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ORIGINAL RESEARCH

Comparison of seven different liquid media for germ tube test for candida albicans

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ABSTRACT

Infections produced by yeast of the genus Candida are the most frequent fungal infections, with Candida albicans being the most prevalent isolated species. The quick identification of this yeast is mostly based on the formation of germ tubes in human or animal serum. This study details the utilisation of seven different liquid media for germ tube development at 2, 2.5, 3, and 4 hours. We looked at 193 yeasts for germ tube formation, including 157 (81.3 percent) C. albicans. At 2 hours, C. albicans germ tube development was most prevalent in human serum (98%), followed by brain heart infusion broth (84%), and tryptic soy broth (65.6%).Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for human serum germ tube formation at 2 h were 98 percent, 100 percent, 100 percent, and 92.3 percent, respectively. An incubation period of more than 2 hours improves sensitivity but decreases specificity, as well as the PPV and NPV of the germ tube test in all tested sera (GTT). In conclusion, with a 2-hour incubation period, human serum was shown to be the most acceptable medium for GTT.

Key words: Germ tube, Candida albicans, Liquid media, Positive predictive value, negative predictive value.

INTRODUCTION

Candida species are identified based on their morphological and biochemical characteristics, such as the appearance and colour of colonies on primary isolation media (Sabouraud or CHROMagar), cell size and shape, production of hyphae and/or pseudohyphae and/or chlamydoconidia, ability to produce germ tubes, sugar fermentation, and nitrate and carbohydrate assimilation [1, 2, 3]. Some traditional identification methods, notably biochemical testing, are time-consuming and labor-intensive to execute [4]. Candida albicans is the most common fungal pathogen isolated in humans. The observation of germ tube formation is a way for assuming C. albicans identification. When yeast cells are cultured in serum at 30–37^0 C for 2–4 hours, they create short, slender tube-like structures (germ tubes) [5]. It is a straightforward, quick, easy-to-implement, low-cost, and well-known method. However, up to 5% of C. albicans isolates have been shown to be germ tube negative [6, 7]. It has also been observed that yeasts other than Candida albicans, such as Candida tropicalis, Candida parapsilosis, Candida dubliniensis, and Cryptococcus gastricus, can generate germ tubes [4, 8, 9].

Other media that stimulate germ tube production, in addition to human serum, include plasma, saliva, sheep serum, foetal bovine serum, rabbit serum, and horse serum. Newer strategies for germ tube generation have been investigated employing serum-free media such

as egg white, YEPD medium, tissue culture medium 199, trypticase soy broth, rice cream agar, 2 percent oxgall broth, rice infusion oxgall Tween 80, Mueller Hinton agar, and several other peptone media [10, 11, 12]. These are safer than the traditional approach, which employs human serum. It may be contaminated with HIV and hepatitis viruses, and it is simple to execute because the time required to produce human serum is reduced.C. albicans identification based on the ability to construct germ tubes at39 C in serum-free YEPD media produced more reliable results than established procedures such as the serum-induced germ tube test, chlamydospore test, and colony colour test in chromogenic media [13, 14].
The purpose of this study was to analyse the use of seven different liquid media for the creation of germ tubes at four different incubation times, as well as to discuss the value of the germ tube test (GTT) for C. albicans species identification.

MATERIAL AND METHODS

CANDIDA STRAINS

C. albicans was found in 157 (81.3 percent) of the 193 strains tested. The isolates were isolated from vagina (37%) blood (25%), urine (22%), and oral specimens (16 percent). All isolates were identified using micromorphology on Dalmau cornmeal agar-Tween 80 and usingVITEK 2 and VITEK MS (MALDI-TOF MS, bioMerieux India Pvt. Ltd.) system. Candida albicans was distinguished from Candida dubliniensis using phenotypic techniques such as chlamydoconidia production on cornmeal agar Tween-80, and growth at 42–45 C. Candida albicans ATCC 10231 was subjected to the same tests as the other strains.

MEDIA

Human serum, tryptic soy broth (TSB), peptone broth, brain-heart infusion broth (BHIB), yeast nitrogen base broth (YNBB), sterile coconut broth and 2% sucrose were used.

