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Multicenter Evaluation of a *Candida albicans* Peptide Nucleic Acid Fluorescent In Situ Hybridization Probe for Characterization of Yeast Isolates from Blood Cultures

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We evaluated aliquots from 244 clinical blood culture bottles that demonstrated yeasts on Gram stain using a *Candida albicans* peptide nucleic acid (PNA) fluorescent in situ hybridization (FISH) probe. The sensitivity, specificity, positive predictive value, and negative predictive value of the *C. albicans* PNA FISH test in this study were 99%, 100%, 100%, and 99.3%, respectively.

Yeasts, long known to cause thrush and vulvovaginal infections, have emerged as a significant cause of nosocomial infections (18, 21). Although *Candida albicans* usually is the most frequently isolated organism from patients with fungemia, other species of *Candida* are also important pathogens in hospitalized patients (18, 21). Furthermore, some of the *Candida* species other than *C. albicans* are inherently resistant to or display dose-dependent susceptibility to fluconazole, the antifungal agent that is most commonly used to treat infections caused by *C. albicans* (18, 23).

The first evidence of fungemia often is the presence of yeasts with or without pseudohyphae in a blood culture bottle that signals positive in an automated system (1). A variety of tests have been developed for the rapid characterization of yeast isolates. These include the rapid assimilation of trehalose by *Candida glabrata*, the detection of rapid urease production or the detection of phenoloxidase by the caffeic acid disk test for *Cryptococcus neoformans*, and the germ tube test or the detection of a colonial fringe on primary isolation media for the identification of *C. albicans* (2, 4–6, 20). A subculture onto specialized CHROMagar is another means of differentiating yeasts (19, 22). All of these tests, however, require subculture rather than direct assessment of the yeast in the positive blood culture bottle. The subcultures from positive blood culture bottles are often not examined and tested until the next day, which may be 18 to 24 h after the blood culture bottle first signaled positive. This delay in identification may affect patient care, since some *Candida* species other than *C. albicans* are much more likely to be resistant to fluconazole; *Candida krusei* is inherently resistant to fluconazole, and *C. glabrata*, a commonly encountered clinical isolate, does not have predictable fluconazole susceptibility and may be resistant or demonstrate dose-dependent susceptibility.

Fluorescent in situ hybridization (FISH) is a well-established diagnostic tool in molecular pathology laboratories and has been used to detect and differentiate microorganisms in histologic sections (9, 10). This technology has been applied to culture and specimens in the clinical microbiology laboratory. Examples include the direct, species-specific identification of bacteria and yeasts from positive blood cultures and the direct identification of the common bacterial pathogens from respiratory specimens of patients with cystic fibrosis (3, 12–14). The rapid differentiation of *Candida* species that are commonly encountered in the hospital setting has been accomplished with traditional fluorescent in situ hybridization (12–14). A peptide nucleic acid (PNA) FISH probe specific for *Candida albicans* has also been demonstrated to be potentially useful on aliquots taken directly from positive blood culture bottles to differentiate *C. albicans* from non-*C. albicans* yeasts (16). This technology has been also been developed and tested for the rapid differentiation of *Staphylococcus aureus* from non-*S. aureus* bacteria that form cocci in clusters (3, 15, 17). We performed a multi-institutional evaluation of the *Candida albicans* PNA FISH assay (AdvanDx, Woburn, MA) on blood culture bottles that signaled positive and demonstrated yeasts on the Gram stain.

The blood culture systems used were the BD BACTEC 9600 and 9240 systems (Becton Dickinson, Sparks, MD) and the BacT/ALERT system (bioMérieux, Durham, NC). The media used were the BD BACTEC resin, standard (aerobic and anaerobic), and the Lytic 10, as well as BacT/ALERT FA, FN, SN, SA, and plastic SN and BacT/ALERT PF (plastic; for investigational use only). More than one blood culture isolate was not accepted from the same patient, unless 7 days or more had elapsed from the detection of the prior isolate. One hundred ten of the positive blood cultures that contained yeasts were collected at the Cleveland Clinic Foundation, where the bioMérieux BacT/ALERT system was used. The remaining 134 blood cultures were collected at Duke University Medical Center and Mayo Clinic. Mayo Clinic provided samples from the BD BACTEC system, whereas Duke University provided samples from both the BD BACTEC and the BacT/ALERT systems.

