Multicenter Evaluation of the *Candida albicans*/*Candida glabrata* Peptide Nucleic Acid Fluorescent In Situ Hybridization Method for Simultaneous Dual-Color Identification of *C. albicans* and *C. glabrata* Directly from Blood Culture Bottles[▽]

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We evaluated the performance of the *Candida albicans*/*Candida glabrata* peptide nucleic acid fluorescent in situ hybridization (PNA FISH) method, a rapid two-color assay for detection of *C. albicans* and *C. glabrata*, in a multicenter study. The assay is designed for use directly from positive blood culture bottles in a FISH format. Intact, fixed cells are labeled fluorescent green (*C. albicans*) or fluorescent red (*C. glabrata*) by rRNA hybridization of fluorophore-labeled PNA probes. Results are available <3 h after cultures signal positive. An evaluation of 197 routine blood culture bottles newly positive for yeast by Gram staining was performed at five hospitals. The sensitivities of detection for *C. albicans*, and *C. glabrata* were 98.7% (78/79) and 100% (37/37), respectively, and the specificity for both components of the assay was 100% (82/82). The assay was also evaluated with 70 fungal reference strains and was challenged in the BacT/ALERT microbiological detection system with spiked blood culture bottles. These results support the use of the assay for rapid, simultaneous identification of *C. albicans* and *C. glabrata* in positive blood culture bottles. This rapid assay may aid in the selection of initial antifungal drugs, leading to improved patient outcomes.

Candida species are the fourth most commonly isolated organisms from nosocomial bloodstream infections (9%), with the highest crude mortality rate (39.2%) (23). The most prevalent of the *Candida* species isolated from blood cultures are *C. albicans* and *C. glabrata*, accounting for up to two-thirds of all blood cultures positive for yeast (9, 16).

Clinical laboratories use a battery of phenotypic, manual, and automated tests to identify yeast isolates to the species level (2). Once the isolate has been detected and subsequently recovered from blood culture bottles onto solid media, there are rapid screening tests for presumptive identification of *C. albicans* (e.g., germ tube and chromo-enzyme assays) and *C. glabrata* (e.g., rapid trehalose) that provide data within a few hours. Colony color and texture on CHROMagar *Candida* can provide presumptive identification for *C. albicans*, *C. tropicalis*, and *C. krusei* within 24 to 48 h. However, carbohydrate assimilation or fermentation patterns, morphology studies, and en-

zyme tests such as those for urease or phenoloxidase can require 24 to 72 h for final identification to species level. These current methods for species determination of *Candida* have been found to be inaccurate in 4.6% of cases, with *C. glabrata* the most frequently misidentified species (9). Where traditional phenotypic methods fail, home-brew molecular methods such as PCR and large-subunit ribosomal DNA sequencing are sometimes required to determine species.

For species identification to have a major impact on decisions regarding antifungals and infection control interventions, the time to identification in the clinical laboratory is critical. Though the susceptibility of a given *Candida* species to a particular drug can vary among isolates, Infectious Disease Society of America guidelines recommend selection of initial antifungal therapy based on species identification (14). Although the guidelines state that fluconazole, amphotericin B, or caspofungin may be used as first-line therapy for *C. albicans*, clinical practice favors the use of fluconazole as the drug of choice. Infections with *C. glabrata* are more of a therapeutic challenge, inasmuch as *C. glabrata* may have reduced susceptibility to fluconazole. There is also the suggestion that resistance to amphotericin B and caspofungin are emerging (5).

Unfortunately, since laboratory identification can take sev-

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eral days by conventional methods, physicians often choose broad-spectrum antifungal therapy as initial treatment for all yeast-positive blood cultures, since delayed therapeutic intervention has been demonstrated to be associated with a poorer outcome (8). The *C. albicans* peptide nucleic acid fluorescent in situ hybridization (PNA FISH) assay was first described in 2002 (19) and later was cleared by the FDA as an in vitro diagnostic kit for identification of yeast directly from positive blood cultures. The assay uses PNA probes targeting *C. albicans*-specific rRNA in a standardized FISH assay format. PNA is a nucleic acid mimic whose hybridization properties make it particularly well-suited for FISH assays (20). *C. albicans* PNA FISH has been shown to have very high sensitivity and specificity (12, 19, 22); the cost-benefit advantage of the assay has been established, with demonstrated savings accrued through avoidance of unnecessary echinocandin therapy (3, 7). *C. albicans/C. glabrata* PNA FISH (AdvanDx, Inc., Woburn, MA), described here, is a novel second-generation in vitro diagnostic test for identifying *C. albicans* and *C. glabrata* from blood culture bottles that have signaled positive and demonstrate yeast on Gram staining. The test differentiates the two *Candida* species by indicating green fluorescence for *C. albicans* and red fluorescence for *C. glabrata*. This study evaluated the use of the PNA FISH assay for accurate real-time identification of *C. albicans* and *C. glabrata* from blood cultures newly positive for yeast.

