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Utility of the Germ Tube Test for Direct Identification of *Candida albicans* from Positive Blood Culture Bottles[▽]

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We compared the germ tube test for the direct identification of *Candida albicans* from positive blood culture bottles, with results obtained from subcultured colonies. The direct germ tube test was 87.1% sensitive and 100% specific for the identification of *C. albicans* when the results obtained from fungal colonies were compared.

Recent evidence has suggested that early institution of appropriate antifungal therapy is a critical factor in improving outcomes during bloodstream infections with *Candida* species (2, 4, 5). Given that most bloodstream isolates of *C. albicans* remain susceptible to azoles such as fluconazole (7, 9), the rapid identification of *C. albicans* is a key step in the diagnostic and treatment algorithm for bloodstream *Candida* infection to guide targeted and cost-effective antifungal strategy (6).

Traditionally, the preliminary identification of *C. albicans* is made through the use of a germ tube test (GTT) performed on a subcultured colony grown on solid agar. Although the test itself is rapid, growth of sufficient colonies on solid agar requires a delay of a minimum of 24 h and up to 72 h before identification can be performed. In a preliminary report, Terlecka et al. performed the GTT directly from 31 BacTAlert blood culture bottles positive for yeast on Gram stain, thirteen of which were *C. albicans* (10). Although the numbers were limited, they observed 100% concordance between the direct GTT and a GTT performed with subcultured organisms grown on solid medium. We report here a 2-year prospective study from two sites investigating the possibility that the GTT could be performed directly from blood culture bottles that had been flagged positive. In addition, to extend these results, 67 yeast isolates previously recovered from candidemic patients were tested in blood culture bottles inoculated with human blood.

Over a 2-year period, all positive blood cultures in which yeast were visualized by Gram staining were identified at two large teaching hospitals in Montreal, Canada. To maximize strain diversity, only the first positive blood culture was tested for each episode of fungemia. At Maisonneuve-Rosemont Hospital, all blood cultures were inoculated into BacTAlert blood culture bottles (bioMérieux, Inc., Marcy l'Etoile, France), while the Bactec system (BD Diagnostics, Oakville, Ontario, Canada) was used at the Royal Victoria Hospital. All positive cultures were subcultured on Sabouraud dextrose agar, and a direct GTT performed. To perform the direct GTT, 10 to 20 μl of the blood culture bottle contents was removed and incubated with 0.5 of rabbit serum for 3 h at 37°C. The presence or absence of germ tubes was recorded. When sufficient growth was obtained on solid agar, a standard GTT was performed by inoculating 0.5 ml of citrated rabbit serum with a loopful of the test strain, followed by incubation at 37°C for 3 h. All isolates were then completely identified using the API20C AUX, the Vitek YBC (bioMérieux), or the Auxacolor 2 (Bio-Rad, Marnes-la Coquette, France) system.

To complement these data, 66 clinical isolates that had previously been recovered from blood cultures were used to inoculate BacTAlert bottles with human blood and evaluated by the direct GTT. All isolates had been identified previously using either the Vitek YBC or the Auxacolor 2 identification systems. Briefly, blood culture bottles were inoculated with 10 ml of whole human blood (Biological Specialty Corp., Pennsylvania) and then infected with 1 ml of sterile water containing between 10 and 50 yeast cells. Seeded bottles were then incubated in the automated BacTAlert system according to the manufacturer's instructions. The direct germ tube was then performed when each sample was flagged as positive and compared to the GTT performed from a subcultured colony.

Sixty-seven positive blood cultures were positive for yeast on Gram stain and prospectively tested by using the direct germ tube method (Table 1). The majority of the blood cultures were found to be positive within the first 48 h of incubation. No false-positive germ tube results were observed for non-*Candida albicans* isolates. Four *C. albicans* isolates were GTT negative when tested directly from blood culture bottles but were subsequently found to be GTT positive when the test was performed directly from a colony. Thus, the calculated sensitivity and specificity of the direct GTT for prospective clinical samples were 87.1% (95% confidence interval [CI] = 69.2 to 95.8%) and 100% (95% CI = 87.9 to 100%), respectively. A 100% concordance between direct and colony GTT results was observed for the experimentally inoculated strains, yielding a sensitivity and specificity of 100% (95% CI = 80.0 to 100% and 90.3 to 100%, respectively) for these isolates (Table 2). For all of the samples tested the overall sensitivity of the direct GTT was 92.2% (95% CI = 80.3 to 97.5%) and the specificity was 100% (95% CI = 94.4 to 100%).