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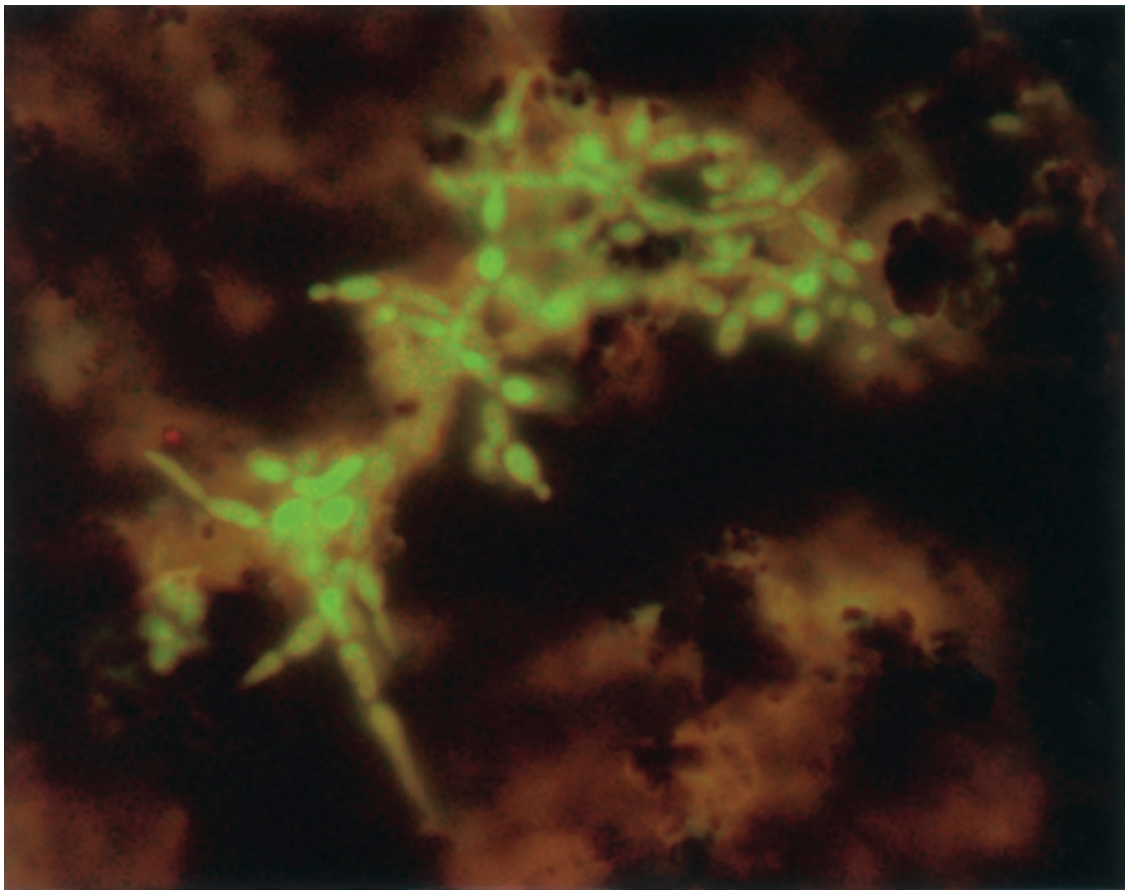

FIG. 1. *Candida albicans* demonstrated bright green fluorescence when hybridized with the *Candida albicans* PNA FISH probe. Magnification, ×400. An FITC filter was used.

At Mayo Clinic, yeast isolates were first assessed after subculture using the germ tube test. If positive, the yeast was identified as *C. albicans*; if negative the yeast was assessed with the API20Caux (bioMérieux, Durham, NC) in conjunction with morphology on cornmeal agar. At the Cleveland Clinic, macroscopic morphology was assessed after subculture onto sheep blood agar for the presence of a colonial fringe. If present, the isolate was characterized as *C. albicans*; the fringe was used as a surrogate for a positive germ tube test. If a colonial fringe was absent, the germ tube test was performed. Yeasts that produced germ tubes were characterized as *C. albicans*, whereas yeasts that did not produce germ tubes were sent to the mycology laboratory and characterized by the Vitek YBC yeast identification card (bioMérieux, Durham, NC) in conjunction with their microscopic morphology on cornmeal agar. At Duke University Medical Center, yeast colonies from positive blood culture bottles were first tested with the *C. albicans* screen (Remel, Lenexa, KS). Isolates that produced a positive reaction according to the manufacturer's guidelines were characterized as *C. albicans*. Additionally, isolates that produced a negative reaction were sent to the mycology laboratory for testing with the API20Caux (bioMérieux), by four sugar fermentation reactions, and by morphological assessment on cornmeal agar.

