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# Evaluation of germ tube test in various media

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**Abstract**
Systemic candidiasis is responsible for a high mortality rate, even after antifungal therapy. *Candida albicans* is the main cause of invasive fungal diseases which is a serious public health issue. Various morphological, biochemical, and molecular methods are available for the identification of *C. albicans* still Germ tube test in a pooled human serum remain the test of choice. It is simple, rapid, and highly reliable test that has been used for many years but handling human serum during test is associated with acquisition of dangerous infection like hepatitis and HIV. In present study we had evaluated germ tube test in various media like 1% nutrient broth, 1% peptone water, 0.5% glucose broth, Glycine (50mg per dL in 0.5% glucose broth), Histidine (50 mg per dL in 0.5% glucose broth) Cystine (50 mg per dL in 0.5% glucose broth) for comparison with recommended standard germ tube testing in human serum.50 strain of *Candida albicans* isolated from 350 samples were subjected to various media for studying production of germ tube. In 98% cases pooled human serum remain the best media. It was observed that, When 72 hour old cultures were used for preparation of test inoculate and *Candida* strain present in it starved for more than 60 minutes chances of germ tube positivity was increased.

**Keywords:** *Candida albicans*, germ tube test, non-albicans *Candida*

## Introduction
Over the past decades there has been a significant increase in the number of reports of mucosal and systemic infections caused by *Candida* species. This is mainly attributed to a dramatic rise in the number of immunocompromised individuals, especially those infected with the human immunodeficiency virus (HIV) and patients receiving immunosuppressive therapy for malignancy and those undergoing transplantation. The wide spread use of broad spectrum antibiotics and the increased use of indwelling catheters and other mechanical devices also are the important contributory factors [1]. *Candida albicans* is the most pathogenic species among the genus *Candida* and is consistently most frequently isolated yeast species from clinical specimen. However, in recent years other species of *Candida* (non-albicans) have been isolated with increasing frequency from suspected cases of infection [2].

*Candida* species are identified by a variety of methods that include germ tube testing, the use of chromogenic media, biochemical and sugar assimilation testing, cornmeal agar morphology. Recently many molecular techniques have been developed for identification of yeast but these methods are expensive and not available routinely in every laboratories [3]. Among all tests, germ tubes production after incubation of yeast colony in human serum was a simple and rapid method for presumptive identification of *Candida albicans* from non-albicans. It is an inexpensive and easy to perform test, no technique expertise required but handling a pooled serum has a danger of acquiring some infections like hepatitis and HIV so inherent safety problems were concerned with its use and even more time required to prepare human serum [4].

The present study was conducted with an aim to evaluate different media like 1% nutrient broth, 1% peptone water, 0.5% glucose broth, Glycine (50mg per dL in 0.5% glucose broth), Histidine (50 mg per dL in 0.5% glucose broth) Cystine (50 mg per dL in 0.5% glucose broth) for comparison with recommended standard germ tube testing in pooled human serum.

## Material and method

Over 350 clinical specimen were collected, from patients admitted in clinical wards of "Shri Chatrapati Shivaji Maharaj Sarvopchar Rugnalaya" Solapur.

The relevant specimen like sputum, urine, pus, or wound discharge from identified clinical sites of infection were collected in sterile containers with all aseptic precaution. All specimens were transported to the laboratory without delay preferably within 30 minutes of collection, if delay was inevitable they were kept at 4 ˚C until transported to the laboratory.

Direct Gram staining was done for preliminary screening of all specimen. After that they were streaked on slants of plain Sabourad's dextrose agar (SDA) and incubated at 37 °C for 48 hours before discarding them as negative. The growth which appeared on SDA slant was identified by conventional method - lactophenol cotton blue mount, Gram staining, germ tube test, growth on corn meal tween 80 agar, carbohydrate assimilation test and carbohydrate fermentation test [5].

*Candida albicans* isolated from specimen were sub-cultured on Sabouraud's dextrose agar and were incubated at 37 $^0$C for 18 to 24 hours before performing the germ tube test. Apart from Germ tube test in pooled human serum it was also attempted in media without serum viz. 1% nutrient broth, 1% peptone water, 0.5% glucose broth, Glycine (50mg per dL in 0.5% glucose broth), Histidine (50 mg per dL in 0.5% glucose. broth), Cystine (50 mg per dL in 0.5% glucose broth) [6, 7, 8].

0.5 ml of all the above media were dispensed into 12x75 mm test tube. The colony was lightly touched with a straight wire and then inoculated in the test tubes containing different media. A positive control (*C. albicans* ATCC 10231) and a negative control (*C. krusei*) were used with each batch of yeasts tested. The test tubes were incubated for 2 hours at 37 $^0$C. For reading the test 1 or 2 drops of content from each test tube was withdrawn with a Pasteur pipette, placed on clean microscopic slide, and examined under objective lens 40X for the presence of germ tube at an interval of 30 minutes, 1 hour and 2 hour. In order to investigate the germ tube structure, the elongated daughter cells from the round mother cell without constriction at their origin were referred to as germ tubes, and constriction hyphae at the round mother cell were referred to as pseudohyphae [9]. A criterion for germ tube positivity was observation of minimum five germ tubes in entire wet mount preparation. Negative results were confirmed by examining at least 10 high power filed [10].