MATERIALS AND METHODS

Reference strains. Nine *C. albicans* and four *C. glabrata* reference strains (including type strains of both species), along with 57 other reference strains representing 31 phylogenetically related or clinically prevalent fungal species and subspecies, were included in the study. Most strains were obtained from the Agricultural Research Service Culture Collection (NRRL), Peoria, IL; the American Type Culture Collection (ATCC), Manassas, VA; and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Strain identities are given in Table 1. Strains were grown overnight at 37°C on YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, 2% agar) (Teknova, Hollister, CA), and a colony was then inoculated into YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose) (Teknova) and incubated overnight at 37°C. Slides were made the following day as described below.

Challenge cultures. Forty-seven blood culture bottles which signaled negative at 7 days were used to challenge the assay. Bottles were selected from those which became available during the study period. Five BacT/ALERT bottle types (10 SA aerobic, 6 PF pediatric, 10 SN anaerobic, 13 FA aerobic, and 8 FN anaerobic) were randomly spiked with representative *Candida* species, other yeast species, and mixtures of yeasts. All of these strains were clinical isolates whose species identification had been confirmed by sequence analysis of 500 base pairs of the 5' end of the nuclear large ribosomal subunit rRNA gene. All seeded bottle assays were performed at Aachen University Hospital using strains from their frozen collection. Strains were thawed and cultured on Sabouraud's dextrose agar (BD, Heidelberg, Germany) overnight at 30°C, and then 100 µl of a yeast suspension in 0.9% NaCl solution was prepared adjusted to a 0.5 McFarland standard, resulting in an inoculum of 2.5×10^4 CFU/ml. The suspensions of mixed yeast species were prepared accordingly; however, the blood culture bottles were inoculated with 50 µl of each yeast suspension. Bottles were returned to the blood culture instrument and incubated until they signaled positive. In addition, 10 uninoculated blood culture bottles were used as negative controls and processed in the same way. Smears were prepared and examined as described below. PNA identification results were compared with those obtained by API ID 32C (bioMérieux) or by sequence analysis.

Clinical specimens. A total of 197 yeast-positive blood culture bottles from five clinical laboratories were included in the study (19 bacterium-positive blood culture bottles were also included as negative controls). The blood culture systems used were the BacT/ALERT (bioMérieux, Durham, NC) (106 samples), the BD BACTEC 9600 (93 samples), and the BD BACTEC 9240 (17 samples) (Becton, Dickinson & Co, Sparks, MD). The blood culture media used were site

TABLE 1. Results of the *C. albicans/C. glabrata* PNA FISH method with 48 reference strains representing *C. albicans*, *C. glabrata*, and phylogenetically related species inoculated into YM broth