When yeasts were detected by routine Gram staining of positive blood culture bottles, duplicate slides were made for *C. albicans* PNA FISH analysis. The slides from all three institutions were submitted for *C. albicans* PNA FISH testing to one author (D.A.W.), in a blinded fashion. The yeast isolates were kept as stock cultures after routine identification for further testing in case of discrepancies between conventional and FISH results.

The *C. albicans* PNA FISH assay (AdvanDx, Inc., Woburn, MA) was performed according to the manufacturer's instructions. Briefly, a 10-μl aliquot from the blood culture bottle that signaled positive and contained yeasts in the Gram stain was mixed with 1 drop of fixation solution. After fixation on a slide warmer for 20 min, slides were immersed briefly in 95% ethyl alcohol. One drop of the *C. albicans* PNA probe was placed on the slide and hybridized at 55°C for 90 min in a humidified atmosphere. The slides were washed in buffer heated to 55°C for 30 min, mounting media were applied, and a coverslip was applied to each slide. The entire PNA FISH procedure required approximately 2.5 h. Slides were read using a fluorescence microscope with both fluorescein isothiocyanate (FITC) and a dual FITC/Texas Red filter(s); this was done to compare filters. The manufacturer, however, recommends only the dual FITC/Texas Red filter. In such preparations, *C. albicans* was expected to appear as fluorescent green yeast cells (Fig. 1), whereas non-*C. albicans* yeasts were nonfluorescent. Positive (*C. albicans*) and negative (*C. glabrata*) control slides were included in each run.

The results of the *C. albicans* PNA FISH test were first

TABLE 1. Initial *Candida albicans* PNA FISH compared to identification by routine laboratory methods

Yeast identification by routine methods	Initial *C. albicans* PNA FISH result	No. of blood cultures
Candida albicans	Positive	93
C. albicans and *C. glabrata*	Positive	1
C. albicans and *C. parapsilosis*	Positive	2
C. albicans[a]	Negative	5
C. glabrata	Negative	74
C. glabrata and *C. parapsilosis*	Negative	1
C. parapsilosis	Negative	18
C. tropicalis	Negative	21
C. krusei	Negative	12
Cryptococcus neoformans	Negative	6
Saccharomyces cerevisiae	Negative	2
S. cerevisiae and *C. glabrata*	Negative	1
Other non-*C. albicans* fungi[b]	Negative	8

[a] See Table 2 for final identification.
[b] These were all read as yeasts in the Gram stain, and species were determined as follows: 1 each of *Candida guilliermondii*, *Candida kefyr*, *Candida lusitaniae*, *Pichia ohmeri*, *Rhodotorula glutinis*, *Fusarium* sp., *Trichosporon* sp., and *Exophiala dermatitidis.*

compared with the results of the routine screening tests used by the three laboratories, as previously described. Concordant results between *C. albicans* PNA FISH and the initial routine identification of the yeast as *C. albicans* or non-*C. albicans* were not investigated further. Any discrepancies were retested with the *C. albicans* PNA FISH test and the API20Caux system (11). If discrepancies could not be clarified, the identification of the isolates was made at Mayo Clinic by DNA sequencing of the D2 region of the large ribosomal subunit, as previously described (8). The collaborator at the Mayo Clinic (L.S.H.), who performed the DNA sequencing, did not know the results of the phenotypic assays or the PNA FISH test. The results of the DNA sequence analysis, if needed, were considered as the definitive identification.

Two hundred forty-four blood cultures from 228 patients that contained yeasts on Gram stain were evaluated by *C. albicans* PNA FISH (Table 1). One hundred one of the 244 positive blood cultures were initially reported to contain *C. albicans* as identified by routine laboratory methods, whereas only 96 were positive by the *C. albicans* PNA FISH test. The repeat *C. albicans* PNA FISH test on aliquots from the five cultures with discrepant results yielded one PNA FISH-positive culture, whereas four remained PNA FISH negative. These four isolates were analyzed with the API 20Caux system for further resolution of the discrepancies. The one isolate that was initially interpreted as PNA FISH negative but was PNA FISH positive on repeat testing was determined to be *C. albicans* by testing with the API20Caux. This isolate was also submitted for DNA sequencing (out of interest), which confirmed the identity as *C. albicans.* The four isolates that were identified as *C. albicans* by the routine laboratory methods but were PNA FISH negative were identified as *Candida dubliniensis* (two isolates), *Candida parapsilosis*, and *C. albicans* using the API20Caux (Table 2). The single isolate that was PNA FISH negative but identified as *C. albicans* by the API20Caux was submitted for DNA sequencing, which revealed it to also be *C. dubliniensis*.

The *C. albicans* isolates highlighted with the *C. albicans* PNA FISH probe were readily demonstrable with both the FITC and dual FITC/Texas Red filters. The yeasts were slightly more obvious when viewed with the dual FITC/Texas Red filter, since the background was red rather than light green.

