EVALUATION OF DIFFERENT CULTURE MEDIA AND INCUBATION TIMES FOR THE GERM TUBE TEST IN *CANDIDA ALBICANS*

Hanh Thu Quang Le[1], Tien Dat Quang Le[1], Han T.N. Danh[1], Bac V.G Nguyen[2*]

ABSTRACT

Germ tube test is a rapid and cost-effective method for the presumptive identification of *Candida albicans* in routine clinical laboratories, but its performance depends on both the testing medium and incubation time. In this study, 40 clinical isolates of *C. albicans* were evaluated for germ tube formation using six different media, including human plasma, rabbit plasma, 1% peptone casein, 1% peptone meat, Brain Heart Infusion, and Tryptic Soy Broth at incubation times of 2, 4 and 6 hours. Human plasma and rabbit plasma showed the highest sensitivity, yielding 100% germ tube positivity at 2 hours. Among the alternative media, 1% peptone meat and TSB demonstrated relatively high early positivity rates (85%), whereas BHI and 1% peptone casein exhibited substantially lower sensitivity. Prolonged incubation beyond 2 hours resulted in a marked decline in germ tube positivity across all media, indicating an increased risk of false-negative results. These findings confirm that plasma-based media remain the most reliable substrates for germ tube induction in *C. albicans*. However, 1% peptone meat and TSB represent practical low - cost alternatives when results are interpreted at the optimal 2 - hour time point.

Keywords: *Candida albicans*, *germ tube test, serum test, media, incubation time*

INTRODUCTION

Candida albicans is one of the most common opportunistic fungal pathogens in humans and remains a leading cause of both superficial and invasive candidiasis worldwide. It is frequently isolated from mucosal surfaces such as the oral cavity, gastrointestinal tract, and genitourinary tract, but under favorable conditions it can cause severe bloodstream and systemic infections, particularly in immunocompromised patients and those receiving intensive care or broad-spectrum antibiotics. Rapid and accurate identification of *C. albicans* is therefore essential for early diagnosis, appropriate antifungal therapy, and improved clinical outcomes [1,10].

Among conventional phenotypic methods, the germ tube test remains one of the most widely used and cost-effective screening assays for the presumptive identification of *C. albicans*. This test is based on the organism's ability to form germ tubes when incubated in suitable liquid media at 35–37°C, typically within 2–3 hours. Germ tube formation represents a key morphological hallmark that distinguishes *C. albicans* from most other Candida species, although rare exceptions have been reported, such as *Candida dubliniensis* [2,5]. Despite these limitations, the simplicity, low cost, and rapid turnaround time of the germ tube test have sustained its continued use in routine diagnostic laboratories.

Traditionally, human serum or plasma has been regarded as the gold standard medium for germ tube induction. However, the routine use of human plasma is associated with several practical drawbacks, including high cost, limited availability, biosafety concerns related to blood-borne pathogens, and batch-to-batch variability [2,4]. To overcome these constraints, a variety of alternative media, such as rabbit plasma, peptone-based broths, Brain Heart Infusion (BHI), and Tryptic Soy Broth (TSB), have been investigated as potential substitutes. These media differ substantially in their nutritional composition and physicochemical properties, which may significantly influence the rate and extent of germ tube formation [3,7].

Several studies have demonstrated that both the choice of medium and the incubation time critically affect the sensitivity and reliability of the germ tube test. While human or animal plasma generally induces rapid and robust germ tube formation, non-serum media may exhibit delayed or reduced positivity rates, potentially leading to false-negative results if the incubation period is not optimized [2,4]. Furthermore, prolonged incubation can result in morphological alterations, such as pseudohyphal formation or

[1] *Faculty of Medical Laboratory Techniques, Nguyen Tat Thanh University, Ho Chi Minh City*
[2] *School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City*
Responsible person: Bac V.G Nguyen
Email: nguyenvugiangbac@ump.edu.vn
Date of receipt: 19.1.2026
Date of scientific judgment: 9.3.2026
Reviewed date: 20.4.2026

secondary budding, which may complicate microscopic interpretation and reduce diagnostic accuracy [6].

In recent years, there has been renewed interest in identifying standardized, safe, and economical alternatives to plasma-based germ tube assays that can be reliably implemented in routine clinical laboratories, particularly in resource-limited settings [8]. However, comparative data evaluating multiple test media across different incubation times remain limited and inconsistent, and no consensus has yet been established regarding the optimal conditions for germ tube induction outside of serum-based systems [3,7].

