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Use of Chromogenic Tube and Methyl Blue-Sabouraud Agar for the Identification of Candida Albicans Strains

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This study was performed to investigate the use of chromogenic tube and methyl blue-Sabouraud agar for the presumptive identification of *Candida albicans.* 124 clinical isolates, including 111 *C.albicans* and 13 *Candida spp* strains, which had been identified by morphology on cornmeal tween 80 agar and Vitek automated identification system, were included. Three different identification procedures, a) germ tube test, b) chromogenic tube test by using CHROMagar Candida and c) methyl blue-Sabouraud agar test, were performed to the strains. 88 of 111 (79.3%) *C.albicans* strains were detected to be positive by germ tube test. 87 (78.4%), 97 (87.4%) and 102 (91.9%) of these isolates were identified as *C.albicans* by chromogenic tube test after 2, 8 and 24 hours of incubation, respectively. 88 (79.3%), 92 (82.9%) and 88 (79.3%) of the isolates were correctly identified as *C.albicans* by methyl blue-Sabouraud agar test after 2, 8 and 24 hours of incubation, respectively. The sensitivity and specificity values were found to be 79.3 and 69.2 for the germ tube test. These values ranged between 78.4-91.9% and 69.2-76.9% for chromogenic tube test and 79.3-82.9% and 76.9-84.6% for methyl blue-Sabouraud agar depending on the incubation period. It can be concluded that the use of chromogenic tube and methyl blue-Sabouraud agar are rapid, simple and objective methods for the identification of *C.albicans* strains.

The identification to the species level of yeasts, cultured from various specimens has become increasingly necessary for clinical laboratories (8). Since *Candida albicans* is the yeast species most often isolated from clinical specimens and is frequently of clinical importance (1), its rapid and true identification is the most important point.

Generally, the yeast identification procedures start with germ tube test in clinical laboratories, which is a rapid method to differentiate *C.albicans* from other Candida species. Although this is a rapid test, it may lead to both false positive and false negative results (4). When the yeast cannot be named with this method, further tests such as culturing on cornmeal agar, carbohydrate assimilation and fermentation tests and automated identification procedures are done (18). These procedures are labor-intensive and can take 18 to 72 hours, which is rather a long period to determine the diagnosis and antifungal therapy.

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Several culture media containing fluorogenic or chromogenic substrates specific for *C.albicans* had been developed for the rapid and reliable identification of *C.albicans* strains. These include Pagano Levin agar, Sabouraud trifeniltetrazolium agar and Albicans ID agar (13,16,20). CHROMagar Candida is a new commercial medium for the identification of Candida species. *C.albicans* strains produce β-N-acetylgalactosaminidase, which interacts with the chromophore (chromonogenic hexosaminidase substrate) incorporated into the agar, and with incubation produces green colonies (2). Another similar type medium is methyl blue-Sabouraud agar. Although the exact reaction between the methyl blue-Sabouraud dye and *C.albicans* is not known, there is a possible reaction with specific cell wall polysaccharides which produces the fluorescent metabolite (6,15). In this study the use of chromogenic tube and methyl blue-Sabouraud agar were evaluated for the presumptive identification of *C.albicans.*

MATERIALS AND METHODS

Candida Isolates:

124 randomly selected clinical isolates, including 111 *C.albicans,* 4 *C.parapsilosis*, 3 *C.kefyr*, 2 *C.krusei*, 2 *C.guilliermondii*, and 2 *C.tropicalis* strains were used in this study. The strains had been identified by morphology on cornmeal tween 80 agar, carbohydrate fermentation tests, and Vitek 32 by using yeast identification card YBC V1303 (BioMérieux). *C.albicans* ATCC 90028, *C.parapsilosis* ATCC 90018 and *C.krusei* ATCC 6258 were used as control strains.

