JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2007, p. 3802–3803 Vol. 45, No. 11
0095-1137/07/$08.00+0 doi:10.1128/JCM.01127-07

Use of Peptide Nucleic Acid-Fluorescence In Situ Hybridization for Definitive, Rapid Identification of Five Common *Candida* Species[▽]

Megan E. Reller, Amanda B. Mallonee, Nicole P. Kwiatkowski, and William G. Merz*

Division of Medical Microbiology, Department of Pathology, the Johns Hopkins Medical Institutions, Baltimore, Maryland

Received 5 June 2007/Returned for modification 3 August 2007/Accepted 27 August 2007

We investigated a 2.5-h peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) assay with five *Candida* species-specific probes to identify *Candida* colonies and compared it to standard 2-h to 5-day phenotypic identification methods. Suspensions were made and slides were prepared and read for fluorescence per the manufacturer's instructions. Sensitivity was 99% (109/110), and specificity was 99% (129/130). PNA-FISH can rapidly identify those *Candida* species isolated most frequently.

Candida spp. are important nosocomial bloodstream pathogens and account for disproportionate morbidity and mortality (2, 3). Although *Candida albicans* remains the most common isolate, nearly half of all invasive infections are now caused by other species (7, 8). Since different species have distinct antimicrobial susceptibility profiles, the prolonged time needed to identify organisms to a species level (up to 5 days) precludes the early use of less expensive, targeted therapy. Peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) is a diagnostic tool that uses fluorescence-labeled probes that hybridize to species-specific rRNA sequences. The PNA-FISH *C. albicans* assay (AdvanDx, Inc., Woburn, MA), which can identify *C. albicans* in ≤3 h directly from positive blood culture bottles (4–6), is now commercially available and has been demonstrated to be cost-effective (1).

We hypothesized that the use of the *C. albicans* probe and the specific PNA probes for *C. glabrata*, *C. krusei*, *C. parapsilosis*, *and C. tropicalis* would reduce the time to identify the colonies on agar plates and slants to a species level. Therefore, we evaluated PNA-FISH probes for rapid identification of the five species of *Candida* most commonly isolated.

We tested 238 recent clinical isolates, including 62 *C. albicans*, 63 *C. glabrata*, 42 *C. tropicalis*, 37 *C. krusei*, 21 *C. parapsilosis*, 7 *C. dubliniensis*, 5 *Cryptococcus neoformans*, and 2 *Saccharomyces cerevisiae*. The isolates were simultaneously identified using standard phenotypic tests such as the germ tube test, morphology on corn meal agar with caffeic acid, 7-carbohydrate fermentation, urease activity, phenoloxidase activity, and API 20-C strips (when required). The isolates were maintained on Sabouraud's dextrose agar at 4°C.

Fresh suspensions of yeast (newly subcultured with Sabouraud's dextrose agar and incubated for 18 to 24 h at 35 to 37°C) were prepared by inoculating the isolate into sterile deionized water to achieve a 0.5 McFarland standard. PNA-FISH probes for five *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *and C. tropicalis*) were stored at 4°C. Slides were prepared according to the PNA-FISH manufacturer's recommendations. One drop of fixative and 10 μl of suspension were added to each slide and gently emulsified. Slides were then air dried and fixed with methanol. Next, 1 drop of PNA-FISH probe was added to each slide, and the slides were incubated at 55 ± 1°C for 90 min. Subsequently, slides were immersed in a preheated (55 ± 1°C), buffered wash solution for 30 ± 5 min to remove unbound probe, air dried, and mounted with a coverslip. A technologist who was not involved with the study coded the slides, including the positive and negative control slides. The slides were then examined by a trained reader (M.E.R. or A.B.M.).

Slides were viewed under a magnification of ×60 or ×100 (low-viscosity oil) with a dual-band filter (fluorescein isothiocyanate/Texas Red) fluorescence microscope to determine the presence or absence of morphologically consistent fluorescent organisms. In the analysis, the performance of the PNA-FISH assay was compared with that of standard phenotypic identification tests.

Finally, we estimated the potential impact of using PNA-FISH to hasten identification of *Candida* obtained from sterile sites at our institution. We searched the 2005 to 2006 laboratory database to identify how many patients with infection by *Candida* isolated from a sterile site could have had a specific diagnosis earlier had species-specific PNA-FISH probes for *Candida* been available.

A total of 238 slides, in addition to positive and negative control slides, were prepared and examined within 2 h of preparation. The five species-specific probes were found to be 99% sensitive (95% confidence interval [CI], 95 to 100%) and 99% specific (95% CI, 96 to 100%) when tested against the matching species and a panel of five to eight other yeasts (Table 1). There was one false negative and one false positive (a *C. albicans* and a *C. parapsilosis* isolate tested with a *C. albicans* probe); both were identified correctly on repeat testing.

