A Simple, Fast, and Reliable Method for the Identification of *Candida albicans*

Environmental Health Insights
Volume 18: 1–5

DOI: 10.1177/11786302241272398

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ABSTRACT

BACKGROUND: Candida is one of the common pathogens in nosocomial infections. Culture is the gold standard for diagnosing candidemia. *Candida albicans* is identified via the germ tube test, which uses serum as the culture medium, which is costly and time-consuming. This study was conducted to evaluate and compare a relatively simple, fast, and reliable method for the detection of *Candida albicans*.

METHODS: We conducted this randomized case study at Taipei City Hospital (TCH) from January 2023 to August 2023, with a total of 30 specimen culture reports collected and confirmed to be cases of *Candida albicans* infection. A germ tube test was performed in a 37°C water bath using serum, plasma, and safe plasma products (Fresh Frozen Plasma, FFP). Further, the same procedures were repeated with the addition of 22% bovine serum albumin (BSA) to the identification/culture.

RESULTS: By adding BSA, more than 50% of the budding phenomenon was observed in 40 minutes, which shortened the diagnosis time compared with the traditional method (2-3 hours). Using BSA can shorten the identification time for early clinical medication and improve the quality of medical care

CONCLUSION: Using safer plasma products for germ tube test of candidiasis not only reduced the risk of infection for medical technicians but could also replace the serum used in traditional methods to increase convenience and save time. This study proposed BSA as a germ tube induction medium enhancer, which reduced the culture time, thereby enabling quicker diagnosis of *C. albicans* infections.

KEYWORDS: *Candida albicans*, serum, germ tube test, Fresh Frozen Plasma (FFP), bovine serum albumin (BSA)

RECEIVED: May 2, 2024. **ACCEPTED:** July 15, 2024.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

Candidiasis is an infection caused by a yeast (a type of fungus) called *Candida*, which commonly infects the skin and other parts of the human body.[1] Common sites of *Candida albicans* infection include vagina, glans, esophagus, oral cavity, and skin. *Candida* has gradually become one of the common pathogenic fungus in nosocomial infections.[2] Severe infections caused by *Candida* are widely recognized as a leading cause of morbidity and mortality in healthcare settings. Approximately 8% of nosocomial infections are caused by *Candida* species.[3] Invasive candidiasis is a major cause of morbimortality, and previous studies have described the high mortality rates.[4] Crude mortality rates of patients with candidemia were in the range of 35% to 60%. *C. albicans* remains the predominant cause of invasive candidiasis in Taiwan and accounts for more than 50% of all cases.[5] Although the majority of cases of candidiasis were not invasive, the incidence is increasing because of the growing complexity of patients.

The currently used identification test for *C. albicans* is the germ tube test, which uses serum as the culture medium.[6] In the test, human serum is routinely used for culture and microscopic examination of *C. albicans*. Although this is a cost-effective method, it carries risks for the spread of the disease.[7] Animal (including rabbit or sheep) serum is safer; however, commercial purchase of these sera is expensive. If human plasma can be used instead of human serum, the time required to perform the test can be reduced. Furthermore, if safer plasma products (Fresh Frozen Plasma, FFP) can be used instead of

human serum or plasma, the risk of disease transmission can be reduced, which also can increase the ease with which these experiments can be performed.

Serum albumin, recognized as a predominant major plasma protein, is distributed among vertebrates, demonstrating versatility and widespread accessibility.[8] Many studies have discussed the composition and attributes of human and bovine serum albumin. Bovine serum albumin (BSA) is regarded as a highly concentrated nutrient that can provide sufficient sources required for cell growth. In this study, we show that adding bovine albumin can shorten the identification time for diagnosis for germ tube test than the traditional (conventional) method (2-3 hours in average).

To evaluate results of the different methods of the germ tube diagnosis of candidiasis, we conducted a retrospective case analysis at Taipei City Hospital (TCH) from January 2023 to August 2023. We aimed to investigate whether the modified culture germ tube culture is an effective and accurate diagnostic method for the early diagnosis of candidiasis, and whether shortening the time frame of diagnosis enables early initiation of clinical medication that might be beneficial in patient care.