Identification Procedures:

Three different identification procedures, a) germ tube test, b) Chromogenic tube test and c) methyl blue-Sabouraud agar test, were performed to the 24 hour cultures of the isolates on Sabouraud dextrose agar. The results were interpreted by two observers blindly.

a) Germ Tube Test: This test was done by using colonies of the isolates grown on Sabouraud dextrose agar at 30°C for 24 hours as described previously (18) and interpreted after 3 hours of incubation at 37°C.

b) Chromogenic Tube Test: This test was performed according to the method of Merlino et al. (9). CHROMagar Candida (CHROMagar Company, France) is a commercial medium supplied as dehydrated powder in preweighed quantities. It was prepared according to the manufacturer's instructions. 1 ml was dispensed into sterile 5 ml tubes with a pipette and allowed to set at room temperature and stored at 4°C for a week. A colony from 24-hour cultures of the *Candida* strains were removed from Sabouraud dextrose agar, then stepped and spread onto the surface of the CHROMagar Candida medium with a sterile straight inoculating wire. Inoculated tubes were placed into an incubator in air at 37°C in the dark and the results were read according to the color and morphology of the colonies after 2, 8, and 24 hours of incubation. Green color development at any stage indicated a positive test for *C.albicans* and any other color was interpreted as being negative for *C.albicans* as described by Odds and Bernaerts (10).

c) Methyl Blue-Sabouraud Agar Test: This fluorometric test was performed according to the methods of Goldschmidt and Schoofs (6, 15). The media was prepared by adding 0.01% methyl blue dye (Color Index: 42780) into Saboraud dextrose agar before autoclaving. Overnight colonies were then transferred to

petri dishes containing this media. Petri dishes were incubated at 37°C and the results were evaluated after 2, 8, and 24 hours of incubation. The colonies were evaluated under UV lamp (wavelength 365 nm). The colonies, which did not fluoresce, were interpreted, as being non *C.albicans* species while the colonies that fluoresced brightly were considered to be *C.albicans* (6, 15).

Statistical analysis:

The sensitivity, specificity, positive and negative predictive values were calculated for the germ tube, chromogenic tube and methyl blue-Sabouraud agar tests (5)

RESULTS

All three-control strains grew in the CHROMagar Candida tubes and *C.albicans* was clearly distinguished from all of the other strains by its green color after 2 hours of incubation as seen in Figure 1. *C.krusei* and *C.parapsilosis* could also be observed as different colors from *C.albicans* after this incubation period.

88 of 111 (79.3%) *C.albicans* strains were detected to be positive by germ tube test. 87(78.4%), 97(87.4%) and 102(91.9%) of these isolates were identified as *C.albicans* by chromogenic tube test after 2, 8 and 24 hours of incubation, respectively. The colonies on methyl blue-Sabouraud agar were evaluated under UV lamp (wavelength 365 nm) and *C.albicans* colonies fluoresced brightly (Figure 2). 88(79.3%), 92(82.9%) and 88(79.3%) of the isolates were correctly identified as *C.albicans* by methyl blue-Sabouraud agar test after 2, 8 and 24 hours of incubation, respectively. The sensitivity, specificity, positive and negative predictive values for germ tube, chromogenic tube and methyl blue-Sabouraud agar tests are shown in Table Ⅰ.

Table Ⅰ. The sensitivity, specificity, positive and negative predictive values for the tests performed

Test Name	Sensitivity (%) (95% Confidence Interval)	Specificity (%) (95% Confidence Interval)	Positive Predictive Value (%) (95% Confidence Interval)	Negative Predictive Value (%) (95% Confidence Interval)
GTT	79.3 (70.3-86.2)	69.2 (38.9-89.6)	95.7 (88.6-98.6)	28.1 (14.4-47.0)
CTT after 2 hours	78.4 (69.4-85.4)	69.2 (38.9-89.6)	95.6 (88.5-98.6)	27.3 (13.9-45.8)
CTT after 8 hours	87.4 (79.4-92.7)	69.2 (38.9-89.6)	96.0 (89.6-98.7)	39.1 (20.5-61.2)
CTT after 24 hours	91.9 (84.8-96.0)	76.9 (46.0-93.8)	97.1 (91.3-99.3)	52.6 (29.5-74.8)
MBSA after 2 hours	79.3 (70.3-86.2)	76.9 (46.0-93.8)	96.7 (90.0-99.1)	30.3 (16.2-48.9)
MBSA after 8 hours	82.9 (74.3-89.1)	84.6 (53.7-97.3)	97.9 (91.8-99.6)	36.7 (20.5-56.1)
MBSA after 24 hours	79.3 (70.3-86.2)	84.6 (53.7-97.3)	97.8 (91.4-99.6)	32.4 (18.0-50.6)

