

Rapid Antifungal Susceptibility Testing Directly from Positive Blood Culture

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Abstract

In 2022, the World Health Organization (WHO) released its inaugural Fungal Priority Pathogens List, designating several *Candida* species as critical or high priority due to their significant threat to public health. *Candida* bloodstream infections (candidemia) carry high morbidity and mortality, underscoring the urgent need for rapid and accurate antifungal susceptibility testing (AFST). However, conventional AFST methods typically require 2 to 10 days from blood culture positivity, delaying effective clinical decision-making.

In this study, we evaluated the LifeScale AST system, an FDA-cleared platform for antibiotic susceptibility testing of Gram-negative bacteria directly from positive blood cultures, for its potential use in rapid AFST of *Candida* species. A total of 14 *Candida* isolates were used to prepare contrived blood cultures. Once the cultures flagged positive, aliquots were tested directly on LifeScale or were subcultured to obtain pure isolates, which were then tested using reference broth microdilution (BMD). BMD tests yielded results in 24-48 hours (mean ~57 hours) from blood culture positivity. By contrast, the LifeScale system produced AFST results in 9 to 25 hours (mean 9.1 hours), achieving an overall essential agreement of >90% compared to reference BMD.

These data demonstrate that LifeScale can rapidly and accurately identify antifungal susceptibility of *Candida* directly from blood cultures, shortening the turnaround time by over 40 hours relative to standard methods. This faster diagnostic approach has the potential to significantly improve patient outcomes by expediting targeted therapy and aiding in efforts to combat the growing threat of antifungal resistance.

Introduction

Fungal infections represent an escalating global health challenge, with rising prevalence and antifungal resistance significantly complicating patient management. The World Health Organization (WHO) has identified *Candida* bloodstream infections (candidemia) as high risk in its Fungal Priority Pathogens List due to their elevated infection and mortality rates. This global increase underscores the urgent need for improved diagnostic strategies.

Antifungal susceptibility testing (AFST) is crucial for guiding targeted treatment. However, conventional AFST methods require 2–10 days after blood culture positivity to deliver results, delaying critical treatment decisions. With limited therapeutic options, clinicians often initiate treatment empirically, increasing the risk of inappropriate antifungal use. The absence of rapid and sensitive diagnostic tools further complicates clinical decision-making and may accelerate antifungal resistance.

Given the high mortality associated with candidemia and the growing rates of resistance, there is a pressing need for a rapid, accurate AFST that facilitates timely, targeted antifungal therapy. This study evaluates the feasibility of a novel rAFST method direct from blood cultures using LifeScale technology, engineered to significantly reduce turnaround times. By shortening the time to actionable results, this approach has the potential to transform the management of invasive fungal infections and strengthen antimicrobial stewardship efforts.

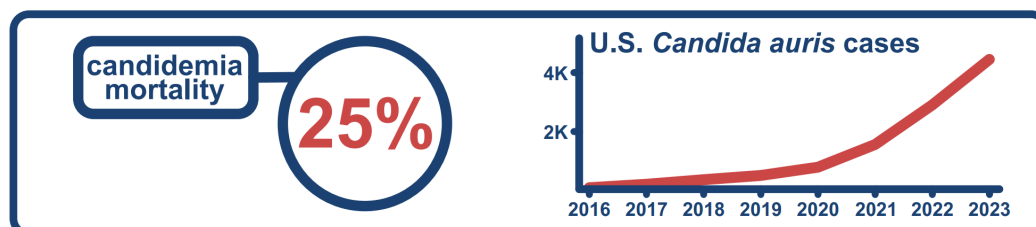


Figure 1, The LifeScale AST system utilizes population profiling to generate MIC results in under 5 hours

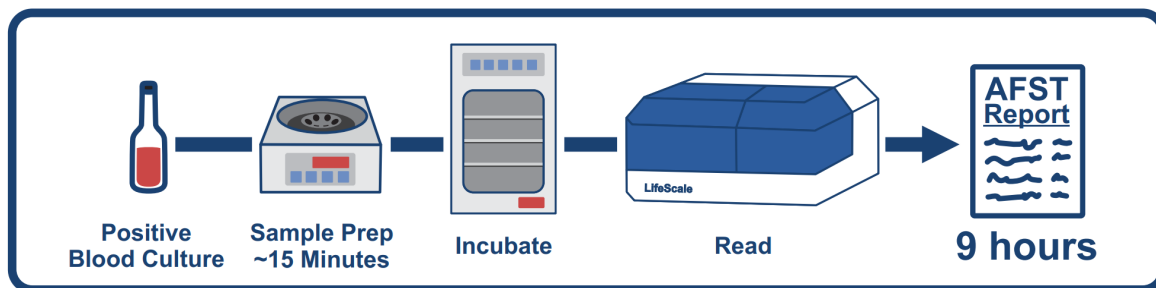


Figure 2, Challenge organisms and antifungal panel

16 *Candida* isolates were evaluated for antifungal susceptibility using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method. The isolates were tested against nine antifungal agents:

1. Amphotericin B AMB
2. Caspofungin CAS
3. Fluconazole FLZ
4. Ibrexafungerp IBX
5. Isavuconazole ISA
6. Micafungin MCF
7. Posaconazole POS
8. Rezafungin RZF
9. Voriconazole VRC

In parallel, these isolates were used to generate contrived MIC values from both methods were compared. Essential agreement was defined as an MIC within two doubling dilutions of the BMD result, while categorical agreement was assessed according to CLSI-established breakpoints.