Materials and Methods

Study period and study population

We conducted this retrospective study at Taipei City Hospital (TCH) from January 2023 to August 2023. A total of 30 specimen culture reports were collected with informed consent obtained, all confirmed as *C. albicans* cases. Germ tube tests were performed in a 37°C water bath to record and compare results obtained using serum, plasma, and FFP. The same procedures were repeated by adding 22% BSA to the second part of identification/culture.

The experiments were carried out by physicians and medical technicians for quality assurance. This study was approved by the Research Ethics Committee, and informed consent was obtained.

Equipment, facilities, and experimental method

While placing a small amount of sample suspected to contain *C. albicans* into a sterile glass test tube for the germ tube test, we added 0.5 mL of serum, mixed well, and place it in a 37°C water bath for 2 to 3 hours. After this, we took a drop of the culture fluid and smeared it on a glass slide and observed it under a microscopic to check for the presence of germ tubes formed by *C. albicans*. The colony identified as *C. albicans* was added to 0.5 mL physiological saline in a sterile test tube containing 20 μL 3% to 5% red blood cell suspension. Further, 1 drop of the mixed solution was taken and added to a tube containing (A) 0.5 mL serum, (B) 0.5 mL plasma, or (C) 0.5 mL FFP. To test the effect of BSA, the same procedures were repeated, with 22% BSA added to the tubes (A, B and C) in the second part of examination. These tubes were marked as A1, B1, and C1, respectively. Each tube of samples was observed, and images were recorded at 30, 35, 40, 60,and 120 minutes. All samples were observed and images were recorded in 10 different visual fields using a microscope (20 μL 3%-5% red blood cell suspension liquid was used to facilitate identification of the focal length). The presence of budding in any one visual field was counted as 1 point. These points were converted into scores and percentage scores after statistical analysis.

Statistical analyses

Specimen source distribution is presented as percentages. The Wilcoxon signed-rank test was performed to compare the time taken for *C. albicans* identification with different culture media (serum, plasma, and FFP with and without BSA). A significance level was defined as $P < .05$. All analyses were performed using SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA).

Results

Samples

In this study, samples were obtained from a total of 30 patients clinically diagnosed with candidiasis. The vagina, urine, and sputum were the most common source of samples from infected patients: catheterizing urine (n = 3; 10.0%), vaginal swab (n = 17; 56.7%), sputum (n = 4; 13.3%), discharge (n = 1; 3.3%), and urine (n = 5;16.7%)

Germination time with different culture methods. The differences in budding and germination time in petri dishes among different methods of the germ tube experiment are shown in Table 1. First, it was seen starting from 30 minutes, the addition of 1 drop (50 μL) of BSA resulted in a significant increase in the rate of sprouting in the petri dish compared with the conventional method. Then, at 40 minutes, the germination ratios of the new method (with BSA) were significantly higher than that of the conventional method for serum, plasma, and FFP. In particular, at 60 minutes, the use of FFP in the new method resulted in 100% germination, whereas the traditional method reached only 70.0%. It showed that the time of sprouting with the new method (adding BSA) is significantly faster than that with the traditional method regardless of the culture method.

The addition of 1 drop of BSA results in >50% of the budding phenomenon being observed in 40 minutes, which reduces the diagnosis time compared with the traditional method (2-3 hours); using FFP can reduce the workload of inspection personnel and infection risk. Plasma or FFP. can be used to replace the serum used in traditional methods to increase convenience and save time. Moreover, using BSA can shorten the time for diagnosis.

Table 1. Observation of the germ tube experiments using different culture methods.

%	A	A1	*P*-VALUE	B	B1	*P*-VALUE	C	C1	*P*-VALUE
30 min	0.0	8.3	.004	0	10.0	.002	0.0	6.3	.005
35 min	11.0	44.0	<.001	10.7	43.0	<.001	0.0	29.3	<.001
40 min	40.3	76.3	<.001	40.0	74.3	<.001	15.0	59.7	<.001
60 min	100	100	-	100	100	-	70.0	100	<.001
120 min	100	100	-	100	100	-	100	100	-

Abbreviation: FFP, Fresh Frozen Plasma.
Tube A: serum Tube B: plasma Tube C: FFP.
Tube A1: serum/albumin Tube B1: plasma/albumin Tube C1: FFP/albumin