## Result

In present study 97 *Candida* species were isolated from 350 sample. Among them 50 were *Candida albicans* and 47 were non albicans *Candida*. Isolated 50 strain of *Candida albicans* were subjected to different culture media for formation of Germ tube. (Table no 1)

Effect of starvation period and growth phase of culture was also evaluated on production of germ tube. (Table no 2 & 3)

**Table 1:** Showing evaluation of different media for Germ tube formation test for *Candida albicans*

| Sr. no | Different media | No. of *Candida albicans* strain showing Germ tube test positive (n=50) |
|---|---|---|
| 1 | Normal Human serum (Pooled) | 49(98%) |
| 2 | 1% Nutrient Broth | 41(82%) |
| 3 | 1% Peptone Broth | 36(72%) |
| 4 | 0.5% Glucose Broth | 10(20%) |
| 5 | 0.5% Glucose Broth | |
| | Containing<br>Glycine<br>Histidine<br>Cysteine | <br>29(58%)<br>25(50%)<br>05(10%) |

**Table 2:** Showing effect of starvation period of Germ tube positivity of *C. albicans*.

| Sr. no | Different Media | *Candida albicans* (n=50) | | |
|---|---|---|---|---|
| | Time in Minutes | 30 | 60 | 120 |
| 1 | Normal Human serum (Pooled) | 10 | 47 | 49 |
| 2 | 1% Nutrient Broth | 11 | 40 | 41 |
| 3 | 1% Peptone Broth | 16 | 33 | 36 |
| 4 | 0.5%Glucose Broth | 10 | 10 | 10 |
| 5 | 0.5% Glucose Broth Containing<br>Glycine<br>Histidine<br>Cysteine | <br>12<br>05<br>04 | <br>29<br>25<br>05 | <br>29<br>25<br>05 |

**Table 3:** Showing effect of growth phase on Germ tube positivity of *C. albicans*.

| Sr. no | Different Media | *Candida albicans* (n=50) | |
|---|---|---|---|
| | | 24 hrs. Old | 48-72hrs.Old |
| 1 | Normal Human serum (Pooled) | 41(82%) | 49(98%) |
| 2 | 1% Nutrient Broth | 25(50%) | 41(82%) |
| 3 | 1% Peptone Broth | 19(38%) | 36(72%) |
| 4 | 0.5%Glucose Broth | 8(16%) | 10(20%) |
| 5 | 0.5% Glucose Broth Containing<br>Glycine<br>Histidine<br>Cysteine | <br>15(30%)<br>18(36%)<br>17(34%) | <br>29(58%)<br>25(50%)<br>5(10%) |

## Discussion

Rapid identification of *Candida* isolates to the species level in the clinical laboratory has become important because the incidence of candidiasis continues to rise in proportion to a growing number of patients at risk. Although various morphological, biochemical, and molecular methods are available for the identification of *C. albicans*, Germ tube test is a simple, rapid, and highly reliable test that has been used since many years [11].

Many workers have highlighted interesting biological property of *Candida* often associated with conclusion of their pathogenicity [7, 8]. There are theories explaining germ tube forming ability of *Candida*, such as effect of yeast cell concentration, [12] starvation of carbon moiety in the milieu, [13] presence of nitrogen containing nutrients, concentrations of amino acid in the milieu, [8] however, no distinct mechanism was confirmed as responsible for this biological behavior.

Hazen and Cutler (1979) [14] have proposed an interesting mechanism designated as "morphogenic auto-regulatory substance" production. In their opinion this is a major influencing factor in determining Yeast-Mycelial (Y-M) conversion in *C. albicans*. They further add that, the other factors like starvation of carbon, presence of nitrogen, relative concentration of amino acids has only supportive or coincidental influence on germ tube formation. However, metabolic pathways governing respiration, glycolysis, anaerobic fermentation, amino acid degradability through amino peptidase could be responsible for state of the flux observed *in vivo* as well as *in vitro*, between yeast and mycelial forms of *Candida*. [7, 8, 13]

In the present study, germ tube formation of *C. albicans* using different support media was carried out to study the factors governing germ tube formation. Pooled normal human serum was found to be best medium in comparison to other media, similar observations are reported by other workers [4, 7, 8, 12, 15, 16]. (Table no 1). In contrast to this Joshi KR *et al.* [17] and Deorukhkar SC [10] found in their studies that trypticase soya broth was a best medium for production of germ tube. Whereas Makwana GE *et al.* [18] found horse serum to be more effective for germ tube production.

Starvation results into change in glucose metabolism and acquisition of pluripotency i.e. to resume growth in either morphological form. In our study starvation period of 120 minutes (2 hours) yielded highest results of germ tube test positivity than 30 and 60 minutes starvation. Other worker have reported starvation period in a range of 20 minutes (Shepared *et al*. 1985) [19], to 60 minutes (Cho *et al*. 1992) [7] to 180 minutes (Holmes *et al*. 1988) [6] in their studies. (Table no 2).

The present study also revealed that inoculum prepared from 48 to 72 hours old *Candida* cultures produce highest germ tube positivity compare to 24 hours old culture, similar results observe in other studies [7, 20].

## Conclusion

In Present study pooled human serum remains the best medium for testing germ tube production inspite of the hazards associated with its use. When starvation period of *Candida* was up to or more than 60 minutes, rate of germ tube test positivity was increased. Also Chances of positivity of germ tube test was enhance when 72 hour old cultures were used for preparation of test inoculate.

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