Species	AMB	CAS	FLZ	IBX	ISA	MCF	POS	RZF	VRC
<i>Candida albicans</i>	0.5	0.03	2	0.016	≤0.008	≤0.008	0.03	≤0.008	0.12
<i>Candida albicans</i>	0.5	0.03	16	0.016	2	≤0.008	0.5	≤0.008	1
<i>Candida albicans</i>	1	≤0.016	0.5	0.016	≤0.008	≤0.008	0.016	≤0.008	≤0.008
<i>Candida auris</i>	2	0.06	256	0.12	0.5	0.12	0.12	0.06	2
<i>Candida auris</i>	2	0.5	256	0.5	0.5	0.12	0.12	0.25	1
<i>Candida auris</i>	2	4	64	0.5	0.03	0.12	0.03	0.5	0.25
<i>Candida glabrata</i>	2	0.5	64	1	2	0.12	1	1	2
<i>Candida glabrata</i>	2	0.5	64	0.25	4	0.12	2	0.06	4
<i>Candida glabrata</i>	1	0.5	128	0.12	>8.0	1	2	0.5	4
<i>Candida guilliermondii</i>	1	0.06	≤0.25	0.5	≤0.008	0.25	0.06	1	≤0.008
<i>Candida krusei</i>	2	0.25	128	0.5	1	0.06	0.25	0.03	1
<i>Candida parapsilosis</i>	2	0.25	≤0.25	0.25	≤0.008	1	0.06	1	≤0.008
<i>Candida parapsilosis</i>	0.5	0.5	≤0.25	0.25	≤0.008	1	0.03	1	0.016
<i>Candida tropicalis</i>	1	2	0.5	4	0.016	0.25	0.06	0.25	0.03
<i>Candida tropicalis</i>	0.5	≤0.016	1	0.016	0.06	≤0.008	0.03	≤0.008	0.03
<i>Candida tropicalis</i>	0.5	≤0.016	0.5	0.016	0.016	≤0.008	0.03	≤0.008	0.03

Susceptible	Non-Susceptible/I/SDD	Resistant
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Figure 3, Challenge organisms and antifungal panel

Results

A panel of 16 *Candida* isolates was evaluated, representing seven species—including three isolates of *Candida auris* and encompassing ten resistant phenotypes as determined by the reference BMD method.

This diverse collection of isolates, selected to cover a broad range of minimal inhibitory concentration (MIC) values, provided a robust challenge for comparing the performance of both antifungal susceptibility testing approaches.

From a positive blood culture, the standard BMD method required an average of **57 hours** to produce final results, with some isolates taking up to 96 hours. In contrast, the LifeScale rAFST method delivered results in an average of **9.1 hours**, representing a dramatic reduction in turnaround time. This significant improvement in time to result could enable earlier clinical intervention and more timely adjustments to antifungal therapy.

Performance metrics further support the utility of LifeScale rAFST. Across all antifungal agents tested, the overall essential agreement **EA was 94.3%** and the overall categorical agreement **CA was 94.8%**. Major errors MAJ occurred at a rate of 2.1%, with no very major errors VMJ observed. For individual agents, EA ranged from 87.5% to 100.0%, while CA ranged from 88.9% to 100.0% where breakpoints were available.

57 Hours



Standard

9.1 Hours



LifeScale

Antifungal	EA	CA	MAJ	VMJ
Amphotericin B	87.5%			
Caspofungin	93.8%	100.0%	0.0%	0/4
Fluconazole	100.0%	90.9%	0.0%	0/4
Ibrexafungerp	93.8%			
Isavuconazole	93.8%			
Micafungin	93.8%	100.0%	0.0%	0/1
Posaconazole	87.5%			
Rezafungin	100.0%	93.3%	7.1%	
Voriconazole	100.0%	88.9%	0.0%	0/1
Overall	94.3%	94.8%	2.1%	0/10

Figure 4, Time to Result performance

Conclusions

This feasibility study of the LifeScale rAFST system indicates that a rapid testing approach can markedly enhance clinical decision-making in the management of invasive fungal infections. By delivering same-day antifungal susceptibility results, rather than the ≥ 48 hour turn-around required by reference methods, rAFST equips clinicians to initiate or escalate targeted therapy during the crucial early window when outcomes are most sensitive to appropriate drug selection.

Beyond shortening time-to-result, the direct-from-blood-culture workflow eliminates subculture steps, conserving laboratory resources and reducing the risk of pre-analytic error. In turn, faster, evidence-based adjustments to therapy can limit unnecessary empiric broad-spectrum antifungal use, curb drug-related toxicity, and slow the emergence of resistance.

References

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