Rapid identification of yeast species by the PCR- and microarray-based Prove-it™ Sepsis

Anne Aittakorpi*, Pentti Kuusela⁺, Pirkko Koukila-Kähkölä⁺, Martti Vaara⁺, Michael Petrou^, Vanya Gant°, Minna Mäki*

* Mobidiag Ltd, Finland, ⁺Division of Clinical Microbiology, Department of Bacteriology, HUSLAB, Helsinki University Hospital, Finland, ^Hammersmith Hospital, Imperial Healthcare NHS Trust, UK, ° Department of Clinical Microbiology, University College London Hospitals NHS Foundation Trust, UK

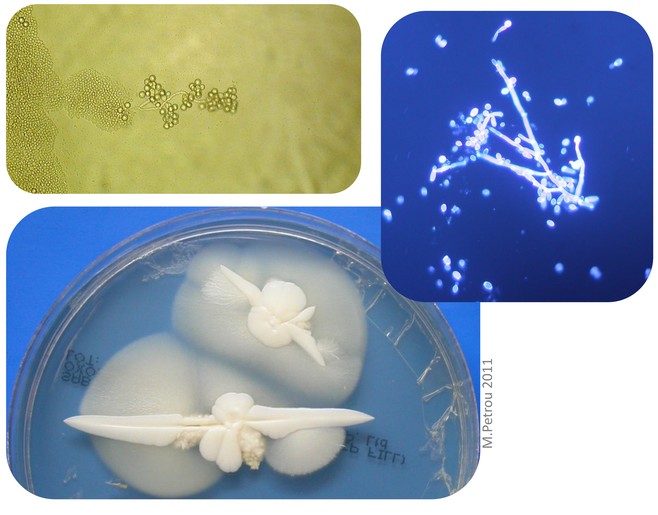
INTRODUCTION

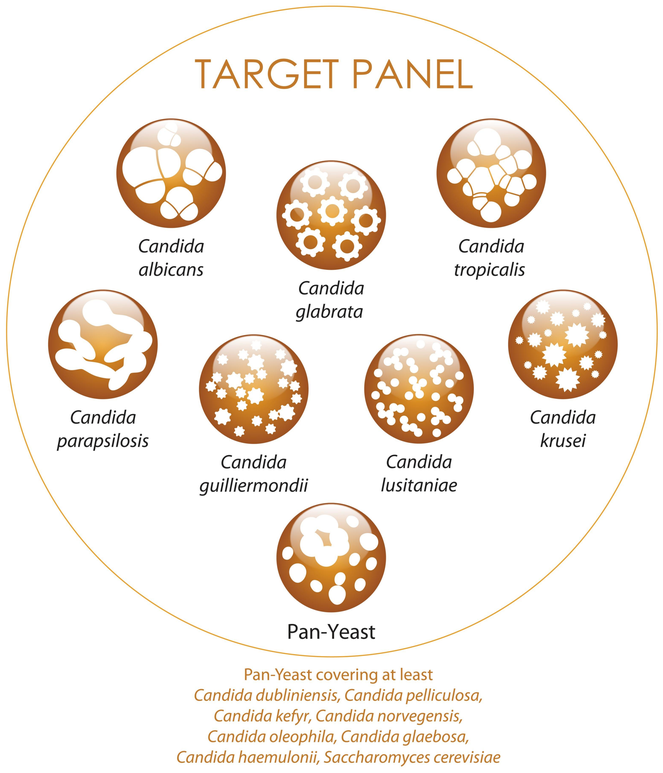
Prove-it™ Sepsis is a rapid PCR- and microarray-based assay platform with proven excellent diagnostic performance for most bacterial pathogens causing sepsis[1]. We have extended this platform's diagnostic range to include 15 yeast species and evaluated its performance against a large number of fungal isolates.

[1] Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, Aittakorpi A, Laakso S, Lindfors M, Piiparinen H, Mäki M, Carder C, Huggett J, Gant V. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study (2010). The Lancet. Jan 16;375(9710):224-230.

METHODS

159 classically speciated (Germ tube, growth on Corn Meal Tween 80, API 20 AUX and API 32C as well as molecular when all failed) clinical fungal isolates were tested. The isolates were cultured on Sabouraud dextrose agar with penicillin for 48 h aerobically at 35 °C and blindly tested using the Prove-it™ Sepsis assay after DNA was extracted with easyMAG (bioMérieux). Original routine identifications of the clinical samples were revealed after the analysis.





CONCLUSIONS

The Prove-it™ Sepsis array platform was modified and extended to detect almost all clinically relevant fungi. According to this study, array's reliability and specificity reached high level and confirmed the quality of the test. As previously reported Prove-it™ Sepsis test for over 60 bacterial species, the addition of rapid and accurate yeast identification to this diagnostic platform will now allow faster, more evidence-based choice of antifungal agent and better patient outcomes.

RESULTS

151 out of 159 samples yielded positive microarray-based results, while 8 samples remained negative. The microarray correctly identified 90% of the samples at species level (143/159) as shown in the *Table 1*. 9% of the organisms was correctly assigned to the pan-yeast group, designed to identify *C. kefyr, C. haemulonii, C. norvegensis, C. dubliniensis, C. oleophila*, *C. glaebosa* and *Saccharomyces cerevisiae*. *Cryptococcus albidus, C. neoformans, Trichosporon asahii, T. mycotoxinivorans, T. mucoides*, and *T. inkin* were not identified by this system. Identification discrepancies were noted at 5% (8/159) of the samples, and they were further studied as shown in *Table 2*. More than one target was identified from three discrepant samples, which may be due to culturing contamination because two different colony morphologies were observed already on the plates. Other discrepancies were sequenced, and sequencing results verified the Prove-it™ result.

Table 1. Prevalence of organisms

Target	%
Candida albicans	30
Candida glabrata	20
Candida parapsilosis	14
Candida tropicalis	9
Candida krusei	4
Candida guilliermondii	3
Candida lusitaniae	1
Pan Yeast	9
Negative	5
Discrepancies	5
	100

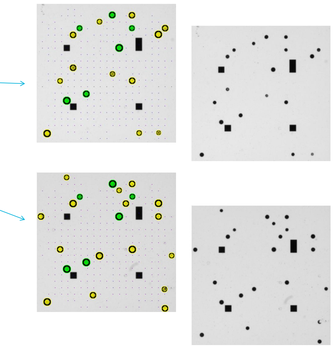


Table 2. Analysis of discrepant results

Culturing	Prove-it result	Comments / sequence result
C.lusitaniae	Pan Yeast	*Candida oleophila*, NCBI 100 %
C.albicans	Pan Yeast	*C.albicans/C.dubliniensis*, NCBI100%
C.albicans	Pan Yeast	*Candida glaebosa*, NCBI100 %
T.mucoides	Pan Yeast	*C.dubliniensis*, NCBI 100 %
C.kefyr	*C.guilliermondii*	*C.guilliermondii,* NCBI 100 %
C.guilliermondii	*C.guilliermondii* + *C.lusitaniae*	Two different colony morphologies on the plate
C.krusei	*C.krusei* + *C.parapsilosis*	Two different colony morphologies on the plate
C.tropicalis	*C.tropicalis* + *C.glabrata*	Two different colony morphologies on the plate

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