This multicenter evaluation of the *C. albicans* PNA FISH assay (AdvanDx) demonstrated that this method is an accurate means of differentiating *C. albicans* from non-*C. albicans* species present in blood culture bottles. Only five discrepancies were observed with the routine screening methods, four of which were initially characterized as *C. albicans* by routine testing, but as non-*C. albicans* by the *Candida albicans* PNA FISH assay. Discrepancy resolution showed that three of these isolates were *C. dubliniensis*, an organism known to produce germ tubes and easily misidentified by this test as *C. albicans* when this test is used alone. Therefore, yeasts that are negative by the *C. albicans* PNA FISH probe but traditionally identified as *C. albicans* may represent *C. dubliniensis* (7). The fourth discrepancy was caused by *C. parapsilosis*, which was thought to have a colonial fringe in the subculture on sheep blood agar. This isolate was examined on a plate after approximately 24 hours of incubation. *Candida parapsilosis*, like many of the other *Candida* species, may produce pseudohyphae after extended incubation, which may explain why this isolate was initially mischaracterized as *C. albicans.* One culture of *C. albicans* that was initially interpreted as negative by the PNA FISH test had to be considered as a false-negative result in this study, even though the repeat PNA FISH test was positive, since repeat testing would not commonly be done in a routine clinical laboratory. The sensitivity, specificity, positive predictive value, and negative predictive value of the *C. albicans* PNA FISH test in this study were 99%, 100%, 100%, and 99.3%, respectively. The overall sensitivity, specificity, positive predictive value, and negative predictive value of the combined routine screening methods used at the various institutions were 100%, 97.3%, 96.0%, and 100%, respectively.

This assay was assessed using a wide variety of the blood culture media that are commercially available and performed well. The *C. albicans* isolates that appeared positive with the *C. albicans* PNA FISH probe were categorized similarly with both the FITC and dual FITC/Texas Red filters. Subjectively it was felt that it was easier to see the fluorescent apple-green yeast cells against the red background displayed with the FITC/Texas Red filter than against the light green background produced by the single FITC filter.

Although the differentiation of *C. albicans* from non-*C. albicans* is useful, additional probes that identify non-*C. albicans* isolates that are or could be resistant to fluconazole, such as *C. krusei* or *C. glabrata*, respectively, would be useful. In addition, the detection of the presence of *C. albicans* in the blood culture does not exclude the possibility, although rare, of the

TABLE 2. Discrepancy resolution

Discrepant isolate	Initial identification	Repeat *C. albicans* FISH	API20Caux identification	DNA sequencing identification
1	*C. albicans*	Negative	*C. parapsilosis*	NA[a]
2	*C. albicans*	Negative	*C. dubliniensis*	NA
3	*C. albicans*	Negative	*C. dubliniensis*	NA
4	*C. albicans*	Negative	*C. albicans*	*C. dubliniensis*
5	*C. albicans*	Positive	*C. albicans*	*C. albicans*

[a] NA, not applied.

presence of a second yeast. Five of the 244 blood culture bottles examined in this study contained two species of yeasts. Only 97/244 (39.8%) of the bottles tested contained *C. albicans*, which reiterates the increasing importance of non-*C. albicans* yeasts as causes of fungemia. In summary, the *C. albicans* PNA FISH assay compares well with routine methods of screening for *C. albicans* but has the advantage of not requiring subculture and overnight incubation prior to analysis.

