Utility of the Germ Tube Test for the Identification of *Candida albicans* Directly from Positive Blood Culture Bottles.

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Abstract. We compared the germ tube test for the identification of *C. albicans* directly 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 compared results obtained from fungal colonies.

Introduction. 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 from 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, and up to 72 hours before identification can be performed. In a preliminary report, Terlecka and colleagues performed the germ tube test directly from 31 BacTAlert blood culture bottles positive for yeast on gram stain, thirteen of which were *C. albicans* (11). Although the numbers were limited, they observed 100% concordance between the direct GTT and the GTT performed from the subcultured organisms grown on solid media. 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.
Methods. During a two year period, all positive blood cultures in which yeast were visualized by gram
stain 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 (bioMerieux
Inc. Marcy l’Etoile, Fr.), while the Bactec system (BD Diagnostics, Oakville, ON) was used at the Royal
Victoria Hospital. All positive cultures were subcultured to Sabouraud-dextrose agar, and a direct germ
tube test performed. To perform the direct germ tube test, 10-20 µl of blood culture bottle contents
were removed and incubated with 0.5 of rabbit serum for 3 hours at 37°C. The presence or absence of
germ tubes was recorded. When sufficient growth was obtained on solid agar, a standard germ tube
test was performed by inoculating 0.5ml of citrated rabbit serum with a loopful of the test strain and
incubating at 37°C for 3 hours. All isolates were then completely identified using the API20C AUX, the
Vitek YBC (bioMerieux Inc.Marcy l’Etoile, Fr) or the Auxacolor 2 (Bio-Rad Marnes-la Coquette, Fr)
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 germ
tube test. All isolates had been identified previously using either the Vitek YBC (bioMerieux Inc.Marcy
l’Etoile, Fr) and or the Auxacolor 2 (Bio-Rad Inc.Marnes-la-Coquette, Fr) identification systems. Briefly,
blood culture bottles were inoculated with 10ml of whole human blood (Biological Specialty
Corporation, PA ), and then infected with 1ml of sterile water containing between 10-50 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 flagged
positive, and compared with the GTT performed from a subcultured colony.
Results: Sixty seven positive blood cultures were positive for yeast on gram stain and prospectively tested using the direct germ tube method (Table 1). The majority of blood cultures were positive within the first 48 hours of incubation. No false positive germ tube results were observed for non-albicans Candida species. 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 was 87.1% (95% confidence interval [CI], 69.2-95.8%) and 100% (95% CI, 87.9-100%) respectively. A 100% concordance between direct and colony GTT was observed for the experimentally inoculated strains, yielding a sensitivity and specificity of 100% (95% CI of 80.0-100%, and 90.3-100% respectively) for these isolates (Table 2). For all samples tested the overall sensitivity of the direct GTT was 92.2% (95% CI, 80.3-97.5%) and the specificity was 100% (95% CI, 94.4-100%).

Discussion: Clinical guidelines for the treatment of candidemia have 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 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 morphologic features of clustered pseudohyphae observed on Gram stain (3). A sensitivity and 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 yeast. Performing the GTT directly from the positive blood culture
bottle greatly reduces the time to reporting of preliminary speciation, as no culture time is required before reporting. From our clinical samples, the direct GTT was highly specific (100% positive predictive value), with sensitivity exceeding 85% when compared with the germ tube test performed directly from fungal colonies. The direct germ tube 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 as the effects of different culture media on germination are undefined.

Although still quite high, our sensitivity of the direct GTT was lower than that reported previously (10). Several factors may have contributed to this observation. First, in the previous report, only thirteen \textit{C. albicans} strains were examined, whereas 51 strains of \textit{C. albicans} were evaluated in this study. 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 stain of the positive blood culture bottle. At least two of the four false negative direct GTT were associated with these slower growing strains. Finally, the current 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.

While not as sensitive as the GTT performed from a fungal colony, reliable identification of over 85% of \textit{C. albicans} 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 \textit{C. albicans} species recovered from blood cultures, and could contribute to improved use of antifungal antibiotics (1).
Table 1. Concordance between the direct GTT and colony GTT for fungal isolates recovered from blood culture bottles. For all four discordant results the direct GTT was negative while the colony test was positive. * Other species include two each *C. neoformans* and *C. lusitaniae*.

<table>
<thead>
<tr>
<th></th>
<th>Concordant</th>
<th>Discordant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Other*</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>63</strong></td>
<td><strong>4</strong></td>
</tr>
<tr>
<td>Strain</td>
<td>Number of isolates</td>
<td>Direct GT+ (%)</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------</td>
<td>----------------</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>20</td>
<td>20 (100)</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>5</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. lipolytica</em></td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>15</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>5</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>2</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 2. Number of isolates and species tested in seeded blood culture bottles. All *C. albicans* strains were GTT positive when tested directly from the colony, and all non-*albicans* species were GTT negative when tested directly from the colony.
References