Clinical guidelines for the treatment of candidemia have

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TABLE 1. Concordance between the direct GTT and colony GTT for fungal isolates recovered from blood culture bottles

Species	No. of isolates	
	Concordant	Discordant[a]
C. albicans	27	4
C. glabrata	15	0
C. parapsilosis	10	0
C. krusei	3	0
C. tropicalis	4	0
Other[b]	4	0
Total	63	4

[a] For all four discordant results the direct GTT was negative, while the colony test was positive.
[b] Other species include *C. neoformans* and *C. lusitaniae* (two each).

TABLE 2. Number of isolates and species tested in seeded blood culture bottles

Species	No. of isolates tested	No. of isolates direct GTT$^+$ (%)[a]
Candida albicans	20	20 (100)
Candida glabrata	15	0 (0)
Candida guilliermondi	1	0 (0)
Candida krusei	5	0 (0)
Candida lipolytica	1	0 (0)
Candida lusitaniae	2	0 (0)
Candida parapsilosis	15	0 (0)
Candida tropicalis	5	0 (0)
Cryptococcus neoformans	2	0 (0)

[a] All *C. albicans* strains were GTT positive (GTT$^+$) when tested directly from the colony, and all non-*albicans* species were GTT negative when tested directly from the colony.

incorporated species-specific recommendations for the choice of antifungal therapy (6). Thus, the rapid identification of *Candida* species from blood cultures is important for the optimal therapy of these critically ill patients.

Several other rapid methods for the identification of yeasts have been described. Most of these techniques, however, require expensive and labor-intensive technologies that are not commonly available in routine microbiology laboratories (1, 8). Widely available technology that is easily incorporated into routine microbiology laboratories would be preferable. Harrington et al. recently reported a method based on the morphological features of clustered pseudohyphae observed upon Gram staining (3). A sensitivity and a specificity of 85 and 97%, respectively, were obtained. Although useful, this technique is heavily dependent on the trained user and has only been evaluated using blood cultures from a single test system (3). The GTT has been a long well-established routine procedure for identification of medically important yeasts. Performing the GTT directly from the positive blood culture bottle greatly reduces the time to reporting of preliminary speciation, since no culture time is required before reporting. From our clinical samples, the direct GTT was highly specific (100% positive predictive value), with a sensitivity exceeding 85% compared to the GTT performed directly from fungal colonies. The direct GTT was simple to perform and was compatible with both major automated blood cultures systems in common use, although extrapolating these results to other blood culture systems should be done with caution since the effects of different culture media on germination are undefined.

Although still quite high, the sensitivity observed for the direct GTT here was lower than that reported previously (10). Several factors may have contributed to this observation. First, in the previous study, only 13 *C. albicans* strains were examined, whereas 51 strains of *C. albicans* were evaluated here. Indeed, although the majority of strains grew to a similar density in blood culture bottles, we observed that some isolates were less abundant upon initial Gram staining of the positive blood culture bottle. At least two of the four false-negative direct GTT results were associated with these slower-growing strains. Finally, the present study was performed as part of the daily flow of the microbiology laboratory, with multiple technicians reading the GTT, rather than a single study investigator, as was previously reported. Both of these factors are likely to increase the chances of a discrepant result but are more indicative of the performance of the direct GTT in the routine clinical laboratory.

Although not as sensitive as the GTT performed from a fungal colony, reliable identification of more than 85% of *C. albicans* isolates on the day of detection of candidemia is a significant clinical improvement over existing fungal identification strategies. Thus, the direct GTT should be considered for inclusion in the algorithm for the rapid presumptive identification of *C. albicans* species recovered from blood cultures and could contribute to the improved use of antifungal antibiotics (1).

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