

# Detection of *Candida albicans* from Positive Blood Culture Bottles

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## Abstract

**Objective:** To determine the efficacy of direct germ tube method in early detections of *Candida albicans*.

**Introduction:** Blood stream infections (BSIs) caused by *Candida* species are on the increase and associated with high mortality rates, which are due in part to delay in the administration of appropriate anti-fungal therapy. Earlier identification of yeast isolates from blood cultures may improve clinical outcomes. Identification of *Candida* as *albicans* or non-*albicans* species depends on the presence or absence of germ tubes. Rapid differentiation of *C. albicans* from non-*albicans* is made on its ability to produce germ tubes after incubation in human serum.

**Materials and Methods:** Germ tube test (GTT) is performed on colonies grown on agar plate after 24-48 h of incubation. In our study, we performed GTT directly on the aliquot taken from positive blood culture bottles and yeast cells seen in Gram stain. The results were compared with GTT using the conventional method.

**Results:** Out of 100 positive blood cultures with yeast like cells on gram stain only eight were positive for *Candida albicans* by both the methods. There was 100 % concordance between the direct GTT and the GTT performed from the subcultured organisms grown on solid media.

**Conclusion:** Early detection of species of *Candida* using the Direct Germ Tube method is of utmost importance for early initiation of appropriate anti-fungal therapy in order to reduce morbidity and mortality of patients with candidemia.

**Key words:** *Candida albicans*, germ tube, identification.

## Introduction

*Candida albicans* forms part of the normal flora of the skin, oral cavity, intestinal tract and vagina. Its isolation is associated with both infection and colonization. It is the most frequently isolated yeast in clinical laboratories.<sup>1</sup> Once considered

primarily nosocomial infection, acquired most commonly in intensive care units (ICUs), candidemia is now being encountered in general medical inpatient units as well as the outpatient setting.<sup>2, 3</sup> Candidemia has been associated with high mortality rates, ranging from 30 to 81%. and prolonged hospital stays.<sup>4,5</sup> *Candida* bloodstream infections are of particular concern for patients who are immunocompromised, admitted in intensive care settings, with central lines, receiving

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parenteral nutrition or on prolonged broad-spectrum antibiotics. A recent study reported that the incidence of candidemia in adults increased by 50% from 2000 to 2005.<sup>6</sup> *Candida albicans* remains the leading cause of *Candida* bloodstream infections.<sup>7</sup> However, the prevalence of non-*albicans* *Candida* infections continues to increase, comprising 35% to 65% of all *Candida* bloodstream infections. The most common non-*albicans* *Candida* infections are *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*.<sup>8</sup> *C. albicans* being the most common cause of fungal blood stream infections in ICUs accounting for 64.2% of the candidemia. The clinical outcome can be improved by early identification of the yeast isolates and prompt administration of appropriate antifungal therapy.<sup>9</sup> *C. albican* remains susceptible to azoles. The widespread inappropriate use of Azoles for the treatment of non-*albicans* *Candida* may encourage proliferation of treatment resistant *Candida* which are either intrinsically resistant to Azoles or develop resistance during treatment.<sup>10 - 12</sup> Germ tube test (GTT) is simple, rapid and easy to perform for the identification and differentiation of *C. albicans* from Non- *albicans* in Microbiology laboratories. Harrington et al recently reported a method based on the morphologic features of clustered pseudohyphae observed on Gram stain.<sup>13</sup> GTT is performed conventionally on colonies grown on agar plate after 24- 48 hours of incubation. Donald et al reported performing GTT directly from Blood culture bottles.<sup>14</sup> In this study GTT was performed directly on aliquot of blood taken from a positive blood culture bottle after yeast cells were seen in Gram stain; preventing 24- 48 hours delay in yeast identification. This will be helpful in timely administration of appropriate antifungal therapy hence reducing patient morbidity and mortality.

## Materials and Methods

This study was conducted in the Microbiology Department of Sindh Institute of Urology and Transplantation Karachi. A total of 100 positive blood cultures in which yeasts were visualized by gram stain were sub-cultured on Sabouraud's Dextrose Agar and a Direct Germ tube test was performed at the same time.

