

## Use of a Panfungal PCR Assay for Detection of Fungal Pathogens in a Commercial Blood Culture System

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**A panfungal PCR assay was used to evaluate the ability of the ESP blood culture system to detect fungemia. The results showed that the ESP system is reliable for the detection of fungi and showed the applicability of using a molecular-based assay as a potential rapid and reliable method for the identification of fungi.**

Fungemia has emerged as a significant cause of morbidity and mortality, especially in immunocompromised patients. Although *Candida albicans* is the major cause of this infection, numerous other fungal species, such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *Cryptococcus neoformans*, and *Malassezia furfur*, have emerged as important causes of serious disease (3, 13). Presently, the major method used to diagnosis fungemia is culture of blood, using a growth-based approach to detect and identify the pathogen (15).

The detection of fungal species in commercial blood culture bottles has been reported to be <90% in comparisons of culture systems (2, 4). This inability to detect fungi, along with the increased time required to phenotypically identify a fungal species, has led to a proposal to consider molecular biology-based methods for testing (1, 6, 8, 16). Numerous continuous-monitoring blood culture systems have been developed, and molecular approaches to detect microbial pathogens in bottles have shown promise (2, 9–12, 14, 15, 17, 18). ESP Culture System II (TREK Diagnostic Systems, Inc., Westlake, Ohio) is one such method. It utilizes a sensitive approach to measure pressure change within the headspace of a culture bottle for microbial detection. The purpose of this study was to evaluate the ability of the ESP blood culture system (ESP system) to detect fungi in the blood of patients and to evaluate the applicability of using a molecular biology-based method for detection and identification.

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Five to 18 ml of blood was collected from patients at the bedside (one sample per patient) and was immediately inoculated evenly into ESP 80A Aerobic and ESP 80N Anaerobic bottles as a part of routine patient care. The anaerobic bottles were not evaluated further as a part of this study. The aerobic bottles were incubated at 35 to 37°C for 14 days or until a bottle was flagged as positive by the ESP instrument. Contents of aerobic bottles flagged as positive were Gram stained and subcultured to Sabouraud dextrose (SAB) agar and to sheep

blood agar (Remel Labs, Lenexa, Kans.) and were then incubated at 30°C for up to 14 days and 37°C for up to 5 days, respectively. Bottles flagged as positive whose contents Gram stained negative for any microbial pathogen were placed back onto the machine for continued monitoring. Aerobic bottles that subsequently contained bacterial pathogens by culture were excluded from further evaluation. No bottles were determined by culture to contain both bacterial and fungal pathogens simultaneously. All bottles that did not flag positive were subcultured to SAB agar and to sheep blood agar on the 14th day of incubation at the date of culture termination. Fungal growth on SAB agar consistent with that of a yeast was assessed for the ability to form germ tubes and, if a negative result was obtained, was tested by using the API 20C (BioMerieux, Hazelwood, Mo.) sugar assimilation strip for identification. A total of 210 aerobic blood culture bottles were included in the evaluation (142 randomly collected bottles from patients at risk for fungemia [56 from neonatal intensive care, 42 from high-dose chemotherapy, 18 from solid organ transplant, and 26 from general medicine patients] and 68 that had flagged positive but were negative for microbes by Gram staining).

Additionally, a 100- $\mu$ l sample of the blood-broth mixture was taken from each bottle at the time of flagging positive or at the time of culture termination for DNA extraction. The process for extraction was as follows: (i) wash the sample twice by adding it to 900  $\mu$ l of sterile water and centrifuging it at 13,000  $\times$  g for 2 min; (ii) after the second wash, resuspend the pellet in 400  $\mu$ l of DNA extraction buffer as described by van Burik et al. (19); (iii) sonicate the sample in a water bath (model 2210; Branson) for 15 min; (iv) heat at 100°C for 5 min; and (v) purify by using the QIAmp blood kit (Qiagen Inc., Valencia, Calif.) with protocols supplied by the manufacturer for crude cell lysates.

The extracted DNA was amplified, sequenced, and analyzed by using the procedures described by Henry et al. (7). Briefly, a PCR assay was performed with fungus-specific primers that amplified the complete internal transcribed spacer 1 and 2 regions of the ribosomal DNA complex (ITS-PCR). Prior to amplification, the extracted DNA was diluted 1:10 and 1:100 to prevent inhibitors in the blood-broth sample from interfering with the PCR assay (5).

Table 1 shows a comparison of blood culture instrumenta-

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TABLE 1. Comparison of methods for the detection of fungal pathogens from blood culture bottles<sup>d</sup>

No. of samples	ESP instrument finding by:		Subsequent finding by:	
	Flag	Gram stain	Culture	ITS-PCR
118 <sup>a</sup>	Neg	Neg	Neg	Neg
65 <sup>b</sup>	Pos	Neg	Neg	Neg
24 <sup>c</sup>	Pos	Pos	Pos	Pos
3 <sup>c</sup>	Pos	Neg	Pos	Pos

<sup>a</sup> True-negative result.

<sup>b</sup> False-positive ESP instrumentation result by culture comparison.

<sup>c</sup> True-positive ESP instrumentation result by culture comparison.

<sup>d</sup> Neg, negative; Pos, positive.

tion, plate culture, and ITS-PCR assay results. ITS-PCR was negative for all 183 samples that were negative by culture while it was positive for all 27 culture-positive samples, for a sensitivity and specificity of 100%. All 27 culture-positive samples were also positive by the ESP system. In 26 cases, the identification by ITS-PCR in combination with sequence analysis and by culture matched (*C. albicans* [8 isolates], *C. parapsilosis* [5 isolates], *Cryptococcus neoformans* [4 isolates], *C. glabrata* [4 isolates], and one isolate each for *M. furfur*, *Candida lusitanae*, *C. krusei*, *C. tropicalis*, and *Aspergillus nidulans*). In one case, phenotypic testing was unable to resolve the identification and called the isolate *Cryptococcus*-like while sequence analysis of the PCR product confirmed this isolate to be *C. neoformans* by a >99% homology to known sequences within GenBank. The inability to identify all the fungal species by using biochemical methods has been reported by others (20).

Only 3 of the 68 positively flagged, Gram stain-negative samples were considered true positives by culture. False-positive results from continuously monitored blood culture systems have been well documented, although the factors causing the false positives are not fully understood (15). Blood bottles from patients at high risk for *M. furfur*- and *Aspergillus* species-caused infections were purposely included in this study, because these fungi are known to be difficult to detect in blood (15). The ITS-PCR analysis showed no evidence of these or any other fungi present in the bottles.

The three positively flagged, Gram stain-negative samples eventually grew in culture and were identified as *C. albicans* (two cases) and *A. nidulans* (one case). The patient from whom *A. nidulans* was detected subsequently died as a result of complications secondary to premature birth, with no postmortem exam being performed. Because *A. nidulans* is a rare cause of aspergillosis and is common as an environmental contaminant, it was unlikely that this organism was the immediate cause of death, although no other etiology was identified as a cause of sepsis in this patient.

These results showed that the ESP system was able to detect fungal pathogens in blood. This study also showed the applicability of using a molecular biology-based assay on blood culture bottles as a potential rapid and reliable method for the identification of fungal species. Future variations in the platforms used for molecular testing will enhance the applicability of this method for patient care.

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