



## Original Research Article

## Utility of non-serum liquid media against conventional human serum in germ tube production test

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## ABSTRACT

**Introduction:** *Candida albicans*, a predominant yeast pathogen can be easily distinguished from other non-albicans species in a diagnostic laboratory based on their property to produce germ tubes using human serum as a routine substrate. As usage of human serum is accompanied with risk of transmission of blood-borne viral infections, several other media were employed by many clinical laboratories. The aim of this study was to evaluate the efficacy of four commonly used non-serum liquid media relative to fresh human serum in germ tube (GT) productivity among various *Candida* isolates.

**Materials and Methods:** A total of 146 *Candida* isolates (96 *C. albicans*; 47 *C. tropicalis*; 3 *C. dubliniensis*) identified by conventional methods were assessed for germ tube production in five types of media namely peptone water, Mueller Hinton broth, Trypticase soy broth, Brain Heart infusion broth and fresh human serum. Results obtained in different media for germ tube production in all *Candida* isolates were analysed for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

**Results:** All the *Candida* isolates showed 100% specificity and PPV in all the five-liquid media. Highest performance in germ tube production (96%) in *C. albicans* isolates was found in Trypticase soy broth followed by fresh human serum (95%). Trypticase soy broth was shown to have 96% sensitivity and 97% accuracy for germ tube formation comparable to human serum with 95% sensitivity and 96.5% accuracy.

**Conclusion:** Trypticase soy broth can be suggested as a choice medium for GT production in diagnostic mycology.

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### 1. Introduction

The rapid presumptive identification of *Candida* species assumes utmost importance in diagnostic mycology due to growing prevalence of candidiasis proportionate to rise in number of patients at risk for fungal infections.<sup>1-4</sup> Candidiasis comprises an entire spectrum of diseases that vary from mild superficial skin involvement to fatal invasive blood stream infections. The common risk factors associated with candidiasis include prolonged use of broad-spectrum antibiotics, cancer patients on chemotherapy, HIV patients, recipients of organ transplants, diabetic patients and pregnancy.<sup>5,6</sup> Though *Candida albicans* is the

predominant species isolated from clinical specimens in the past two decades, infections with other *Candida* species are frequently reported in recent years.<sup>7,8</sup> Speciation of *Candida* is a useful tool in guiding treatment options as *albicans* and non-*albicans* strains differ in their susceptibility patterns.<sup>9,10</sup>

One of the widely employed rapid technique readily available in most clinical laboratories for presumptive identification of *Candida albicans* from other species is germ tube production test first described by Reynold and Braude in 1956.<sup>11</sup> Germ tubes denote true hyphae produced by *Candida* isolates when incubated at 37°C in human serum for two hours visible microscopically as short, slender tubes arising from mother yeast cell without

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constriction at their point of origin.<sup>12</sup> Besides *C. albicans*, *C. dubliniensis* and *C. africana* also form germ tubes.<sup>13,14</sup> This test serves as an economically feasible alternative to many other expensive phenotypic and genotypic methods unavailable in resource poor settings.<sup>11</sup>

Handling human serum as a substrate for germ tube test in routine laboratory practice is associated with the risk of contamination with blood borne viruses like HIV, Hepatitis B and HCV.<sup>13</sup> To obviate this limitation, few researchers have evaluated certain other media compared to human serum regarding their performance in germ tube production test.<sup>15,16</sup> These include several media like sheep serum, egg white, peptone media, tryptic soy broth and Mueller Hinton agar. Due to scant studies available in this area of research from our place the present work was undertaken with an objective to compare the utility of four commonly used non-serum substrates against the pooled human serum to perform the germ tube test.

## 2. Materials and Methods

The study included a total of 146 *Candida* strains (96 *C. albicans*; 47 *C. tropicalis*; 3 *C. dubliniensis*;) obtained from diverse clinical samples processed in our microbiology laboratory during the year 2019. Institutional ethical committee approval was sought. Speciation of *Candida* isolates was done by culturing all the strains on HiChrom *Candida* agar and performing sugar fermentation and assimilation tests;<sup>17</sup> chlamydoconidia formation on corn meal agar (Hi Media laboratories, Mumbai). All *Candida* strains being tested were subcultured on Sabouraud's dextrose agar and incubated for 24 hours at 37°C to obtain fresh growth to inoculate in appropriate media to carry out GT test.

Four different non-serum liquid media – peptone water, Mueller Hinton broth, Trypticase soy broth, Brain Heart infusion broth along with fresh human serum were prepared and 0.5ml of each media was dispensed in five different test tubes. Single colony from 24hour old culture was lightly picked up by a straight wire and inoculated in test tubes containing separate media followed by incubation at 37°C for two hours. *C. albicans* ATCC 10231 and *C. krusei* were used as positive and negative controls respectively<sup>11</sup>. Soon after two hours of incubation a drop of liquid from each test tube was mounted on a clean glass slide with a cover glass and examined under light microscope (X 40). A positive test was read if a minimum of five filamentous extensions were noticed in the whole mount, arising laterally from mother yeast cell- without constriction at their point of origin; 3times longer and one-half in width to the parent yeast cell.<sup>18</sup> The test was read negative when no germ tubes could be seen in at least 10 high power fields examined in the entire wet mount.

Germ tube production of *Candida* isolates in various liquid media was evaluated with different performance

indicators namely sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

## 3. Results

Germ tube test (GTT) results were positive in 82 of 96 (85%) strains of *Candida albicans* in peptone water; 89 of 96 (93%) in Mueller Hinton broth; 92 of 96 (96%) in Trypticase soy broth, 88 of 96 (92%) in Brain Heart infusion broth and 91 of 96 (95%) in fresh human serum (Table 1). All the 3 isolates of *Candida dubliniensis* showed true germ tube formation in all the four types of liquid media except peptone water in which only 2 isolates were found positive. None of the *C. tropicalis* isolates tested, formed true germ tubes in any of the five types of liquid media. Pseudohyphae produced by *C. tropicalis* strains could be clearly differentiated from the true germ tubes of *C. albicans* and *dubliniensis* species due to presence of constriction at their origin from the mother yeast cell.