Fungal isolate	Strain ^f	<i>C. albicans/C. glabrata</i> PNA FISH result ^a
<i>Aspergillus niger</i>	ATCC 16404	— ^b
<i>Candida (Clavicipora) lusitanae</i>	NRRL Y-11827 ^T	—
<i>Candida albicans</i>	NRRL Y-12983 ^T	+G ^c
<i>Candida albicans</i>	NRRL Y-17967	+G
<i>Candida albicans</i>	NRRL Y-17968	+G
<i>Candida albicans</i>	NRRL Y-17974	+G
<i>Candida albicans</i>	NRRL Y-17976	+G
<i>Candida albicans</i>	NRRL Y-27022	+G
<i>Candida albicans</i>	NRRL YB-3898	+G
<i>Candida albicans</i>	NRRL Y-7873	+G
<i>Candida albicans</i>	ATCC 20735	+G
<i>Candida bracarensis</i>	Lab strain ^e	+R
<i>Candida bracarensis</i>	CBS 10154	+R
<i>Candida dubliniensis</i>	NRRL Y-17512	—
<i>Candida dubliniensis</i>	NRRL Y-27201	—
<i>Candida glabrata</i>	NRRL Y-65 ^T	+R
<i>Candida glabrata</i>	ATCC 66032	+R
<i>Candida glabrata</i>	ATCC 15126	+R
<i>Candida glabrata</i>	NRRL Y-17815	+R
<i>Candida guilliermondii</i>	ATCC 6260 ^T	—
<i>Candida guilliermondii</i>	NRRL Y-324	—
<i>Candida haemulonii</i>	GHP2531	—
<i>Candida haemulonii</i>	CBS 5149 ^T	—
<i>Candida krusei</i>	ATCC 14243	—
<i>Candida krusei</i>	NRRL Y-7550	—
<i>Candida krusei</i>	NRRL Y-27441	—
<i>Candida krusei</i>	NRRL Y-94	—
<i>Candida krusei</i>	ATCC 6258 ^T	—
<i>Candida lodderae</i>	NRRL Y-17317 ^T	—
<i>Candida nivariensis</i>	CBS 9983	+R
<i>Candida nivariensis</i>	CBS 9984	+R
<i>Candida nivariensis</i>	CBS 9985	+R
<i>Candida orthopsilosis</i>	Lab strain ^e	+G ^c
<i>Candida palmioleophila</i>	CBS 8109	—
<i>Candida palmioleophila</i>	CBS 7418	—
<i>Candida palmioleophila</i>	CBS 8346	—
<i>Candida parapsilosis</i>	ATCC 22019 ^T	—
<i>Candida parapsilosis</i>	NRRL Y-12969	—
<i>Candida parapsilosis</i>	NRRL Y-543	—
<i>Candida parapsilosis</i>	NRRL YB-415	—
<i>Candida pseudointermedia</i>	GHP2103	—
<i>Candida pseudointermedia</i>	GHP2339	—
<i>Candida sojae</i>	NRRL Y-17909 ^T	—
<i>Candida sojae</i>	NRRL Y-27145	—
<i>Candida tropicalis</i>	ATCC 750 ^T	—
<i>Candida tropicalis</i>	NRRL Y-12968	—
<i>Candida utilis</i>	ATCC 9950	—
<i>Candida viswanathii</i>	NRRL Y-6660 ^T	— ^d
<i>Candida viswanathii</i>	NRRL Y-27370	—
<i>Candida wickerhamii</i>	CBS 2928	—
<i>Candida zeylanoides</i>	NRRL Y-1774 ^T	—
<i>Cryptococcus neoformans</i>	ATCC 204092	—
<i>Debaryomyces hansenii</i> var. <i>fabryi</i>	NRRL Y-17914 ^T	—
<i>Debaryomyces hansenii</i> var. <i>hansalii</i>	NRRL Y-1454	—
<i>Kazachstania barnettii</i>	CBS 5648	—
<i>Kazachstania bovina</i>	CBS 2678	—
<i>Kazachstania bovina</i>	CBS 2760	—
<i>Kazachstania telluris</i>	GHP1669	—
<i>Kluyveromyces marxianus</i>	GHP6	—
<i>Kluyveromyces marxianus</i>	GHP2153	—
<i>Kluyveromyces marxianus</i>	GHP2209	—
<i>Kluyveromyces marxianus</i>	GHP2330	—
<i>Kluyveromyces marxianus</i>	GHP2438	—
<i>Lodderomyces elongisporus</i>	NRRL Y-27304	—
<i>Lodderomyces elongisporus</i>	NRRL Y-7681	—
<i>Nakaseomyces bacilliporus</i>	CBS 7753	—
<i>Nakaseomyces delphensis</i>	ATCC 24205 ^T	+R
<i>Pichia norvegensis</i>	NRRL Y-7687 ^T	—
<i>Saccharomyces cerevisiae</i>	ATCC 9763	—
<i>Trichosporon mucoides</i>	ATCC 201382	—

^a +G, positive green; +R, positive red; — negative.

^b Weak heterogeneous red-orange signal, negative.

^c Weakly positive.

^d A few green cells were observed.

^e Johns Hopkins Medical Institutes laboratory strain.