During 2005 to 2006, *Candida* species were isolated from a total of 192 sterile-site specimens from the same number of patients. The use of the five *Candida* species-specific probes could have identified 190 of 192 (99%) yeasts recovered from sterile sites, since probes are still not available for two species (*C. dubliniensis* and *C. lusitaniae*). Excluding the 118 isolates of *C. albicans* for which other rapid tests (germ tube or rapid enzymatic assay) are available for identification, the use of species-specific

* Corresponding author. Mailing address: Medical Microbiology Division, Department Pathology, Meyer B1-193, the Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21287-7093. Phone: (410) 955-5077. Fax: (410) 614-8087. E-mail: wmerz@jhmi.edu.

▽ Published ahead of print on 5 September 2007.

TABLE 1. Identification of *Candida* by PNA-FISH versus standard phenotypic identification

PNA-FISH probe	No. of isolates found positive/no. isolates tested[a]								Test characteristics	
	C. albicans	*C. dubliniensis*	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*	*S. cerevisiae*	*C. neoformans*	% of sensitivity (95% CI)	% of specificity (95% CI)
C. albicans	32/33 (97%)	0/3	0/9	0/6	0/6	1/2	0/1	0/1	99 (84–100)	100 (82–100)
C. glabrata	0/10	0/1	34/34 (100%)	0/6	0/6	0/3	ND	0/1	100 (90–100)	100 (87–100)
C. tropicalis	0/8	0/2	0/11	21/21 (100%)	0/7	0/2	ND	0/1	100 (75–100)	100 (87–100)
C. krusei	0/6	0/1	0/7	0/6	13/13 (100%)	0/5	ND	0/1	100 (66–100)	100 (81–100)
C. parapsilosis	0/5	ND	0/2	0/3	0/5	9/9 (100%)	0/1	0/1	100 (84–100)	100 (88–100)

[a] ND, not done.

PNA-FISH probes still would have hastened definitive identification of 72/74 (97%) isolates from sterile sites.

We found that PNA-FISH can provide rapid and accurate identification of *Candida* at the species level from colonies, thereby enabling more directed early treatment for patients. At our institution, the number of patients from whom one of these five common *Candida* species is recovered from a sterile body site is ~100 per year. PNA-FISH could allow identification of common non-*Candida albicans* species on average 24 h sooner than is possible with current phenotypic tests. Most clinical laboratories have access to a fluorescence microscope; therefore, the assay requires only a specialized heating chamber. Since consistent interpretation of fluorescence is necessary, parallel reading by two technologists for an initial period might be helpful; however, once readers attain competency, the assay has outstanding sensitivity and specificity. Importantly, the detection of positive fluorescence by PNA-FISH provides a final identification; therefore, tedious confirmatory testing is not necessary. Confirmation of improved clinical outcomes with early targeted treatment enabled by definitive identification of *Candida* at the species level from sterile sites using PNA-FISH, as well as the assay's cost effectiveness, will require prospective study.

We thank the staff of the Johns Hopkins Hospital Clinical Mycology Laboratory for their help and Mark Fiandaca of AdvanDx, Inc., Woburn, MA, for providing the PNA-FISH reagents.

REFERENCES

1. **Alexander, B. D., E. D. Ashley, L. B. Reller, and S. D. Reed.** 2006. Cost savings with implementation of PNA FISH testing for identification of Candida albicans in blood cultures. Diagn. Microbiol. Infect. Dis. **54:**277–282.
2. **Bassetti, M., E. Righi, A. Costa, R. Fasce, M. P. Molinari, R. Rosso, F. B. Pallavicini, and C. Viscoli.** 2006. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect. Dis. **6:**21.
3. **Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema.** 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. **37:**1172–1177.
4. **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.
5. **Rigby, S., G. W. Procop, G. Haase, D. Wilson, G. Hall, C. Kurtzman, K. Oliveira, S. Von Oy, J. J. Hyldig-Nielsen, J. Coull, and H. Stender.** 2002. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. J. Clin. Microbiol. **40:**2182–2186.
6. **Wilson, D. A., M. J. Joyce, L. S. Hall, L. B. Reller, G. D. Roberts, G. S. Hall, B. D. Alexander, and G. W. Procop.** 2005. Multicenter evaluation of a *Candida albicans* peptide nucleic acid fluorescent in situ hybridization probe for characterization of yeast isolates from blood cultures. J. Clin. Microbiol. **43:**2909–2912.
7. **Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond.** 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. **39:**309–317.
8. **Wisplinghoff, H., H. Seifert, S. M. Tallent, T. Bischoff, R. P. Wenzel, and M. B. Edmond.** 2003. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. Pediatr. Infect. Dis. J. **22:**686–691.