GTT: Germ tube test

CTT: Chromogenic tube test

MBSA: Methyl blue-Sabouraud agar

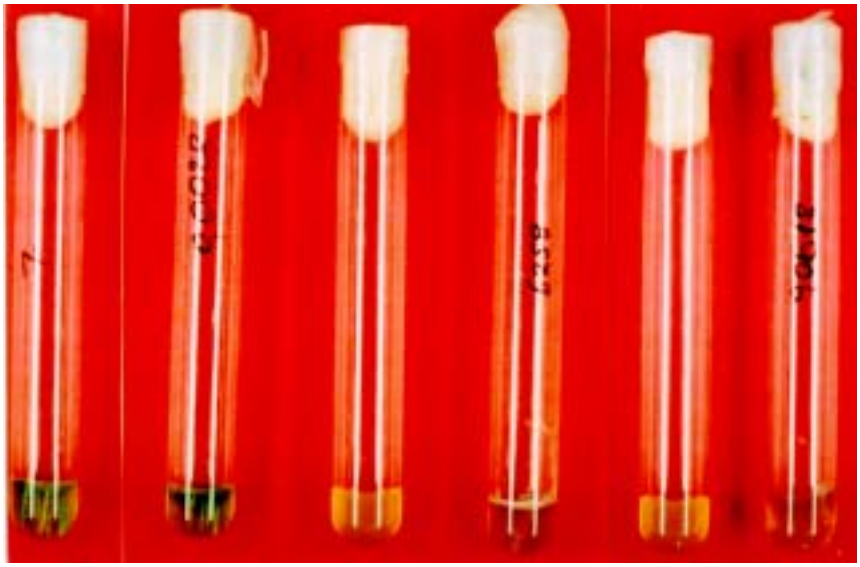

Figure 1. A clinical isolate of *C.albicans*, *C.albicans* ATCC 90028, uninoculated tube, *C.krusei* ATCC 6258, uninoculated tube and *C.parapsilosis* ATCC 90018 (From left to right)

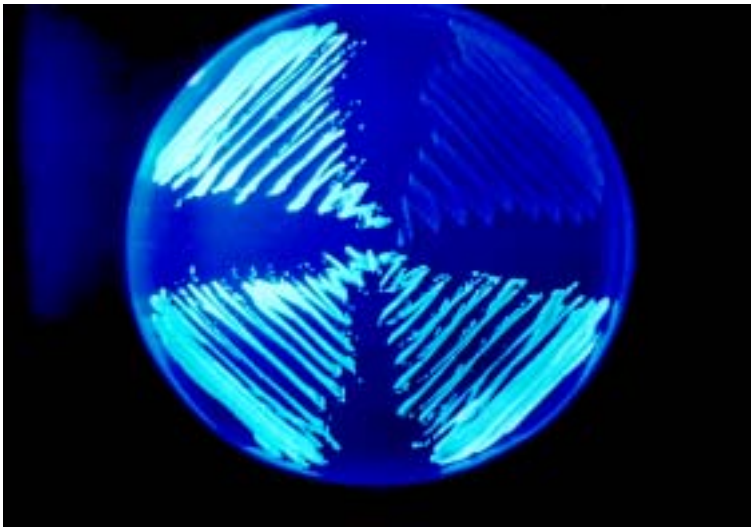

Figure 2. The appearance of 3 *C.albicans* and one *C.parapsilosis* strain on methyl blue agar

