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Identification of *Candida albicans* and *Candida glabrata* within 1.5 Hours Directly from Positive Blood Culture Bottles with a Shortened Peptide Nucleic Acid Fluorescence In Situ Hybridization Protocol[▽]

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***Candida albicans* and *Candida glabrata* can be identified in blood culture bottles within 2.5 h using peptide nucleic acid fluorescence in situ hybridization. A 1.25-h protocol was compared to the standard with 40 positive (clinical and spiked) blood culture bottles tested in batches of 5. All *C. albicans* (15) and *C. glabrata* (16) isolates, alone or mixed, were identified correctly using both protocols, whereas 18 isolates (five other species) were negative by both protocols. This shortened method will significantly reduce the time to identification.**

The rapid, accurate identification of yeasts is important for optimal patient care. Since antifungal susceptibility/resistance is species associated (1, 8), the selection of appropriate targeted antifungal regimens for patients with serious, opportunistic yeast infections (most commonly invasive candidiasis) is often based on the identification of yeast recovered from sterile anatomic sites, including blood, to the species level. In vitro testing requires growth on solid medium and then an additional 24 to 48 h to determine MICs; therefore, the rapid and accurate identification of yeasts could improve patient management.

Rapid, accurate identification is extremely important for outbreak analysis both in detecting the transmission of yeast strains among hospitalized patients and in assessing the success of infection control interventions. In addition, accurate identification is necessary to monitor changes in epidemiology such as shifts in pathogenic species, emergence of new species (especially those that are resistant), and emergence of resistant strains (8).

There are, however, relatively few commercially available rapid (<4-h) assays, phenotypic or molecular, for the identification of medically important yeasts. Rapid phenotypic tests include morphologically based assays such as a germ tube test for *Candida albicans*/*Candida dubliniensis* (2, 3), rapid enzyme-based assays such as colorimetric assays with chromogenic substrates for *C. albicans* (4), or a rapid trehalose test for *C. glabrata*. (5, 9). In addition, there are chromogenic agars such as ChromAgar Candida (Becton, Dickinson, and Co., Sparks, MD) for the presumptive identification of four commonly encountered *Candida* species based on colony colors (6, 7). Other identification tests, however, require isolated colonies on agar surfaces for inocula, increasing the time to identifications by 24 to 48 h, including identifications from positive blood culture bottles. In addition, many assays provide only a presumptive identification, and additional time-consuming assays are needed for final identifications. Rapid molecular-based assays have the right properties including accuracy, generation of final identifications, and the ability to develop rapid formats. The peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) technology has all of those properties, and kits are currently commercially available as a single probe for *C. albicans* and also as a dual probe for both *C. albicans* and *C. glabrata*. Advantages of PNA FISH include both the ability to be performed directly using aliquots from positive blood culture bottles and the finding that the identifications are final due to the highly species-specific PNA probes. The "standard protocol," as defined in the package insert by the manufacturer (AdvanDx, Woburn, MA) takes approximately 2.5 h for the identification of *C. albicans* and *C. glabrata* (10).

From empirical evidence, we hypothesized that a shortened PNA FISH protocol could be designed to yield identifications in significantly less time than the standard protocol without affecting accuracy. The hybridization (staining) step was reduced significantly in the shortened protocol compared to the standard protocol. This protocol was then tested and compared with the standard protocol with 40 blinded positive blood culture bottles (15 spiked and 25 clinical) for the accurate identification of *C. albicans* and *C. glabrata*. A high concentration of yeast was used to spike the blood culture bottles for comparison of the protocol methods and not for sensitivity of detection.