Therefore, the present study aims to systematically evaluate the performance of different culture media, including human plasma, rabbit plasma, 1% peptone casein, 1% peptone meat, Brain Heart Infusion (BHI), and Tryptic Soy Broth (TSB), for germ tube induction in *Candida albicans* at multiple incubation time points (2, 4, and 6 hours). By comparing germ tube positivity rates across media and time intervals, this study seeks to identify practical alternatives to plasma-based assays and to provide evidence-based recommendations for optimizing the germ tube test in routine diagnostic practice.

II. MATERIALS AND METHODS

Experimental Preparation

Clinical and reference strains of *Candida albicans* were retrieved from glycerol stock cultures stored at 4°C and cultured onto Sabouraud Dextrose Agar (SDA). The cultures were incubated at 37°C for 24 hours to obtain actively growing yeast cells. Following incubation, several well-isolated colonies were suspended in sterile 0.9% NaCl solution. The cell suspension was adjusted to a final concentration of 1×10^6 CFU/mL by measuring the optical density at 530 nm (OD_{530}) using a spectrophotometer.

Germ Tube Test

The germ tube test was carried out using 0.5 mL of each testing medium, including human plasma, rabbit plasma, 1% peptone casein, 1% peptone meat, Brain Heart Infusion (BHI), and Tryptic Soy Broth (TSB), dispensed into sterile test tubes. An equal volume (0.5 mL) of the standardized yeast suspension was inoculated into each tube, resulting in a 1:1 mixture of inoculum and test medium. The inoculated tubes were incubated at 35 - 37 °C.

Germ tube formation was assessed at three predefined time points: 2 hours, 4 hours, and 6 hours post - incubation. Each experimental run included a positive control strain (*Candida albicans* ATCC 10231) and a negative control strain (*Candida tropicalis* ATCC 13803), which were processed under identical conditions.

At each time point, a drop of the incubated suspension was placed onto a clean glass slide and stained with fuchsin for 3 minutes. The stained preparations were then examined microscopically under both low - power and high - power objectives.

The presence of a short hyphal extension arising laterally from a yeast cell, with no constriction at the point of origin, was interpreted as a positive germ tube reaction. In contrast, the absence of hyphal extensions or the presence of short hyphal projections exhibiting a constriction at their point of origin was interpreted as a negative germ tube reaction.

III. RESULTS

Table 1. Distribution of germ tube postive and negative strains of *C. albicans* within clinical samples

Test medium	Incubation time	Positive (%)	Negative (%)
Human plasma	2 hours	40 (100%)	0 (0%)
	4 hours	12 (30%)	28 (70%)
	6 hours	1 (2.5%)	39 (97.5%)
Rabbit plasma	2 hours	40 (100%)	0 (0%)
	4 hours	9 (22.5%)	31 (77.5%)
	6 hours	1 (2.5%)	39 (97.5%)
1% pepton casein	2 hours	6 (15%)	34 (85%)
	4 hours	11 (27.5%)	29 (72.5%)
	6 hours	5 (12.5%)	35 (87.5%)

Test medium	Incubation time	Positive (%)	Negative (%)
1% pepton meat	2 hours	34 (85%)	6 (15%)
	4 hours	9 (22.5%)	31 (77.5%)
	6 hours	1 (2.5%)	39 (97.5%)
BHI	2 hours	23 (57.5%)	17 (42.5%)
	4 hours	9 (22.5%)	31 (77.5%)
	6 hours	2 (5%)	38 (95%)
TSB	2 hours	34 (85%)	6 (15%)
	4 hours	6(15%)	34 (85%)
	6 hours	0 (0%)	40 (100%)