Discussion

Candidiasis is a common infection and has gradually become one of the common pathogenic fungi in nosocomial infections. Several previous studies indicated that the incidence of candidemia is increasing and patients are developing serious infections. Invasive candidiasis has become a serious problem in patients in the intensive care unit, with attributable mortality rates.[9] Consequently, rapid and correct identification of species can play an important role in the management of candidiasis. Early initiation of appropriate treatment in confirmed invasive candidiasis is an important determinant of survival, and utilizing the golden time for treatment is crucial.[10] Conventional methods for identification of *Candida* species are based on morphological and physiological attributes. However, accurate identification of all isolates from clinical samples is often complex and time-consuming.

There are many laboratory methods for the identification of *Candida*, and the most commonly used is cultural microscopic exam. The presence of clustered pseudohyphae on Gram stain had a high sensitivity, specificity, positive predictive value, and negative predictive value for *C. albicans*; therefore, the presence of pseudohyphae clusters on Gram staining is useful in distinguishing *C. albicans*.[11] Other than Gram staining, there are many different staining methods, including Wright's–Giemsas, periodic acid Schiff stain (PAS), May–Grünwald Stain, and Liu's stain. In our lab, we adopted the Wright's–Giemsas stain (Figure 1) and the Liu's stain (Figure 2). Both stains clearly show WBC swallowing *Candida*. The patterns of fungi and mycelium can be directly observed in the blood of patients infected with candidemia and the medical examiner must be highly alert to these features to facilitate differential diagnosis and early treatment.

The germ tube test is routinely used for the rapid identification of *Candida albicans* and its variants, and is generally thought to be specific for these organisms.[12] There is evidence to demonstrate that hyphae of *Candida* have a sense of touch that enables them to grow along grooves and through pores (thigmotropism). This may aid infiltration of epithelial surfaces during tissue invasion. Hyphae are also aerotropic and can form helices when contacting solid surfaces. *Candida* species form germ tubes when incubated in serum up to 3 hours, which can be observed under a microscope. Currently, there are also polymerase chain reaction (PCR) and pulse-field gel electrophoresis analysis methods. PCR of whole-blood samples had perfect sensitivity and specificity for patients with candidemia when compared with healthy controls.[13] It offers attractive method for early diagnosis of specific *Candida* spp.; however, that requires 1 to several days and cannot be effectively used in fast clinical testing. Moreover, biochemical tests including DNA hybridization or enzyme-linked immunosorbent assay (ELISA) are accurate,[14] and some are suitable for large-scale screening; however, these methods are not easy to apply, and are less convenient and quick compared with the germ tube test.

C. albicans forms germ tubes in vitro in the presence of serum. In our study, the use of plasma products (Fresh Frozen Plasma, FFP) instead of serum can reduce the risk of infection (The FFP. provided by the blood donation center is a low-risk infectious material) for medical examiners during inspection in the lab; therefore, we decided to study plasma products. The use of plasma/FFP. to replace the serum used in conventional cultural methods could increase convenience in the hospital lab setting.[15] In our study, it showed significance among Tube B (plasma) and Tube C (FFP) compared with tube A (serum). Using safer plasma products instead, it not only reduced the risk of infection for medical technicians at work, but also can be used to replace the serum used in traditional methods to increase convenience and saved time, respectively.

Adding 22% BSA could shorten the identification time enabling early initiation of clinical medication, thereby improving the quality of medical care. BSA is widely used in laboratory tests and experiments. BSA solution is intended to be used as a high protein enhancer and, it plays the following roles including: maintaining protein stability, providing good thermal stability, and acting as an ideal protein protectant.[16] This study proposed BSA as a stable and less expensive germ tube induction medium enhancer, showing that the use of BSA leads to reduced time for culture/intubation and can be used without safety concerns. Adding BSA is found to be more

