Evaluation of PNA-FISH Yeast Traffic Light for Rapid Identification of Yeast Directly from Positive Blood Cultures and Assessment of Clinical Impact

N. R. H. Stone, R. L. Gorton, K. Barker, P. Ramnarain, C. C. Kibbler

Department of Microbiology, Royal Free Hospital, London, United Kingdom

The PNA-FISH Yeast Traffic Light assay was performed on 54 clinical isolates of yeasts inoculated into blood culture bottles. The assay showed high sensitivity (*Candida albicans*/*C. parapsilosis*, 100%; *C. glabrata*/*C. krusei*, 92.3%; *C. tropicalis*, 100%) and specificity (*C. albicans*/*C. parapsilosis*, 100%; *C. glabrata*/*C. krusei*, 94.8%; *C. tropicalis*, 100%). Case note review estimated a change in therapy in 29% of cases had the PNA-FISH result been available to the clinician.

Candidemia is associated with a mortality of up to 40% (1) and requires early and appropriate antifungal therapy (2, 3). Current diagnostic methods for identifying the species causing candidemia are limited by their relatively long turnaround time, which can lead to a delay in appropriate therapy. The PNA-FISH (peptide nucleic acid fluorescent *in situ* hybridization) Yeast Traffic Light assay (AdvanDx, Woburn, MA) is capable of distinguishing the five commonest *Candida* species directly from blood cultures within 90 min. The method employs fluorescently labeled probes to complement species-specific rRNA sequences (4). *Candida albicans* and *C. parapsilosis* fluoresce green, *C. tropicalis* fluoresces yellow, and *C. glabrata* and *C. krusei* produce red fluorescence, when viewed under a fluorescence microscope. The assay cannot distinguish between *C. albicans* and *C. parapsilosis* or between *C. glabrata* and *C. krusei*. Other species do not produce any characteristic fluorescence. This study evaluates the sensitivity, specificity, turnaround time, and potential clinical impact of the assay.

Fifty-four yeast isolates, recovered from 52 different patients' blood cultures, were used. Two clinical specimens contained two different yeast species (Table 1). All had been identified to species level using AuxaColor 2 (Bio-Rad Laboratories, Hercules, CA).

A suspension of each isolate of a 0.5 McFarland standard was made in sterile distilled water. One hundred microliters of the suspension was diluted into 8 ml of water to make suspension B. Ten microliters of suspension B, corresponding to 60 to 100 CFU, was inoculated into aerobic and anaerobic bottles of negative blood cultures (which had previously been incubated for 5 days before being declared negative) and placed onto the Bactec 9000 blood culture system (BD, Oxford, United Kingdom). When cultures were flagged as positive, PNA-FISH was performed by a researcher blinded to the identity of the yeast.

The PNA-FISH Yeast Traffic Light assay was performed according to the manufacturer's instructions. This involved heat fixing a drop of blood on a glass slide with fixation solution, followed by addition of a drop of the PNA-FISH probe and incubation for 30 min at 55°C ± 1°C. The slide was then immersed in wash solution for 30 min at 55°C ± 1°C before being viewed under a fluorescence microscope. A control slide was used each time that the assay was performed. The case notes for the patients from whom the yeasts had been isolated were retrospectively reviewed. The predicted choice of antifungal therapy was estimated on the basis that identifying *C. albicans*, *C. parapsilosis*, or *C. tropicalis*

would have resulted in fluconazole therapy and that identifying *C. glabrata* or *C. krusei* would have led to the use of caspofungin as initial therapy.

Specificity and sensitivity were determined for each probe. A true positive was defined as green fluorescence in the presence of *C. albicans* or *C. parapsilosis*, yellow fluorescence in the presence of *C. tropicalis*, and red fluorescence with *C. krusei* or *C. glabrata*. A true negative was defined as the absence of fluorescence of each probe when the target yeast was not present. The gold standard against which the probe was compared was the AuxaColor 2 assay.