^f T, type strain; NRRL, Agricultural Research Service Culture Collection; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; GHP, lab strain from University Hospital RWTH, Aachen, Germany.

specific, and the yeast-positive samples included 158 aerobic and 39 anaerobic culture bottles; 77 bottles contained activated charcoal, and 15 contained resins. Specimens were coded and patient identifiers removed.

Routine identification of fungi. Fungi were identified by routine methods, including germ tube analysis and testing by commercial identification systems. Yeast isolates recovered from blood cultures were tested with the Vitek II (bioMérieux Inc., St. Louis, MO) and cornmeal Tween 80 agar for identification to species level. Isolates were also grown on CHROMagar Candida (Becton Dickinson, Baltimore, MD) at 37°C for 48 h to determine species identification by colony color. At one site, *C. dubliniensis* isolates were identified by PCR and sequence analysis of the entire ITS1-D2R region (1,160 bp) using previously described universal fungal primer pairs designated ITS5 and DR2R (10).

Preparation of smears. Smears to be stained were made by adding 1 drop of fixation solution to the well of a slide combined with 30 µl of a fresh overnight YM broth culture for the reference strains studies or 1 drop of blood culture broth for the clinical studies. The smears were heat fixed by placing them on a 55°C heat block for 20 min, placed in reagent alcohol or 96% (vol/vol) ethanol for 5 to 10 min, and then air dried. For the clinical validation, positive control slides were prepared with a 1:1 mixture of overnight cultures of *C. albicans* (Y-17968) and *C. glabrata* (ATCC 15126). For negative controls, *Saccharomyces cerevisiae* (ATCC 9763) or *C. krusei* (ATCC 14243) smears were used. *Aspergillus niger* (ATCC 16404) slides were prepared by inoculating culture media in the slide well and incubating in a 35°C humidified incubator for 18 h and then fixed as described above. To ensure quality, all smear preparations of reference strains were tested in the same assay format with a universal eukaryotic PNA probe (15).

Slides were tested and scored at the individual laboratories. Heat blocks, water baths, slide handling equipment, and microscope specifications were standardized for all AdvanDx PNA FISH assays. AdvanDx 60X wash buffer, mounting medium, fixation solution, and positive and negative control slides were provided to each site by AdvanDx.

***C. albicans/C. glabrata* PNA FISH method.** A standardized protocol and standardized equipment (13, 19), differing from those of other AdvanDx PNA FISH assays only by the PNA probe reagent, were used. Hybridization with the PNA probes was performed on slides using 1 drop of *C. albicans/C. glabrata* PNA probe mixture and incubated for 90 min at 55°C. Excess PNA probe mixture was removed by immersion of the slides into preheated 1× wash solution (55°C) for 30 min. Air-dried slides were mounted using 1 drop of mounting medium and covered with a coverslip. Microscopic examination was conducted using a fluorescence microscope equipped with a fluorescein isothiocyanate/Texas Red dual band-pass filter. *C. albicans* cells were identified as multiple fluorescent green cells in multiple fields of view, and *C. glabrata* cells were identified as multiple fluorescent red cells in multiple fields of view. Each smear was scored positive or negative for both potential outcomes (red or green fluorescence).

RESULTS

Performance with reference strains. All (9/9) *C. albicans* strains stained green. However, one strain of *C. albicans* had a slightly weaker fluorescence signal than the others, presumably due to a point mutation in the target sequence, as previously reported (11, 19). The *Candida orthopsilosis* strain (formerly called *Candida parapsilosis* type II) cross-reacted to produce a weakly positive green signal; however, none of the *C. parapsilosis* strains (0/4) produced a green signal. Of two *Candida viswanathii* strains, one strain had heterogeneous staining, with very rare weakly positive green fluorescent cells that were interpreted as negative. All (4/4) *C. glabrata* strains were positive, showing red fluorescence. *Nakaseomyces delphensis* (1/1), *Candida bracarenensis* (2/2), and *C. nivariensis* (3/3) strains cross-reacted to produce strong red signals. All other reference strains were negative (nonfluorescent) for both potential outcomes (green and red). The results are displayed in Table 1.

The detection limits for *C. albicans* and *C. glabrata* were both determined to be approximately 10⁵ CFU per ml by serial dilutions of positive cultures (data not shown). This is consistent with the analytical sensitivity of other slide-based staining techniques (4).

