (VME) and major (ME) discrepancies were determined by comparing the LifeScale result to the SOC resulted generated for the mixed culture component identified by Verigene. For MEs, the LifeScale result was compared to the SOC result for the second isolate in the mixed culture and were considered resolved if agreement was found. For VMEs, reference microbroth dilution (BMD) was performed on both isolates (when available) and were considered resolved if the reference BMD results indicated they were susceptible.

Figure 2. Detection and Analysis of Polymicrobial Samples



RESULTS

1. Sixteen polymicrobial samples were not detected by Gram stain (Table 2). These were tested on LifeScale and recovered on subculture. Of these, Verigene identified four samples that are not contained in the LifeScale data base. Results were not reported for these samples.
2. For the remaining 12 polymicrobial samples, LifeScale reported results for 157 organism/drug combinations. SOC AST results were available for 127 of these combinations, a reduced number because of the absence of Meropenem/Vaborbactam on the SOC AST system.
3. As shown in Table 3, seven samples produced at least one VME or ME when LifeScale results were compared to SOC for the isolates identified by Verigene.
4. Thirteen MEs were encountered with four samples in which LifeScale reported a resistant result versus a susceptible one reported by SOC for the Verigene-identified isolate. For the seven MEs associated with samples 167 and 210 (Table 3), a resistant result was obtained from the SOC AST for the second isolate. For sample 251, reference BMD showed that the Verigene-identified sample was resistant. For 3 of the 5 MEs associated with sample 128, the SOC's expert rules yielded susceptible results while raw MICs were greater than the resistant breakpoint. In summary, 11 of the MEs, the LifeScale results were consistent with the most resistant isolates from each polymicrobial sample. The remaining 2 MEs could not be resolved because SOC results for the second isolate (Stenotrophomonas maltophilia) were not reported.
5. Four VMEs were encountered with 4 samples in which LifeScale reported a susceptible result while the SOC AST for the Verigene-identified isolate was resistant. Reference BMD showed that these isolates were categorically susceptible or intermediate, thus resolving the VMEs.

CONCLUSIONS

1. In this study, LifeScale reported results for 12 polymicrobial cultures not screened by Gram stain. The associated organism/antibiotic combinations represent 11.5% (157/1365) of the study total.
2. Of the organism/antibiotic combinations for which SOC data were available, 13% (17/128) of the combinations showed an initial error when comparing LifeScale results to SOC results for Verigene-identified isolates. All 4 VMEs were resolved by reference BMD which showed that the mixed culture constituents were susceptible, and the discrepancies were due to MEs of the SOC AST.
3. For organism/antibiotic combinations reported by LifeScale to be resistant, SOC and/or reference BMD results (when available) showed that at least one constituent was resistant. This indicates that the resistant constituent determined growth of the mixed culture on LifeScale.
4. If an AST is performed on a mixed culture that includes at least one resistant constituent, a resistant result carries the least risk with respect to patient treatment. If the trend observed in this study proves robust on further testing, this may justify the reporting of rapid LifeScale AST results for positive blood cultures without the need for separate confirmation that a sample is monomicrobial.
5. Additional screening, such as rapid identification methods that flag mixed cultures, could be incorporated into the workflow to reduce the number of mixed samples reported by rapid ASTs.

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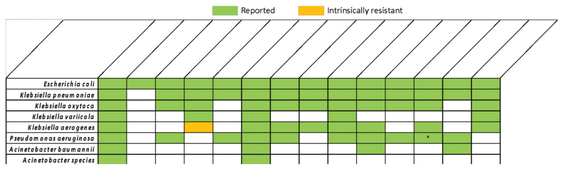
Table 1. LifeScale Reported Species and Antibiotics



All blank cells are off-label for the antibiotic

**There are currently no CLSI breakpoints for P. aeruginosa - Meropenem/Vaborbactam*

Table 2. Polymicrobial Samples Tested on LifeScale

	ISOLATE 1		ISOLATE 2	
LifeScale Results Available	*Acinetobacter*	*lwoffii*	*Corynebacterium*	*striatum*
	**Acinetobacter*	*lwoffii*	*Acinetobacter*	*variabilis*
	Acinetobacter	*variabilis*	*Enterobacter*	*cloacae*
	Escherichia	*coli*	*Escherichia*	*coli*
	Escherichia	*coli*	*Proteus*	*mirabilis*
	Escherichia	*coli*	*Enterobacter*	*bugandensis*
	Klebsiella	*pneumoniae*	*Acetobacter*	*orientalis*
	Klebsiella	*pneumoniae*	*Staphylococcus*	*capitis*
	Klebsiella	*oxytoca*	*Enterobacter*	
	Klebsiella	*pneumoniae*	*Stenotrophomonas*	*maltophilia*
	Pseudomonas	*aeruginosa*	*Enterococcus*	*faecalis*
	Pseudomonas	*aeruginosa*	*Klebsiella*	*pneumoniae*
	Pseudomonas	*aeruginosa*		
Not Reported by LifeScale	*Bacteroides*	*fragilis*	*Escherichia*	*coli*
	Enterobacter	*cloacae*	*Proteus*	*mirabilis*
	Stenotrophomonas	*maltophilia*	*Staphylococcus*	*edidermidis*
	Panotea	*agglomerans*	*Pseudomonas*	*putida*

**A third component of mixed culture was detected (a second A. variabilis)*

Table 3. Errors *via* Comparison of *LifeScale* to SOC AST for Isolate Identified by Verigene, and Resolution Comparing to SOC and Reference BMD.

Sample	Organism	ME	VME	# Resolved	Antibiotics	SOC 1	SOC 2	Ref BMD
LV-100167	*E. coli*	6	0	6	Ampicillin, Aztreonam, Cefazolin, Cefepime, Gentamicin, Ceftazidime	x	x	
LV-100128	*P. aeruginosa*	5	0	3*	Aztreonam, Ceftazidime/avibactam, Cefepime, Ceftazidime, Piperacillin/tazobactam	x	3 of 5	
LV-100210	*K. pneumoniae*	1	0	1	Cefazolin	x	x	
LV-100251	*K. oxytoca*	1	0	1	Cefazolin	x		x
LV-100214	*P. aeruginosa*	0	2	2	Aztreonam, Cefepime	x	x	x
LV-100170	*E. coli*	0	1	1	Piperacillin/tazobactam	x	x	x
LV-100211	*A. variabilis*	0	1	1	Trimethoprim/Sulfa	x	x	x
	TOTAL	**13**	**4**	**15***				

"x" signifies that data were available

**Two MEs could not be resolved due to lack of SOC data for 2nd isolate (Stenotrophomonas maltophilia)*