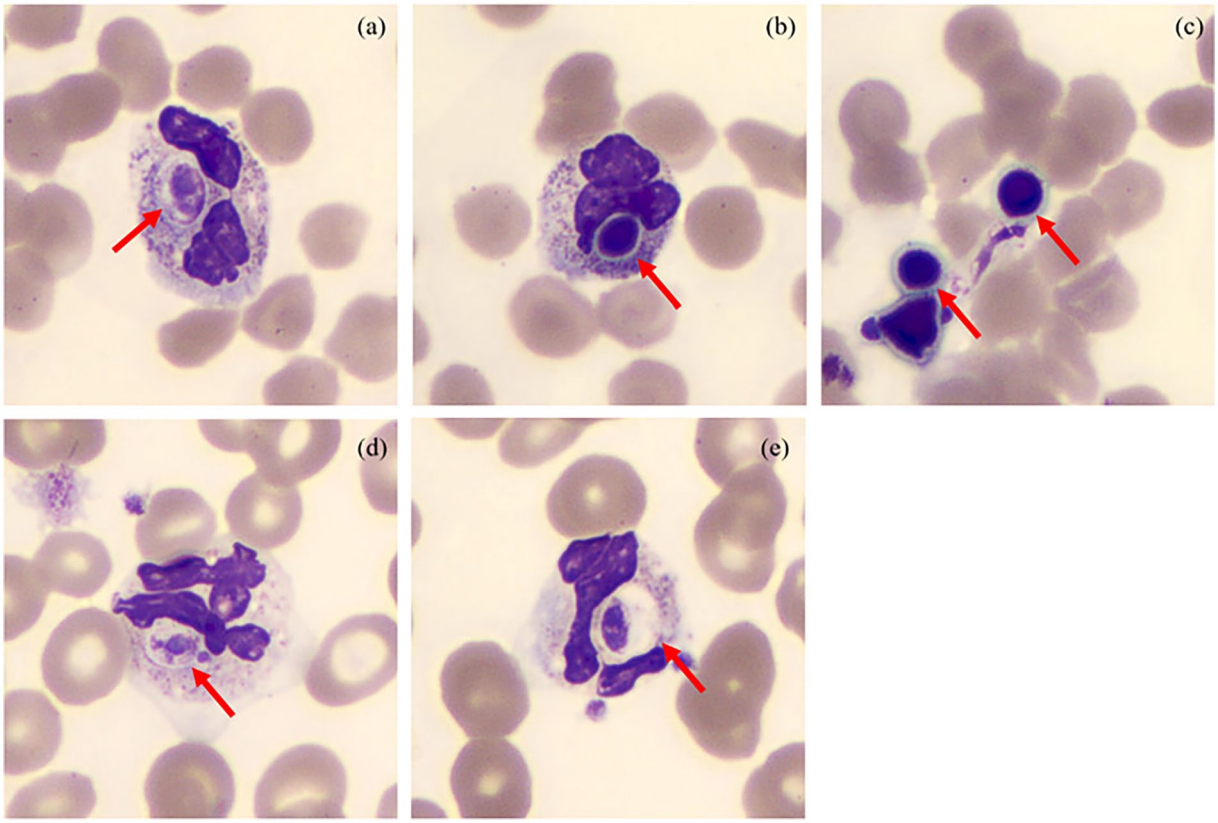

Figure 1. Wright's-Giemsas stain used to confirm the diagnosis of Candida. Different staining methods show different colors of cells which all indicate the presence of yeasts in the patient's blood pictures of white blood cell swallowing *Candida* can be clearly seen (x1000). (a) Yeast appears light purple to dark purple in Wright's-Giemsa stain. The most distinctive feature of the fungus is that the outer membrane has a transparent layer resembling a loop. In this picture, it is clear that a neutrophil has engulfed 1 yeast cell, which is light purple. The yeast is in the middle (light purple), the dense and dark upper and lower bands are the nucleus of the neutrophil, and the background shows numerous red blood cells. (b) This image shows a neutrophil white blood cell that has engulfed a dark purple yeast cell. There is a transparent loop in the outer membrane. (c) This picture shows 2 yeasts (round shape) scattered in the blood and stained dark purple. Both have a transparent ring in their outer membrane and have not yet been swallowed by white blood cells. (d) This image depicts a neutrophil white blood cell that has swallowed a yeast cell. The stained light purple yeast (indicated by an arrow) has a transparent outer membrane. Many red blood cells and platelets are visible on the upper left side. (e) A neutrophil has swallowed a stained purple yeast cell in the middle, with an outer membrane resembling a transparent ring. There are 2 platelets (small dots) attached to the neutrophil.