TABLE 2. Results of *C. albicans/C. glabrata* PNA FISH method with different yeast species alone and in various combinations inoculated into 47 negative blood culture bottles, compared to routine identifications

Species spiked (n)	Green signal	Red signal	Bottle type(s) ^a
<i>C. albicans</i> (3)	+	—	FA, SA, SN
<i>C. albicans-C. dubliniensis</i> (1)	+	—	FA
<i>C. albicans-C. guilliermondii</i> (1)	+	—	FA
<i>C. albicans-C. krusei</i> (1)	+	—	FN
<i>C. albicans-C. lusitanae</i> (1)	+	—	SA
<i>C. albicans-C. parapsilosis</i> (1)	+	—	SA
<i>C. albicans-C. tropicalis</i> (1)	+	—	PF
<i>C. albicans-S. cerevisiae</i> (1)	+	—	SN
<i>C. albicans-T. asahii</i> (1)	+	—	FA
<i>C. dubliniensis</i> (2)	—	—	FA, SA
<i>C. albicans-C. glabrata</i> (2)	+	+	FN, SN
<i>C. glabrata</i> (3)	—	+	FA, SA, SN
<i>C. glabrata-C. dubliniensis</i> (1)	—	+	SA
<i>C. glabrata-C. guilliermondii</i> (1)	—	+	FA
<i>C. glabrata-C. krusei</i> (1)	—	+	SN
<i>C. glabrata-C. lusitanae</i> (1)	—	+	FA
<i>C. glabrata-C. parapsilosis</i> (1)	—	+	SN
<i>C. glabrata-C. tropicalis</i> (1)	—	+	SA
<i>C. glabrata-S. cerevisiae</i> (1)	—	+	FN
<i>C. guilliermondii</i> (1)	—	—	FA
<i>C. krusei</i> (2)	—	—	FN, SA
<i>C. lusitanae</i> (2)	—	—	FA, SN
<i>C. parapsilosis</i> (2)	—	—	PF, SN
<i>C. tropicalis</i> (2)	—	—	FA, PF
<i>S. cerevisiae</i> (2)	—	—	FN, PF
<i>T. asahii</i> (1)	—	—	FA
None (5) ^b	—	—	FA, FN, PF, SA, SN

^a SA, standard aerobic; PF, pediatric FAN; SN, standard anaerobic; FA, FAN aerobic; FN, FAN anaerobic.

^b Run in duplicate with same result.

Performance with negative blood cultures spiked with yeast.

The assay was challenged by spiking various mixtures of yeast species into negative blood cultures of five commonly used BacT/ALERT bottle types. As can be seen in Table 2, all samples in all bottle types were correctly identified. Although variations in positive signal strength were noted, they did not correlate to bottle type or to presence/absence of a coinoculated species.

Clinical performance. The assay was performed with 197 blood culture bottles positive for yeast by Gram staining (Table 3). By using routine methods, seventeen different fungal pathogens, including nine *Candida* species, were identified. Some genera, such as *Fusarium*, *Rhodotorula*, and *Scedosporium*, were not identified to the species level. The most frequently identified yeasts were *C. albicans* (40.1%), followed by *C. parapsilosis* (22.8%), *C. glabrata* (18.8%), *C. tropicalis* (4.6%), and *C. dubliniensis* (4.6%). *Candida albicans*-positive samples included two containing either *C. parapsilosis* or *C. tropicalis*. PNA FISH-positive results, either green or red fluorescence, were reported 58.4% of the time (115/197). *Candida dubliniensis*, which is frequently misidentified as *C. albicans* due to positive germ tube test results, was correctly determined negative in all nine samples tested using the PNA FISH assay.