All the five media in the present study showed 100% specificity for GT production. Trypticase soy broth was found to produce better results relative to other media for germ tube productivity with higher sensitivity (96%) and accuracy (97%) almost resembling fresh human serum with 95% sensitivity and 96.5% accuracy. Mueller Hinton broth demonstrated 93% sensitivity and 95% accuracy while Brain Heart infusion broth depicted 92% sensitivity and 94.5% accuracy in germ tube production. Least performance was revealed by peptone water with 84% sensitivity and only 89% accuracy. (Table 2). None of the liquid media could produce germ tubes in all the 99 isolates of *Candida albicans*.

## 4. Discussion

The germ tube test was a widely accepted, easy and reliable technique classically used in diagnostic mycology for quick presumptive identification of *C. albicans* and *C. dubliniensis*. Germ tube production by yeast cells indicates their morphological adaptation to filamentous forms during unfavourable conditions.<sup>13</sup>

Using human serum as a substrate in GTT however was associated with many short comings as it should be freshly prepared or stored frozen and the inoculum size needs to be minimal (< 10<sup>7</sup> cells/ml) because heavy inoculum was known to inhibit germ tube production. The major concern regarding handling human serum is the threat of infection with HIV or Hepatitis virus. Variability in the performance noticed with different batches of serum and biological inhibitors present in pooled human sera might increase the chances of false negative results<sup>11,14</sup>. Mackenzie DWR<sup>19</sup> reported that storage of human serum at 4°C for 15 days reduced its ability to generate germ tubes by 50%.

According to present study, maximum number (96%) of *C. albicans* strains produced germ tubes in Trypticase soy

**Table 1:** Positive GTT results shown by Candida isolates tested in various media.

Media	isolates with positive GTT			Total (n=146)
	<i>C. albicans</i> (n=96)	<i>C.dublinsiensis</i> (n=3)	<i>C.tropicalis</i> (n=47)	
Peptone water	82	2	0	84
Mueller Hinton Broth	89	3	0	92
Trypticase soy broth	92	3	0	95
Brain Heart Infusion broth	88	3	0	91
Fresh human serum	91	3	0	94

GTT – Germ tube test.

**Table 2:** Efficacy indicators for various tested media in GTT positivity.

Media used	Sensitivity	Specificity	PPV	NPV	Accuracy
Peptone water	84%	100%	100%	75%	89%
Mueller Hinton Broth	93%	100%	100%	87%	95%
Trypticase soy broth	96%	100%	100%	92%	97%
Brain Heart Infusion broth	92%	100%	100%	85%	94.5%
Fresh human serum	95%	100%	100%	90%	96.5%

GT – Germ tube; PPV – Positive Predictive Value; NPV – Negative Predictive Value

broth compared to 95% positivity of the same species in fresh human serum. Mueller Hinton broth and Brain Heart infusion broth showed positive results in 93% and 92% of *C. albicans* isolates respectively. All the tested liquid media excluding peptone water proved to be equally good for production of germ tubes in all the three isolates of *C. dubliniensis*. In Peptone water only 85% and 67% of *C. albicans* and *C. dubliniensis* strains respectively could form germ tubes. This could be due to lower nutritive value of peptone water relative to other media.

The present study showing 96% GT positive results in all *Candida albicans* isolates tested in Trypticase soy broth<sup>20</sup> correlated with a study by Joshi et al., who demonstrated 100% GT positivity in the same species. Deorukhkar et al., also indicated Trypticase soy broth to be more effective option than pooled human serum in germ tube test for *C. albicans* and *C. dubliniensis*.<sup>11</sup> A new germ tube induction medium composed of 3 parts of EDTA added rabbit coagulase plasma and 2 parts of Trypticase soy broth was developed by Berardinelli S and Opheim DJ for effective germ tube production in *C. albicans*.<sup>21</sup> In contrast to such findings, Arora DR et al., and Makwana GE et al., reported Trypticase soy broth to be less efficient than human serum and horse serum respectively in GT production.<sup>15,16</sup> Mattei AS et al., recommended Mueller Hinton broth or agar as a preferred media to human serum in germ tube production test.<sup>22</sup> Role of other media like animal serum, Sabouraud's broth, RPMI-1640 broth and peptone water in GT formation was evaluated and found to be less productive.<sup>23</sup>

As pooled human serum was associated with the risk of contamination with blood borne viruses, Trypticase soy broth can be suggested as a better substitute for GTT due to its easy availability, safety and storage stability at 4°C up to a period of 30 days. All five different types of Media revealed 100% specificity and positive predictive

value (PPV) for GT production in all *Candida* isolates tested indicating their precision in detecting true positives. In addition, a higher negative predictive value was noted with Trypticase soy broth used in GTT compared to other media denoting that it is more likely to be accurate in picking up true negatives. Overall accuracy rate was found to be better for Trypticase soy broth making it a good surrogate medium to human serum in GT productivity.

## 5. Conclusion

Trypticase soy broth could replace conventional human serum used as a routine substrate for GTT in daily laboratory practice to accomplish rapid presumptive identification of *C. albicans* and *C. dubliniensis*. It can be recommended as a feasible, safe and economical choice to human serum in germ tube production test.

## 6. Source of funding

None.

## 7. Conflict of interest

None.

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