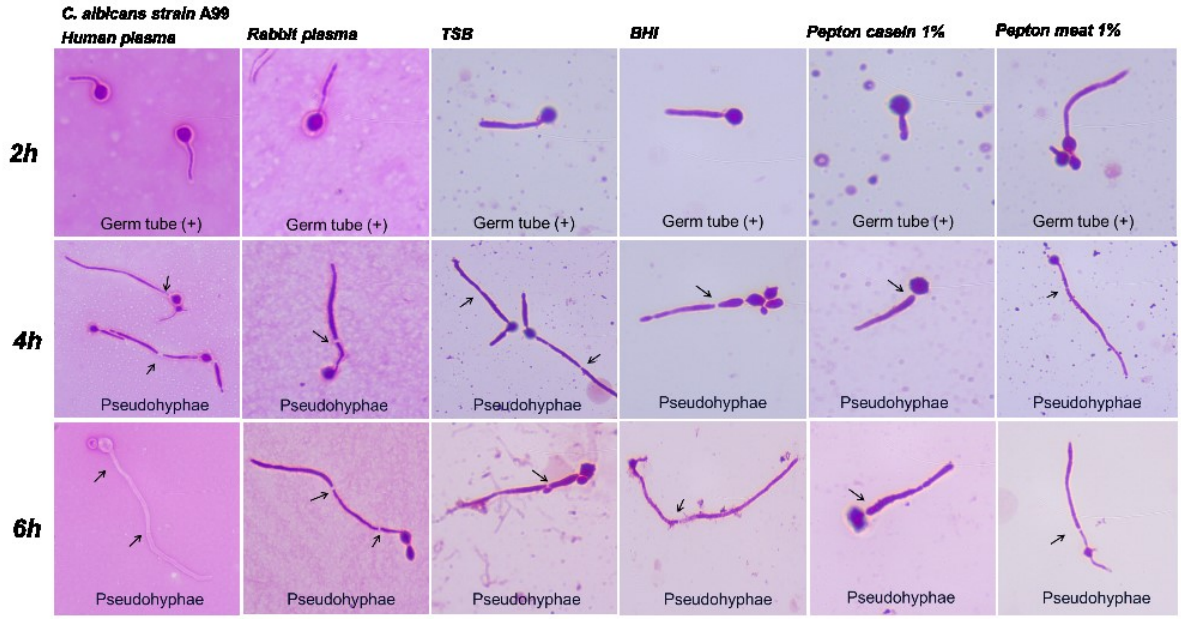

Figure 1. Germ tube test of *Candida albicans* strain A99

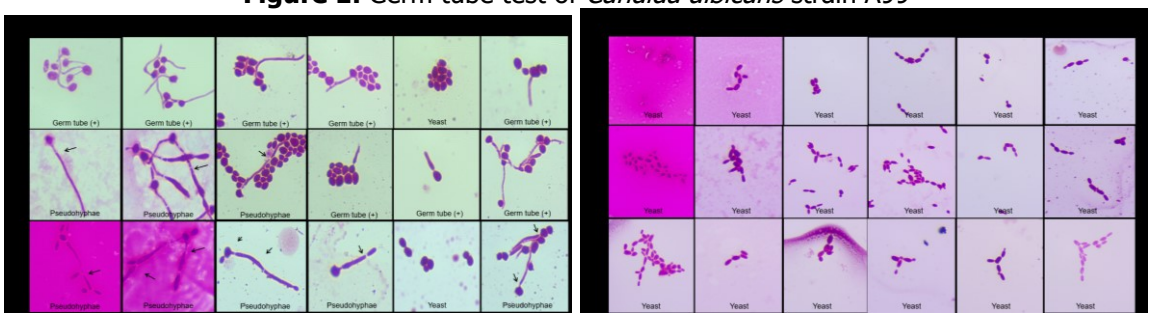

Figure 2. Germ tube test of positive control *Candida albicans* ATCC10231 and negative control *Candida tropicalis* ATCC13803

A total of 40 clinical isolates of *Candida albicans* were evaluated for germ tube formation using six different testing media (human plasma, rabbit plasma, 1% peptone casein, 1% peptone meat, Brain Heart Infusion [BHI], and Tryptic Soy Broth [TSB]) at three incubation time points (2, 4, and 6 hours). Germ tube positivity rates varied markedly depending on both the type of medium and the duration of incubation (Table 1).

At the 2-hour incubation time point, human plasma and rabbit plasma demonstrated the highest sensitivity, with all 40 isolates (100%) showing positive germ tube formation in both media. Among the non-plasma alternatives, 1% peptone meat and TSB yielded relatively high positivity rates of 85%, while BHI showed a moderate positivity rate of 57.5%. In contrast, 1% peptone casein exhibited the lowest early

performance, with only 15% of isolates producing germ tubes at 2 hours.

Prolonged incubation beyond 2 hours resulted in a marked decline in germ tube positivity across all media. At 4 hours, positivity rates decreased substantially in both plasma-based media, falling to 30% in human plasma and 22.5% in rabbit plasma. Similar declines were observed in non-plasma media, with positivity rates of 27.5% in 1% peptone casein, 22.5% in 1% peptone meat and BHI, and 15% in TSB. These findings indicate that delayed result interpretation significantly reduces the apparent sensitivity of the germ tube test.

At 6 hours of incubation, germ tube formation was rarely observed in any of the tested media. Positivity rates were 2.5% in both human plasma and rabbit plasma, 12.5% in 1% peptone casein, 2.5% in 1% peptone meat, and 5% in BHI. Notably, no germ tube formation was detected in TSB at this time point (0%). This uniform decline across all media suggests that extended incubation may adversely affect germ tube stability or promote morphological changes that complicate interpretation.