The diagnostic sensitivity of the assay was 99.1% (115/116). Separation of the assay into its two potential positive outcomes (green or red fluorescence) reveals diagnostic sensitivities of

TABLE 3. Performance of the *C. albicans*/*C. glabrata* PNA FISH method with 197 blood cultures positive for yeast on Gram stain and tested at five hospital laboratories, comparing routine identification methods to PNA FISH results

Routine identification (no. of samples)	No. with the following PNA FISH result:		
	<i>C. albicans</i>	<i>C. glabrata</i>	Negative
<i>Candida albicans</i> (77)	76	0	1 ^a
<i>Candida albicans</i> and <i>Candida parapsilosis</i> (1)	1	0	0
<i>Candida albicans</i> and <i>Candida tropicalis</i> (1)	1	0	0
<i>Candida glabrata</i> (37)	0	37	0
<i>Candida parapsilosis</i> (44)	0	0	44
<i>Candida dubliniensis</i> (9)	0	0	9
<i>Candida tropicalis</i> (8)	0	0	8
<i>Candida lusitanae</i> (3)	0	0	3
<i>Cryptococcus laurentii</i> (2)	0	0	2
<i>Cryptococcus neoformans</i> (2)	0	0	2
<i>Fusarium</i> spp. (2)	0	0	2
<i>Malassezia furfur</i> (2)	0	0	2
<i>Rhodotorula</i> spp. (2)	0	0	2
<i>Scedosporium</i> spp. (2)	0	0	2
<i>Candida guilliermondii</i> (1)	0	0	1
<i>Candida kefyr</i> (1)	0	0	1
<i>Candida krusei</i> (1)	0	0	1
<i>Saccharomyces cerevisiae</i> (1)	0	0	1
<i>Trichosporon asahii</i> (1)	0	0	1

^a Positive on retest.

98.7% (78/79) for identification of *C. albicans*, and 100% (37/37) for identification of *C. glabrata*. The one false-negative sample was determined to be *C. albicans* positive by repeat assay and culture. The diagnostic specificity of the assay was 100% (81/81), as all culture-negative samples tested negative. The positive predictive value for *C. albicans* was 100% (78/78), and that for *C. glabrata* was 100% (37/37). The negative predictive value for the assay was 98.8% (81/82). Performance characteristics determined from each clinical site are shown in Table 4.

Positive signals were characterized by bright fluorescent yeasts often found in clusters. The pseudohyphae of *C. albicans*, when present, also stained bright green. Some variation was typically observed from cell to cell. At high magnification ($\times 600$ to $\times 1,000$), negative yeasts were often easily visible, though they were generally uncolored or weakly yellowish (representative images are displayed in Fig. 1).

Additional testing was performed at two sites with blood

FIG. 1. Smears made from blood culture bottles spiked with *C. albicans* (A), *C. glabrata* (B), both *C. albicans* and *C. glabrata* (C), and *S. cerevisiae* (D) tested with the *C. albicans*/*C. glabrata* PNA FISH method. All cultures grown for 6 h at 37°C in BacT/ALERT FN medium (bioMérieux, Durham, NC).

cultures positive for bacteria only, and all tested negative by the assay. The 19 samples included 5 gram-negative rods (no species identification), 4 *Staphylococcus aureus* isolates, 3 *Staphylococcus epidermidis* isolates, 2 coagulase-negative staphylococci, 2 *Escherichia coli* isolates, 1 *Klebsiella pneumoniae* isolate, 1 *Enterobacter cloacae* isolate, and 1 mixed sample containing *Enterococcus faecalis* and *Klebsiella oxytoca*.

DISCUSSION

The results with the reference strains, spiked negative blood cultures, and multicenter clinical evaluation all support the accuracy and specificity of the assay for simultaneous detection of *Candida albicans* and *Candida glabrata*. A major advantage is the ability to perform the assay directly from blood culture bottles without the need to isolate colonies on agar media. Accordingly, definitive identification of these two species could be available within 3 h of a positive Gram stain or within a shift, if tests are batched. Other identification assays such as rapid trehalose, urease, and enzymatic screening formats all require isolated colonies, and most provide presumptive rather than final identifications. Even molecular assays, in most cases, require a colony and 1 to 2 days before identifications are

TABLE 4. Performance statistics for the *C. albicans*/*C. glabrata* PNA FISH method at five clinical laboratories