PROCEDURE

As an inoculum, freshly produced yeasts on Sabouraud glucose agar after 24 hours at 37 C were employed. At first, each isolate was coded and tested blindly, and the data were gathered at the end of the study. All strains were tested for their capacity to produce GTT at the same time. Germ tubes were described as filamentous outgrowths from blastoconidium that were at least twice as long as the parent cell and did not constrict at the junction. The isolated yeast colony was inoculated in glass tubes with 1 ml of various media at a concentration of roughly 105–106 cells ml), then incubated at 37 C for 2, 2.5, 3, and 4 hours. A drop of yeast inoculum was then placed on a clean microscope slide, followed by a coverslip. If at least 100 yeast cells did not have germ tubes, the examination of the wet mount preparation under the 40x microscope objective was reported negative. All experiments were carried out in duplicate.

STATISTICAL ANALYSIS

The specificity (number of false positives + number of true negatives/numbers of true negatives), sensitivity (number of false negatives + number of true positives/numbers of true positives), negative predictive value (number of false negatives + number of true negatives/numbers of true negatives) and positive predictive value (number of false positives + number of true positives/numbers of true positives) of the seven liquid media were compared and analysed.

RESULTS

The efficiency of GTT for yeast of the genus Candida was tested in seven different liquid media using natural and synthetic culture media across time periods ranging from 2 to 4

hours. C. albicans formed germ tubes that were not constricted at their points of origin on the parent cells and were longer than the latter at 2 h. C. albicans germ tubes were enlarged at their places of origin, i.e., pseudohyphae, at 2.5 h. (Fig. 1a, b). Both species displayed extended germ tubes and hyphae/pseudohyphae at the third and fourth hour.Candida albicans produced the most germ tubes in human serum (98 percent), followed by BHIB (84 percent) and tryptic soy broth (65.6 percent) at 2 hours. Other sera and liquid mediums performed less well (Table 1). At 2 h, the sensitivity, specificity, PPV, and NPV for human serum germ tube formation were 98 percent, 100 percent, 100 percent, and 92.3 percent, respectively. An incubation period of more than 2 hours improves sensitivity but decreases specificity, PPV, and NPV of GTT in all studied sera (Table 2).

DISCUSSION

Rapid identification of Candida albicans as the primary agent of candidosis is a serious concern for mycology laboratories. In the last two decades, a range of technologies for recognising C. albicans from clinical specimens have been commercialised for this purpose. When compared to the GTT, these tests were reported to be faster and more sensitive in the identification of Candida albicans. However, because GTT is a simple and inexpensive alternative to other rapid test procedures, it may be preferred by laboratories looking to save money.

Table 1: Germ tube production of Candida albicans in 7 liquid media at different incubation times

	C. albicans (n = 157)			
Sera	**2 h**	**2.5 h**	**3 h**	**4 h**
Pooled human serum	154	156	157	157
Peptone water	90	97	101	108
Yeast nitrogen base broth	93	125	126	137
2% sucrose	55	71	93	118
Brain heart infusion broth	132	137	143	146
Trypticase soy broth	103	108	112	134
Sterile coconut water	67	107	114	116

For many years, the observation of germ tube production in serum has been widely used as a method for presumptive identification of Candida albicans, and the results have been quite reliable [15, 16]. Even though this is a rapid test, misinterpretation of elongated blastoconidia as germ tubes or lack of germ tube production in some strains of Candida albicans, as well as positive results observed for other species due to increased incubation time and false negatives due to heavy inoculum, may pose a problem [17]. Because of the health risks associated with handling pooled sera, the test necessitates a moderate to high level of skill.