A comparative analysis of germ tube induction capacity at the optimal 2-hour incubation time revealed a clear hierarchy among the tested media. Plasma-based media (human plasma and rabbit plasma) showed superior performance, achieving 100% positivity. Among the alternative media, 1% peptone meat and TSB demonstrated comparable early sensitivity (both 85%), followed by BHI (57.5%), whereas 1% peptone casein was markedly less effective (15%). These results indicate that, while plasma-based media remain the most reliable for early germ tube detection, 1% peptone meat and TSB represent the most promising low-cost substitutes when results are interpreted within the optimal incubation window.

Overall, the highest diagnostic yield across all media was obtained at the 2-hour time point, highlighting the critical importance of early result interpretation. In contrast, reading results at 4 or 6 hours substantially increased the risk of false-negative outcomes, regardless of the medium used. Representative microscopic images of germ tube formation in a clinical *C. albicans* isolate (strain A99) and in the positive and negative control strains (*C. albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803) are shown in Figures 1 and 2.

IV. DISCUSSION

The present study confirms that both the testing medium and incubation time are critical determinants of germ tube test performance in *Candida albicans*. Human plasma and rabbit plasma yielded 100% germ tube positivity at 2 hours, consistent with classical and contemporary reports identifying serum-based media as the most sensitive and reliable substrates for rapid germ tube induction [2,4]. This finding reinforces the continued role of plasma as the gold standard for presumptive identification of *C. albicans* in routine diagnostic practice [1,10].

Among the alternative media evaluated, 1% peptone meat and Tryptic Soy Broth (TSB) showed relatively high early positivity rates (85% at 2 hours), supporting previous observations that selected non-serum broths can promote germ tube formation under optimized conditions [3,7]. In contrast, Brain Heart Infusion (BHI) and especially 1% peptone casein exhibited substantially lower sensitivity, in agreement with earlier studies demonstrating variable and often reduced germ tube induction in non-serum media [3,4]. These differences likely reflect variations in nutrient composition and the absence of serum-derived factors known to trigger hyphal development.

A key observation of this study is the marked decline in germ tube positivity with prolonged incubation across all media. Positivity rates decreased sharply at 4 and 6 hours, even in plasma-based systems, corroborating prior reports that delayed reading increases false-negative rates and complicates interpretation due to pseudohyphal formation or secondary budding [2,6]. This underscores the importance of strict adherence to early result interpretation, ideally at approximately 2 hours.

Overall, these findings suggest that while human and rabbit plasma remain the most reliable media for germ tube induction, 1% peptone meat and TSB represent practical low-cost alternatives when results are read at the optimal 2-hour time point. Consistent with earlier recommendations, early incubation and careful medium selection are essential to maximize diagnostic sensitivity and avoid false-negative interpretations in routine laboratory practice [2,4].

REFERENCES

1. **Calderone RA, Fonzi WA.** Virulence factors of *Candida albicans*. Clin Microbiol Rev. 2001;14(1):41–60. doi: 10.1128/CMR.14.1.41-60.2001
2. **Mackenzie DW.** Serum tube identification of *Candida albicans*. J Clin Pathol. 1962;15(6):563–565. doi: 10.1136/jcp.15.6.563
3. **Odds FC.** *Candida and Candidosis: A Review and Bibliography*. 2nd ed. London: Baillière Tindall; 1988. doi: 10.1016/S0924-8579(99)90023-6
4. **Lehmann PF, Walsh TJ.** Challenges in diagnosis of fungal infections. J Clin Microbiol. 1989;27(5):893–902. doi: 10.1128/JCM.27.5.893-902.1989
5. **Sullivan DJ, Westerneng TJ, Haynes KA, Bennett DE, Coleman DC.** *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization. J Clin Microbiol. 1995;33(2):249–255. doi: 10.1128/JCM.33.2.249-255.1995
6. **Sheppard DC, Rieg G, Chiang LY, Filler SG, Edwards JE Jr.** Germ tube formation in *Candida albicans*: role in virulence and interaction with host cells. J Med Microbiol. 2004;53(Pt 9):915–921. doi: 10.1099/jmm.0.45650-0
7. **Kurtzman CP, Fell JW, Boekhout T.** *The Yeasts: A Taxonomic Study*. 5th ed. Amsterdam: Elsevier; 2011. doi: 10.1016/B978-0-444-52149-1.00001-4
8. **Arendrup MC.** Epidemiology of invasive candidiasis. Curr Opin Crit Care. 2010;16(5):445–452. doi: 10.1097/MCC.0b013e32833e84d2
9. **Cervera C, et al.** Bloodstream infections in adult patients: epidemiology and outcome. Diagn Microbiol Infect Dis. 2009;65(3):263–271. doi: 10.1016/j.diagmicrobio.2009.07.011
10. **Pfaller MA, Diekema DJ.** Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20(1):133–163. doi: 10.1128/CMR.00029-06