Laboratory	Blood culture system(s)	% (no./total)				
		Sensitivity		PPV	NPV	Specificity
		<i>C. albicans</i>	<i>C. glabrata</i>			
A	BacT/Alert, BACTEC	100 (10/10)	100 (5/5)	100 (15/15)	100 (3/3)	100 (3/3)
B	BACTEC	100 (25/25)	100 (5/5)	100 (30/30)	100 (27/27)	100 (27/27)
C	BacT/Alert	92.30 (12/13)	100 (3/3)	100 (5/5)	90.0 (9/10)	100 (10/10)
D	BacT/Alert	100 (17/17)	100 (11/11)	100 (28/28)	100 (25/25)	100 (25/25)
E	BacT/Alert, BACTEC 9240	100 (14/14)	100 (13/13)	100 (27/27)	100 (17/17)	100 (17/17)
Total		98.7 (78/79)	100 (37/37)	100 (115/115)	98.8 (81/82)	100 (82/82)

available. An additional benefit of the PNA FISH assays is the ability of the technologist to view an organism's microscopic morphology as well as fluorescence, which provides an added quality control. The PNA FISH technology, following the same basic procedure, is available for detection of other infectious pathogens and does not require major capital equipment expenditures.

Screening of 70 reference strains representing closely related yeast species and of 10 bacterial species frequently isolated from blood culture demonstrated excellent specificity for clinically relevant organisms. Four clinically rare yeasts showed cross-reactivity with this assay. *N. delphensis*, *C. bracarensis*, and *C. nivariensis* tested positive for *C. glabrata*, and *C. orthopsilosis* was weakly positive for *C. albicans*. It has been reported that *N. delphensis* and *C. glabrata* are closely related species by rRNA analysis and that *N. delphensis* is nonpathogenic (11). The clinical relevance of the recently described species *C. bracarensis*, *C. nivariensis*, and *C. orthopsilosis* is unclear, since they are likely to be misidentified as *C. glabrata* and *C. parapsilosis*, respectively, by current routine methods (1, 6, 21).

The assay was validated with blood cultures spiked to simulate the real milieu and allow testing of detection of specimens containing two yeast species. These results support the detection of both *C. albicans* and *C. glabrata* with the assay for detection in the presence of other yeast species. In addition, these experiments supported the use directly from blood cultures. The spiked blood culture experiments also demonstrate the validity of testing directly from various blood culture bottle types from a commercially available and widely used blood culture system. Positive results were detected in all (5/5) bottle types tested. Positive and negative signal quality was not affected by the type of blood culture media used, even though some of the bottles contained charcoal.

The combined prevalence of *C. albicans* and *C. glabrata* was 58.9% [(79 *C. albicans* + 37 *C. glabrata*)/197] in this study. Other reports suggest a combined prevalence of 70% (9, 17). A weakness of this study is that it was performed on de-identified blood culture bottles, and no effort was made to exclude repeat samples from a given patient. Thus, it is impossible to know if the observed species distribution reflects true prevalence rates. This study therefore had a high incidence of *C. parapsilosis* and *C. dubliniensis* compared to published values (16). All *C. dubliniensis* isolates in this study came from one hospital.

The performance data reported in this study are comparable to those previously reported for *C. albicans* PNA FISH as well as other PNA FISH assays for other blood pathogens (13, 19). PNA FISH has proven to be remarkably specific due to the high specificity of PNA probes (18) combined with the use of rRNA, a well-established phylogenetic marker, as the target molecule (24). The PNA FISH technique mimics traditional staining methods, and results are read by fluorescence microscopy, a concept that is easily adaptable to routine clinical microbiology settings. The combination of fluorescence and morphology increases the confidence of reporting (20). The dual color concept may provide an increased reliability of positive test results, since identification requires accurate reactions of both the green and red probe sets. The definitive nature of the test results allow both *C. albicans* and *C. glabrata* to be directly reported by the blood bench technologists, eliminating the need for further costly and time-consuming workup.

Early and appropriate therapy can critically affect patient outcomes (8); however, antifungal therapy for patients with candidemia is still largely empirical, taking into account patient symptoms, history, and hospital trends. Clinicians and hospital administrators must take into account cost and patient safety issues when considering the use of broad-spectrum antifungals and/or combination therapies. One of the missing links to date has been a rapid method for the determination of the infecting *Candida* species, which provides important information with respect to largely predictable antifungal susceptibility profiles.

In summary, the *C. albicans/C. glabrata* PNA FISH assay is an accurate diagnostic assay to guide optimal therapy in a timely fashion. It replaces identification by slow and subjective phenotypic profiles with the rapid, objective detection of genetic signatures. Rapid identification of yeast to the species level from newly positive blood cultures will provide clinicians with actionable information for antifungal therapy days earlier than current methods.

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