

# Rapid antifungal susceptibility testing direct from positive blood culture

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## Abstract

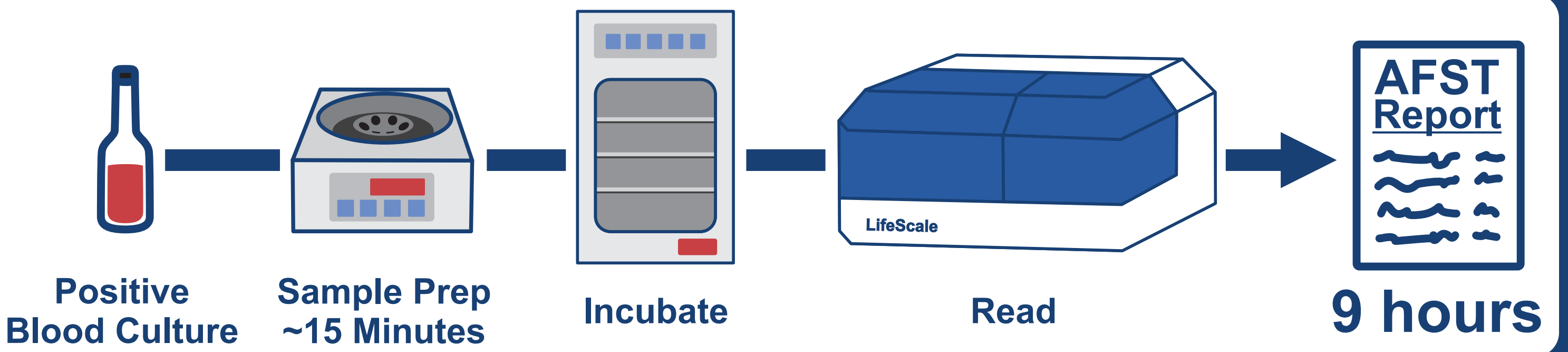
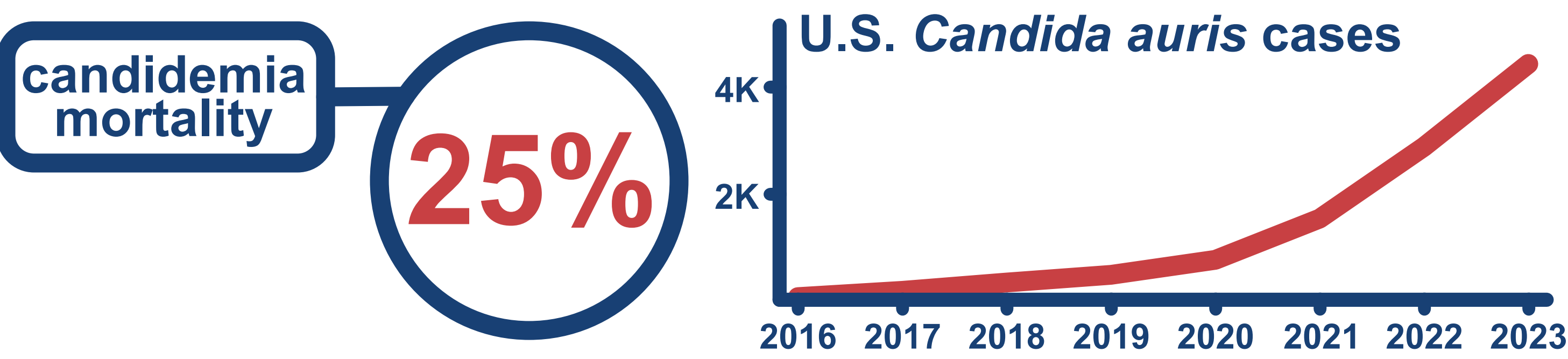
**Background:** In 2022, the World Health Organization (WHO) released its first fungal priority pathogens list, classifying many *Candida* species as critical or high priority based on public health risk. The CDC reports that the in-hospital all-cause mortality rate for candidemia exceeds 25%. The typical time to obtain antifungal susceptibility testing (AFST) results is 3 days for on-site testing and 7-10 days for off-site testing, underscoring the need for rapid and accurate tests. In this study, we demonstrate a proof-of-principle of an accurate rapid AFST performed directly from positive blood culture.

**Methods:** Contrived blood cultures were generated from 16 isolates of *Candida* species. Each culture was processed using two methods in parallel. To mimic the clinical standard of care, isolates were derived from solid media culture and tested using reference broth microdilution. For rapid testing, blood cultures were tested directly using the LifeScale rAFST System.

Minimal inhibitory concentration (MIC) discrepancy, time to result, and total workflow turnaround time were compared between the standard method and LifeScale technology.

**Results:** The mock standard method provided AFST results >48 hours following blood culture positivity, while LifeScale delivered results in ~9 hours. LifeScale results had an overall essential agreement of >90% compared to reference.

**Conclusions:** The LifeScale rAFST is a promising technology to accurately diagnose fungal infections significantly faster than current methods. Further clinical studies are warranted to evaluate the impact of LifeScale's rapid diagnostic capabilities on patient mortality, treatment efficacy, and the development of antifungal resistance. The potential to deliver AFST results direct from blood culture marks an exciting step forward in improving patient outcomes and combating fungal 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.

## 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.

## Challenge organisms and antifungal panel

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			

## Methods

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: Amphotericin B **AMB**, Caspofungin **CAS**, Fluconazole **FLZ**, Ibrexafungerp **IBX**, Isavuconazole **ISA**, Micafungin **MCF**, Posaconazole **POS**, Rezafungin **RZF**, and Voriconazole **VRC**.

In parallel, these isolates were used to generate contrived blood cultures that were monitored continuously until positivity. Once positivity was confirmed, samples were processed and run on the LifeScale rAFST platform according to the manufacturer's instructions.

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.

## Time-to-result and performance

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

## Conclusion

This feasibility study of the LifeScale rAFST system indicates that a rapid testing approach has the potential to significantly enhance clinical decision-making in the management of invasive fungal infections. By delivering same-day antifungal susceptibility results—as opposed to the two days or more required by current methods—rapid technologies can enable more timely, targeted interventions.

Overall, the streamlined workflow of the LifeScale rAFST system supports earlier treatment adjustments, representing a meaningful advancement in patient care and antifungal stewardship.

## References

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