REFERENCES

1. **Baron, E. J.** 1998. Processing and interpretation of blood cultures, p. 58–62. *In* H. D. Isenberg (ed.), Essential procedures for clinical microbiology. ASM Press, Washington, D.C.
2. **Cartwright, R. Y.** 1976. A simple technique for observing germ tube formation in *Candida albicans*. J. Clin. Pathol. **29:**267–268.
3. **Chapin, K., and M. Musgnug.** 2003. Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. J. Clin. Microbiol. **41:**4324–4327.
4. **Fenn, J. P., E. Billetdeaux, H. Segal, L. Skodack-Jones, P. E. Padilla, M. Bale, and K. Carroll.** 1999. Comparison of four methodologies for rapid and cost-effective identification of *Candida glabrata*. J. Clin. Microbiol. **37:**3387–3389.
5. **Freydiere, A. M., R. Guinet, and P. Boiron.** 2001. Yeast identification in the clinical microbiology laboratory: phenotypical methods. Med. Mycol. **39:** 9–33.
6. **Freydiere, A. M., F. Parant, F. Noel-Baron, M. Crepy, A. Treny, H. Raberin, A. Davidson, and F. C. Odds.** 2002. Identification of *Candida glabrata* by a 30-second trehalase test. J. Clin. Microbiol. **40:**3602–3605.
7. **Gutierrez, J., P. Morales, M. A. Gonzalez, and G. Quindos.** 2002. *Candida dubliniensis*, a new fungal pathogen. J. Basic Microbiol. **42:**207–227.
8. **Hall, L., S. Wohlfiel, and G. D. Roberts.** 2003. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for identification of commonly encountered, clinically important yeast species. J. Clin. Microbiol. **41:** 5099–5102.
9. **Hayden, R. T., X. Qian, G. W. Procop, G. D. Roberts, and R. V. Lloyd.** 2002. In situ hybridization for the identification of filamentous fungi in tissue section. Diagn. Mol. Pathol. **11:**119–126.
10. **Hayden, R. T., X. Qian, G. D. Roberts, and R. V. Lloyd.** 2001. In situ hybridization for the identification of yeastlike organisms in tissue section. Diagn. Mol. Pathol. **10:**15–23.
11. **Hazen, K.** 1998. Yeast identification, p. 304–316. *In* H. D. Isenberg (ed.), Essential procedures for clinical microbiology. ASM Press, Washington, D.C.
12. **Hogardt, M., K. Trebesius, A. M. Geiger, M. Hornef, J. Rosenecker, and J. Heesemann.** 2000. Specific and rapid detection by fluorescent in situ hybridization of bacteria in clinical samples obtained from cystic fibrosis patients. J. Clin. Microbiol. **38:**818–825.
13. **Jansen, G. J., M. Mooibroek, J. Idema, H. J. Harmsen, G. W. Welling, and J. E. Degener.** 2000. Rapid identification of bacteria in blood cultures by using fluorescently labeled oligonucleotide probes. J. Clin. Microbiol. **38:** 814–817.
14. **Kempf, V. A., K. Trebesius, and I. B. Autenrieth.** 2000. Fluorescent In situ hybridization allows rapid identification of microorganisms in blood cultures. J. Clin. Microbiol. **38:**830–838.
15. **Oliveira, K., S. M. Brecher, A. Durbin, D. S. Shapiro, D. R. Schwartz, P. C. De Girolami, J. Dakos, G. W. Procop, D. Wilson, C. S. Hanna, G. Haase, H. Peltroche-Llacsahuanga, K. C. Chapin, M. C. Musgnug, M. H. Levi, C. Shoemaker, and H. Stender.** 2003. Direct identification of *Staphylococcus aureus* from positive blood culture bottles. J. Clin. Microbiol. **41:**889–891.
16. **Oliveira, K., G. Haase, C. Kurtzman, J. J. Hyldig-Nielsen, and H. Stender.** 2001. Differentiation of *Candida albicans* and *Candida dubliniensis* by fluorescent in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. **39:**4138–4141.
17. **Oliveira, K., G. W. Procop, D. Wilson, J. Coull, and H. Stender.** 2002. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. **40:**247–251.
18. **Pfaller, M. A., D. J. Dickema, R. N. Jones, H. S. Sader, A. C. Fluit, R. J. Hollis, S. A. Messer, and The SENTRY Participant Group.** 2001. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1887 through 1999 in the SENTRY Antimicrobial Surveillance Program. J. Clin. Microbiol. **39:**3254–3259.
19. **Powell, H. L., C. A. Sand, and R. P. Rennie.** 1998. Evaluation of CHROMagar Candida for presumptive identification of clinically important *Candida* species. Diagn. Microbiol. Infect. Dis. **32:**201–204.
20. **Roberts, G. D., C. D. Horstmeier, G. A. Land, and J. H. Foxworth.** 1978. Rapid urea broth test for yeasts. J. Clin. Microbiol. **7:**584–588.
21. **Safdar, A., V. Chaturvedi, E. W. Cross, S. Park, E. M. Bernard, D. Armstrong, and D. S. Perlin.** 2001. Prospective study of *Candida* species in patients at a comprehensive cancer center. Antimicrob. Agents Chemother. **45:**2129–2133.
22. **Willinger, B., and M. Manafi.** 1999. Evaluation of CHROMagar Candida for rapid screening of clinical specimens for *Candida* species. Mycoses **42:**61–65.
23. **Yang, Y. L., Y. A. Ho, H. H. Cheng, M. Ho, and H. J. Lo.** 2004. Susceptibilities of *Candida* species to amphotericin B and fluconazole: the emergence of fluconazole resistance in *Candida tropicalis*. Infect. Control Hosp. Epidemiol. **25:**60–64.