As a result, it appears reasonable to study whether there are any conditions that allow for 100 percent specificity and 100 percent sensitivity. GTT was reported to have sensitivities ranging from 94.1 percent to 99.1 percent and specificities ranging from 94.4 percent to 100 percent in the literature. Heelan et al. [3] discovered that four drops of rabbit plasma and TSB each increased the sensitivity and specificity of the GTT by 100 percent. Kim et al. [17] reported that Candida albicans was the only species (including C. dubliniensis) capable of producing germ tubes at 39 C for 1 hour on serum-free yeast extract peptone dextrose media. According to Arora et al. [20], human serum remains the best with 100 percent positive for C. albicans identification, followed by horse serum (76.3 percent), peptone water (61.8 percent), and TSB (61.8 percent). As a result, we set out to discuss the utility of GTT in seven liquid mediums, with a particular focus on the identification of Candida albicans. Initially, phenotypic methods were used to distinguish C. albicans strains from C. dubliniensis. Pooled human serum (98 percent) was shown to be the best medium for the GTT, followed by BHIB

(84 percent), whereas 2% sucrose (35 percent) was found to be the poorest at 2 h. Human serum with a 2-hour incubation period yielded the highest sensitivity and specificity, as well as the highest PPV and NPV. An incubation duration of more than 2 hours, on the other hand, increased sensitivity while decreasing specificity of GTT(Table 1). As a result, at the third and fourth hours, specificity and NPV were discovered to be zero (Table 2).

Table 2: Specificity, sensitivity, NPV and PPV of germ tube production in seven liquid media at different incubation times

	Specificity				Sensitivity				NPV				PPV			
Sera	**2h**	**2.5 h**	**3 h**	**2h**	**2.5 h**	**3h**	**4h**	**4 h**	**2h**	**2.5 h**	**3h**	**4h**	**2h**	**2.5 h**	**3 h**	**4 h**
Pooled human serum	100	80.5	0	100	95.7	81.3	81.3	0	98.0	99.3	100	100	92.3	96.6	0	0
Peptone water	100	83.3	0	100	94.1	73.7	75.0	0	57.3	61.7	64.3	68.7	34.9	33.3	0	0
Yeast nitrogen base broth	100	80.5	0	100	94.6	77.7	79.1	0	59.2	79.6	80.2	87.2	36.0	47.5	0	0
2% sucrose	100	83.3	0	100	92.2	72.0	76.6	0	35.0	45.2	59.2	75.1	26.0	25.8	0	0
Brain heart infusion broth	100	80.5	0	100	95.1	79.8	80.2	0	84.0	87.2	91.0	92.9	59.0	59.1	0	0
Tryptic ase soy broth	100	80.5	0	100	93.9	75.6	78.8	0	65.6	68.7	71.3	85.3	40.0	37.1	0	0
Sterile coconut water	100	80.5	0	100	93.8	76.0	76.3	0	42.6	68.1	72.6	73.8	28.5	36.7	0	0

According to Bruatto et al. [21], filamentous outgrowth in some strains of C. tropicalis cannot be confused with germ tubes because they have a constriction at their place of origin and a bigger diameter. In our investigation, we also saw the development of pseudohyphae cells that resembled germ tubes in serum. C. glabrata could be distinguished from other species in our experience by the absence of germ tube production at any incubation time and the presence of small and oval blastoconidium, whereas C. krusei and C. kefyr produced longer blastoconidium and presented larger and longer germ tubes and pseudohyphae at 2.5 h or later compared to, and thus distinguished from, C. albicans.

In this investigation, pooled human serum exhibited the maximum sensitivity (95.7 percent), which could be attributed to inhibitors in the human serum, yeast cell concentration, and serum storage conditions. Furthermore, YNBB medium had 94.6 percent sensitivity, while Kim et al. [17]found 100 percent positive at 39^0C. The incubation temperature and time may be the reason of variability in germ tube positive rate. In our study, trypticase soya broth exhibited a sensitivity rate of 93.9 percent, which is similar to the findings of Arora et al. [20].

In contrast, Deorukhkar et al. [23] showed greater sensitivity rates of 100% and 94% in Trypticase soya broth, respectively. In this investigation, 36.0 percent of C. albicans isolates were positive for germ tube test in peptone water. Similarly, Deorukhkar et al. [23] showed 94.1 percent sensitivity in peptone water. Germ tube formation in 2 percent Sucrose solution

was only 92.2 percent among the less suitable medium, almost same to how Raghunath and Kumari [22]reported a greater sensitivity rate of 80 percent. This could be because the initial pH permitted germ-tube production to proceed, while a subsequent reduction in pH could prevent germ-tube formation. In conclusion, our findings indicate that human serum was the optimal medium for the GTT, with the best evaluation time at the second hour.

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