DISCUSSION

Isolation and identification of the pathogenic yeasts are required for early diagnosis and therapy. Rapid presumptive identification of *C.albicans* has been based on the demonstration of germ tube formation in serum (17). It is a simple and rapid test however; it uses potentially dangerous sera and problems with misinterpretation and false positive results due to *C.tropicalis* are not rare (4). Furthermore, it has been stated that 5% of *C.albicans* isolates do not produce germ

tubes (12). The sensitivity and specificity values obtained for germ tube test in our study were 79.3 and 69.2 %, respectively. Ainscough et al. (1) found these values as 84.6 and 100%, respectively. In the same study 2 isolates produced negative germ tube results but were found to be *C.albicans* by biochemical identification. In another study these percentages were calculated to be over 97% for this test (7). In Merlino et al's (9) study, all *C.albicans* isolates were found to be positive by germ tube test however 2 *C.tropicalis* gave false positive by germ tube test. Our values are lower than the results of the other studies, which may result from using human sera, different strains. Another reason might be that most of our strains had been isolated from immunocompromised patients or from patients who were under antifungal therapy. In our study, different Candida species (1 *C.kefyr,* 1 *C.guilliermondii and 2 C.tropicalis*) were misinterpreted as positive by germ tube test.

Several studies investigated the usefulness of CHROMagar Candida media for the identification of yeast and many researchers have found CHROMagar Candida plates to be effective for the presumptive identification of *C.albicans, C.tropicalis* and *C.krusei* (1,3,7,10,11,14,19). The sensitivity and specificity values obtained for *C.albicans* strains ranged between >97-100% and 100%, respectively (1,3,7,11,14,19). In these studies CHROMagar Candida plates were used however in our study CHROMagar Candida tubes were used. The sensitivity and specificity values were found to be between 78.4-91.9 and 69.2-76.9 depending on the incubation period. Merlino et al (9) detected two false positive results due to *C.tropicalis* strains after 2.5, 4 and 8 hours of incubation but neither false positive nor false negative results were obtained after 24 and 48 hours of incubation. In our study both sensitivity and specificity values increased in parallel to the incubation period. While the sensitivity was 78.4% after 2 hours of incubation, it was 91.9% after 24 hours. We also found similar results for the specificity. After 2 and 8 hours of incubation 2 *C.parapsilosis*, 1 *C.tropicalis, and* 1 *C.kefyr* isolates were false positive. After 24 hours, while *C.parapsilosis* and *C.tropicalis* isolates remained the same; *C.kefyr* isolate turned to be negative. Our sensitivity and specificity values for CHROMagar Candida test were lower than the other studies which might be due to the fact that in this study we used CHROMagar tubes instead of CHROMagar petri dishes which gave the opportunity of observing the colonies in detail.

Our study show that when a small volume of CHROMagar Candida medium was used, the inocula provided a high concentration of species-specific enzymes which results in a shorter incubation period for identification. This allows a rapid identification of *C.albicans* isolates. CHROMagar Candida tubes decrease the cost so effectively by using very small volumes. Merlino et al (9) added that CHROMagar Candida was labor intensive during interpretation. It has also been reported that there was also the possibility to leave the medium overnight to be able to get more precise results (9). CHROMagar Candida tubes were found to be very helpful, allowing identification of *C.albicans* colonies. Thus this easy to use, inexpensive and time saving medium appears to be well suited for routine purposes in clinical microbiology laboratories.

Organic dyes have been used for a long time in diagnostic microbiology to differentiate species by color reactions. Methyl blue-Sabouraud agar is another dye containing media used to differentiate *C.albicans* from other species. The dye is autoclavable, so this medium is easy to prepare and evaluate. However this test is hard to evaluate with naked eye and requires the use of a UV lamp. The sensitivity

and specificity values were found to be between 79.3-82.9 and 76.9-84.6 for our strains depending on the incubation period. Higher values were obtained for both sensitivity and specificity after 8 hours. There seemed to be no possible reason why these 4 strains fluoresced at 8 hours of incubation but didn't fluoresce after 24 hours. These strains gave the same result when the test was repeated. Goldschmidt et al (6) found these values as 98.0 and 99.5%, respectively after 24-hour incubation. Our values are somewhat lower than the ones found by Goldschmidt, this may be due to different strains or interpretation.

It can be concluded that the use of chromogenic tube and methyl blue-Sabouraud agar are rapid, simple and objective methods for the identification of *C.albicans* strains.

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