### Direct Germ Tube Test:

20 µl of the contents was withdrawn from positive blood culture bottle and incubated with 0.5 ml of human serum for 3 hours at 37°C. The presence or absence of germ tubes was recorded. When sufficient growth was obtained on Sabouraud's Dextrose agar a standard germ tube test was performed by inoculating 0.5ml of human plasma with a loopful of the test strain and incubating at 37°C for 3 hours. All isolates were also inoculated on Cornmeal and Chromagar for further identification of *Candida* species by observing colonial morphology. The isolates with a positive germ tube test were also confirmed by using API 20 C AUX.

## Results

Out of the 100 positive blood cultures tested using the direct Germ tube method 9 isolates were germ tube positive. All these 9 isolates were also Germ tube positive using the conventional Germ tube method. Isolates negative for direct germ Tube test were also negative using conventional GTT method performed from the growth obtained on Sabouraud Agar. There was 100 % concordance between Direct and conventional GTT. No false positive germ tube tests were obtained using the Direct GTT from non - *albicans* species. The colony morphology of all the isolates on Cornmeal and Chromagar were consistent with the germ tube findings of the isolates.

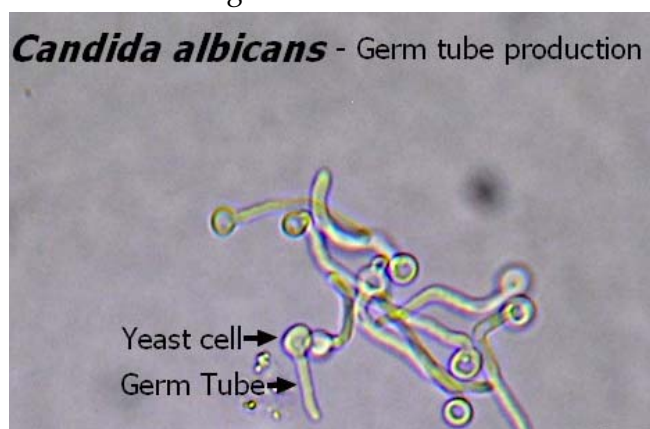
Table 1 showing the distribution of various *Candida* species isolated.

Species	Positive	Negative
<i>Candida albicans</i>	9	-
<i>Candida lusitaniae</i>	-	42
<i>Candida parapsilosis</i>	-	45
<i>Candida glabrata</i>	-	2
<i>Candida tropicalis</i>	-	2

Table 2 Showing the Result of Germ tube formation both by Direct and Conventional methods

Species	Number	Percent
<i>Candida albicans</i>	9	9
<i>Candida lusitaniae</i>	42	42
<i>Candida parapsilosis</i>	45	45
<i>Candida glabrata</i>	2	2
<i>Candida tropicalis</i>	2	2

Picture 1 showing the formation of Germ Tube.



Results obtained by API Aux were also consistent with the Direct GTT. All isolates with positive Direct GTT were identified as *Candida albicans*.

## Discussion

The GTT is a routine procedure for identification of medically important yeast. Performing the GTT directly from the positive blood culture bottle greatly reduces the reporting of preliminary speciation, as no subculture time is required saving 24-48 hours before reporting. The direct GTT is equally specific and sensitive (100%) compared with the germ tube test performed from fungal colonies obtained after 24 - 48 hours of incubation. The test is simple to perform, and compatible with the automated blood culture systems in common use saving precious time of identification.

Reliable identification of *C. albicans* on the day of detection of candidemia is a significant 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 contributing to earlier initiation of appropriate antifungal agents. Guidelines for the treatment of candidemia recommend a species specific approach so various attempts are being made to device procedures for early speciation of candida. Harrington et al reported another method for early detection of *Candida* species which is heavily dependent on the trained user.<sup>13</sup> The GTT has been a long well established routine procedure. Our results are in concordance with the studies done by Donald et al.<sup>14</sup>

## Conclusion

Early detection of species of *Candida* using the Direct Germ Tube method is of utmost importance for early initiation of appropriate antifungal therapy in order to reduce morbidity and mortality of patients with candidemia

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