Fifteen clinical aerobic blood culture bottles (BacTAlert; bioMérieux, Durham, NC) with negative final culture results were spiked with 24 h of growth from Saboraud's dextrose agar plates (1.0 ml of a suspension made in 0.8% aqueous NaCl with a turbidity equivalent to a 0.5 McFarland standard). Final concentrations in the bottles were $\sim 1.0 \times 10^5$ log CFU/ml. Bottles were incubated at 37°C for 18 to 24 h, and two PNA FISH slides per Gram stain-positive bottle were prepared (blinded/coded) by an individual not involved in the hybridizing (staining) or reading of the slides. Six negative blood culture bottles were spiked with *C. albicans*, *C. glabrata*, *C. tropi-*

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calis, *C. krusei*, *C. parapsilosis*, and *C. lusitaniae*, and nine were spiked with combinations of different *Candida* spp. Twenty-five positive blood culture bottles (4 aerobic, 6 anaerobic, and 15 FA aerobic BactAlert bottles) with positive Gram stain from the clinical laboratory were also tested. Quality control slides were also prepared from spiked bottles that were inoculated with reference strains of *C. albicans* and *C. glabrata* as positive controls and *C. tropicalis* as a negative control. Quality control was performed with each batch of test slides and with each protocol.

Two PNA FISH slides were prepared from each positive bottle by mixing 10 μl of the specimen sample with a drop of PNA fixative on the slides. They were then allowed to dry on the PNA FISH workstation for 2 to 10 min. Once dry, they were fixed with methanol for 2 min. One drop of PNA probe solution was added to each slide of each set. One slide per set was incubated at 55°C for 90 min using the standard method, and the second slide was incubated at 55°C for 30 min using the shortened method. Mounting medium (supplied in the PNA FISH kit) and a cover glass were applied to each slide, and the slides were examined for fluorescent cells using a fluorescein isothiocyanate/Texas Red dual-band filter on a fluorescent microscope equipped with a 100× oil immersion objective within 2 h of preparation. The prepared slides were examined for fluorescence; the presence of bright green fluorescent cells was considered to be a positive result for *C. albicans*, and the presence of bright red fluorescent cells was considered to be a positive result for *C. glabrata*. A lack of fluorescent cells with other yeast species was considered to be a negative result.

There was 100% agreement for the results of the shortened and the standard protocols with the 15 spiked blood cultures. All bottles with *C. albicans* ($n = 4$) and *C. glabrata* ($n = 5$) either singly or mixed were detected and identified correctly, and all bottles ($n = 10$) with other species, but not *C. albicans* or *C. glabrata*, were negative. There was also 100% agreement for the results of the shortened and the standard protocols with the 25 positive clinical blood cultures. All *C. albicans* ($n = 11$) and *C. glabrata* ($n = 11$) strains were correctly identified, whereas the three specimens positive for *Cryptococcus neoformans* were negative. Fluorescence of both species was not reduced with the rapid protocol. The shortened protocol worked with all three types of BactAlert blood culture bottles and with both single *C. albicans* probes and dual probes. These results support our hypothesis that the standard PNA FISH protocol in the package insert could be shortened without any reduction in fluorescence or loss of sensitivity leading to false-negative results. The reduction in time was accomplished by shortening the hybridization step from 90 min to 30 min. The washing step (30 min) was crucial and could not be shortened (data not shown). The rapid protocol could be used with different BactAlert blood culture bottle types and with the different PNA FISH probes. The data from the 25 positive cultures from the clinical laboratory support the validity of the shortened protocol with clinical specimens, and the data from the 15 spiked blood cultures confirmed the specificity with four other commonly encountered *Candida* spp. and the accurate identification of these two species even in a single positive blood culture.

Seventy percent of episodes of candidemia in our institute are caused by *C. albicans* and *C. glabrata*, and hopefully, quicker identifications will be used to target initial antifungal decisions. We have validated and implemented the shortened protocol. The limitations of this study include the following: this was a single-center study, the limited number of species recovered from clinical specimens required spiking of blood culture bottles, and no dose-dependent study was performed to look at concentrations needed for detection. The assay is being performed once per shift (every 24 h/7 days) on the first blood culture positive for yeast per patient and again upon subsequent positive results from the same patient with blood specimens collected 1 week later. Even though the PNA FISH identification is a final, and not presumptive, identification, all bottles positive for any yeast are subcultured with ChromAgar Candida to detect multiple species in the same culture or for use as inocula for identifications of those from PNA FISH-negative specimens. Reflexive in vitro susceptibility testing is performed on the first isolate per patient, as is the PNA FISH assay. Rapid identifications using PNA FISH and reflexive in vitro susceptibility will provide data in less time than is currently possible and, hopefully, will contribute to better patient care.

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