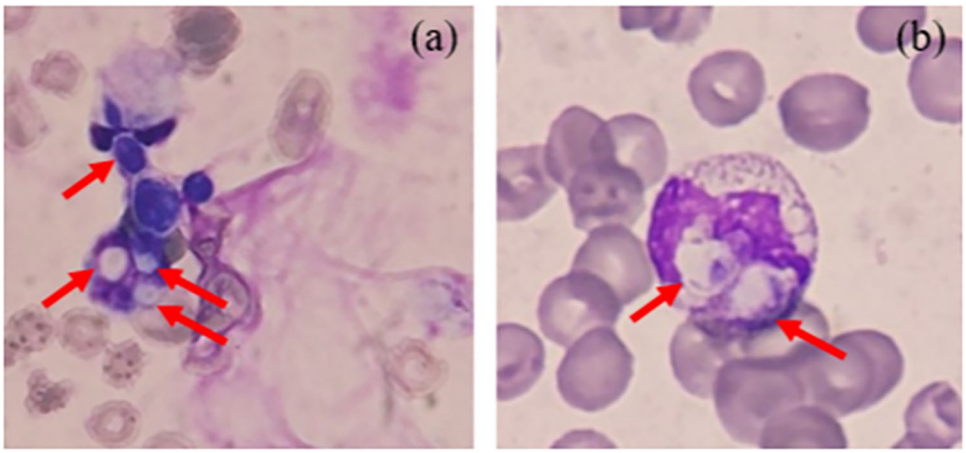

Figure 2. Liu's stain used to confirm the diagnosis of Candida. White blood cell swallowing *Candida* can be clearly seen (x1000). (a) The image shows 2 neutrophils. One neutrophil in the lower left has swallowed 3 yeasts, which are clearly visible as light purple, round shapes with a bubbly appearance (indicated by arrows). Another neutrophil in the upper left swallowing dark purple color yeast exhibit budding phenomenon. (b) A white blood cell has completely swallowed 2 yeasts, stained light purple with thick transparent ring

effective than serum only for induction of germ tubes by *C. albicans* isolates.

In our study, using safer plasma products instead of the conventionally used serum for germ tube test of candidiasis not only reduced the risk of infection for personnel at work, but also increase convenience and saved time. This study proposed BSA as a germ tube induction medium enhancer, showing that less time was required for culture. It is found to be more effective than serum only for induction of germ tubes by *C. albicans* isolates.

Conclusions

Several global surveillance studies indicate that the incidence of candidemia is increasing. Despite limited sensitivity (21%-71%), culture remains the gold standard for diagnosing candidemia. Early initiation of appropriate treatment in confirmed invasive candidiasis is an important determinant of survival. The use of safer plasma products (Fresh Frozen Plasma, FFP) can reduce the risk of infection for personnel, and plasma/FFP. can be used to replace the serum used in traditional methods to increase convenience. Using 22% BSA in the germ tube test can shorten the identification time, provide opportunities for early clinical medication, reduce mortality, and improve the quality of medical care. Our modified test is a fast, efficient, and reliable method.

Acknowledgements

The authors are grateful to the members of the Research Office for Health Data, Department of Education and Research, Taipei City Hospital, Taiwan for their valuable contributions in data management and statistical analysis.

Author Contributions

Su Jane Fan Chiang, Mei-Kuei Chien and Chang-Yi Tsai: Data curation, Formal Analysis and Project administration.

Su Jane Fan Chiang, Jui-Chang Hsiao, Fan-Hlan Koo: Supervision and Validation.

Su Jane Fan Chiang, Yung - Feng Yen, Yi-Chang Chou, Chih-Chien Cheng: Original draft writing, review & editing.

All authors contributed to drafting the manuscript and revising it critically for important intellectual content, and read and approved the final version.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The Institutional Review Board of the Taipei City Hospital approved this study (TCH Research Ethics Committee approval) and individual consent is not required and waived as all the identification data were encrypted (TCHIRB- -11112007-E).

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