Results by probe are summarized in Table 2. The sensitivity for the *C. albicans*/*C. parapsilosis* probe was 100% (28/28), that for the *C. glabrata*/*C. krusei* probe was 92.3% (12/13), and that for the *C. tropicalis* probe was 100% (6/6). The specificity for the *C. albicans*/*C. parapsilosis* probe was 100% (24/24), that for the *C. glabrata*/*C. krusei* probe was 94.8% (37/39), and that for the *C. tropicalis* probe was 100% (46/46). Two false positives for the *C. glabrata*/*C. krusei* (red) probe were observed—one with *Cryptococcus neoformans* and the other with *Blastoschizomyces capitatus*. One *C. glabrata* isolate was missed in a mixed culture. The average turnaround time for the PNA-FISH assay was 90 min. Case note review suggested that had the PNA-FISH result been available to the clinician, there would have been no change in antifungal therapy in 32 cases (61.5%), a switch from caspofungin to fluconazole in 11 cases (21.2%), and a switch from fluconazole to caspofungin in 4 cases (7.7%). In 5 of the 52 cases, there was insufficient documentation to make an assessment.

The assay was found to be highly sensitive and specific and also rapid. However, the two false positives observed with the red probe were of concern. One isolate was in fact *Cryptococcus neoformans*, and the other was *Blastoschizomyces capitatus*. This may be explained by operator factors, with background debris reported as fluorescence. Repeat assays of the discrepant results showed no fluorescence. This also highlights the limitation of the assay to the

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Address correspondence to N. R. H. Stone, neil.stone@nhs.net.

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TABLE 1 Number and species of isolates used

Yeast species	No. of isolates
<i>Candida albicans</i>	18
<i>Candida parapsilosis</i>	10
<i>Candida glabrata</i>	9
<i>Candida tropicalis</i>	6
<i>Candida krusei</i>	4
<i>Candida guilliermondii</i>	2
<i>Candida lusitanae</i>	1
<i>Candida pelliculosa</i>	1
<i>Cryptococcus neoformans</i>	2
<i>Blastoschizomyces capitatus</i>	1
Total	54

detection of five yeast species detected by the probe. Although they encompass the most commonly encountered *Candida* species, the inability to identify *Cryptococcus* is a weakness. However, other, readily available diagnostic techniques, such as cryptococcal antigen, combined with a suggestive clinical history, usually make the identification of *Cryptococcus* species relatively straightforward.

The cost of the assay currently limits its adoption in routine clinical practice in many health care settings, although this could be offset by the cost savings of reducing use of echinocandins. However, newer methods such as matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis may in future provide an advantage over PNA-FISH in terms of time and cost in the diagnosis of candidemia, although methods of extracting yeast from blood cultures for MALDI-TOF analysis are undergoing refinement (5, 6).

This study provides a clinical context, unlike the only other published evaluation of the Yeast Traffic Light assay (7). Additionally, the yeasts used for the study were genuine clinical isolates which had been recovered from patients with candidemia rather than reference strains. The study is limited, however, as the clinical impact assessment was based on a retrospective review of clinical notes. Documentation of when or which antifungal was prescribed (and given) was not always clear, and the information for some patients was not available. Additionally, the number of isolates tested was small, particularly those of species that the assay claims not to be able to identify ($n = 7$). Where possible, PNA-FISH was performed as soon as the blood cultures flagged positive; however, in some cases the positive-blood-culture bottles were placed in refrigeration for up to 48 h and batched before probing, in which cases it is possible that the yeasts would continue growing in the blood culture bottles, and this may have affected the sensitivity results. Additionally, there may have been material in the negative blood cultures used, such as antifungal drugs, which may have interfered with the growth of yeast.

In conclusion, this study finds the assay to be highly sensitive and specific, with a short turnaround time. The cost of the assay

TABLE 2 Results summary by probe

Routine identification (AuxaColor 2)	No. of isolates by probe				Negative (no fluorescence)
	Total	<i>C. albicans/</i> <i>C. parapsilosis</i> (green)	<i>C. tropicalis</i> (yellow)	<i>C. glabrata/</i> <i>C. krusei</i> (red)	
<i>C. albicans</i>	17	17			
<i>C. parapsilosis</i>	10	10			
<i>C. tropicalis</i>	4		4		
<i>C. krusei</i>	4			4	
<i>C. glabrata</i>	8			8	
<i>C. glabrata</i> + <i>C. tropicalis</i>	1		1		
<i>C. albicans</i> + <i>C. tropicalis</i>	1	1	1		
<i>C. guilliermondii</i>	2				2
<i>C. lusitanae</i>	1				1
<i>C. pelliculosa</i>	1				1
<i>B. capitatus</i>	1			1	
<i>C. neoformans</i>	2			1	1

remains relatively high, but the assay could lead to cost savings from reduction in the use of echinocandins and shorter hospital stays. Favorable outcomes in prospective, randomized trials could lead to more widespread adoption of this assay in routine clinical laboratories.

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