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# Comparison of Several Types of Plasma as Media in the Germ Tube Test for Identification of *Candida albicans*

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**ABSTRACT** *Candida albicans* is a commensal on the oral mucosal surfaces of healthy individuals but can be pathogenic in immunocompromised individuals. The germ tube test is an inexpensive, faster, and easier method used to identify and differentiate it from other yeast species. Generally, the test is carried out using serum as the medium. Plasma overall has the same characteristics as serum and differs only in the content of clotting factors. In addition, plasma is easier to obtain than serum in most healthcare services. This study aims to demonstrate three types of plasma as alternative media for germ tube tests. CPDA (Citrate Phosphate Dextrose Adenine), EDTA (Ethylenediaminetetraacetic), and Sodium Citrate plasma were compared to serum in producing of germ tubes. Each plasma and serum was tested in five repetitions. The experimental method was carried out in Mycology Laboratory at the Health Polytechnic of Jambi. The data were observed based on the germ tube formation time and analyzed with a one-way ANOVA test. The average germ tube formation times observed in this study were 70 minutes (serum), 129 minutes (CPDA plasma), 164 minutes (EDTA plasma), and 146 minutes (Citrate plasma). The lengths were significantly different between serum and the three plasma ($P>0.05$). Nevertheless, CPDA plasma showed the average time which is at the minimum incubation time limit according to the standard protocol of the test. The CPDA plasma was faster than other plasma in the formating time of the germination. Therefore, CPDA plasma can be recommended as a substitute for serum in the germ tube test for it was easier to obtain, and considered safer to use.

**INDEX TERMS** *Candida albicans*, germ tube test, serum, plasma.

## I. INTRODUCTION

Candidiasis is an infection caused by Candida yeast microorganisms, especially *Candida albicans*. This fungus attacks the nails, skin, and mouth [1]. *Candida albicans* is a polymorphic yeast species that often forms part of the commensal gastrointestinal mycobiota of healthy individuals. It is also an important opportunistic pathogen [2]. *Candida albicans* is ubiquitous, dimorphic yeast which resides in the oral cavity as commensal. But it also acts as pathogen under certain specific circumstances and causing most common infection of oral cavity called oral thrush [3]. Candida affects all ages and genders. From several cases that occur in various countries, candidiasis occurs in approximately 70% in women [4]. One of the normal floras in the human body is Candida, which is found in the digestive tract, mucous membranes of the respiratory tract, vagina, urethra, skin, and under the fingers and toes. However, in these places this fungus will become dominant and cause pathological conditions when the immune system decreases [1]

There are several predisposing factors that can change the properties of Candida sp from saprophytic to pathogens, such as using of corticosteroids, and overusing or misusing of antibiotics that can lead to secondary infections. As an opportunistic pathogen of oral and vulvovaginitis, *Candida albicans* is a major cause of systemic invasive candidemia, especially in critically ill and immunocompromised patients with AIDS, autoimmune and transplant recipients. Invasive candidiasis accounts for up to 15 to 30% of all nosocomial infections in critically ill patients. Management of these severe infections is challenging due to the lack of rapid and reliable diagnostic methods, which results in delays in initiating appropriate antifungal therapyids and cytostatic drugs, pregnancy, use of anti-pregnancy pills, high humidity, and Diabetes Mellitus [5].

*Candida albicans* is a single cell fungus, round to oval in shape. *Candida albicans* reproduces by forming blastospores. Blastospores will continue to each other and increase in length to form pseudohyphae. The pseudohyphae form is more dangerous and invasive than the spore. This is because the pseudohyphae are larger, making it more difficult for macrophages to phagocytose [6-7].

Germ tube is an endogenous germination of *Candida albicans* yeast cells, has parallel walls and there is no constriction on the blastospore stem cell. Germ tube formation is affected by temperature, presence of protein (serum), neutral pH, carbon dioxide, and N-acetylglucosamine [8]. *Candida albicans* cells were induced by serum at 37 ºC to produce germ tubes with optimum pH between 7.0 and 8.0 [9]. According to research by Moya-Salazar & Rojas (2018), which analysed comparative studies for the identification of *Candida albicans* with the germ tube test in human serum and plasma, the results of the germ tube test on plasma and serum were sensitivity 100%, specificity of percentage of 99,3%, positive predictive value of 99.3%, and negative predictive value of 100% [10].

This study demonstrated that plasma was comparable to serum in producing the germ tube. Plasma is the liquid part of the blood that does not contain blood cells but still contains blood clotting factors. Plasma is obtained by separating blood cells from the blood (whole blood) by centrifugation. The plasma has a different composition of clotting factors according to the type of anticoagulant added [11]. Serum is the liquid part of blood that does not contain blood cells and blood clotting factors. Other coagulation proteins and proteins unrelated to haemostasis remain in serum at similar levels in plasma [12]. By knowing that serum almost entirely has the same content as plasma, the authors are interested in observing plasma as an alternative medium for serum substitutes for media in germ tube test. Germ tube test on CPDA, Citrate plasma, and EDTA plasma, then compared to serum for the length of germ tube formation time.

Serum as a medium for germ tube tests is often an obstacle in practice in clinical laboratories related to the availability and safety of its use. This study aims to demonstrate the potential use of several types of plasma which are widely available for various purposes in healthcare facilities. The residues of these substances are expected to be used as a source of availability of effective germination media in germ tube tests. This certainly can facilitate the implementation of tests at the identification stage of Candida albicans which may cause secondary infections in patients under certain conditions.

## II. METHODHOLOGY

The research used an experimental method with a quantitative approach, and the data observed in this study were obtained from the results of examinations on the formation of germ tubes on the four tested media. Each media as the experimental treatment was observed five times in repetition. The time for the formation of the germ tube starts from the culturing of the yeast colonies into serum or plasma media until the formation of the specified germ tube. Time was recorded after the length of germ reached three times of the stem cell (yeast) diameter. The data were then analyzed statistically using a one-way ANOVA test. The study was conducted at Mycology Laboratory, Medical Laboratory Technology Department, Health Polytechnic of Jambi. This study was reviewed and approved by the Ethics Committee of Health Polytechnic of Jambi (Reference No. LB.02.06/2/263/2022 dated June 27, 2022).

### *A. MATERIALS*

Cultures of *Candida albicans* was obtained from the culture collection of Mycology Laboratory, Health Polytechnic of Jambi. The strain was subcultured and incubated at 37°C overnight for 48h on Potato Dextrose Agar (PDA) medium. Plasma CPDA was obtained from the collection of the blood bank unit at Raden Mattaher Hospital in Jambi. Meanwhile, plasma citrate and EDTA were obtained from the collection of blood samples left after the hematology course by students of MLT Department at Health Polytechnic of Jambi. All plasma was examined for glucose levels before being used as research material.

### 1. PROCEDURE FOR *CANDIDA ALBICANS* CULTURE

a) Tools, materials, and reagents: ose, spiritus, PDA media, Culture of *Candida albicans*.
b) Heat the ose over the spiritus, take the *Candida albicans* culture with ose and planted on PDA media with the scatter and scrape technique, incubation at 37°C for 2-3 days and viewed macroscopically, *Candida albicans* macroscopically in PDA media, the surface of colonies is smooth, slippery, yellowish white in colour and smells of yeast.

### 2. PROCEDURE OF GERM TUBE TEST

a) Tools, materials, and reagents: ose, tube, Pasteur pipette, objects glass, deck glass, spiritus, incubator, microscope, stopwatch, EDTA plasma, CPDA plasma, citrate plasma, serum, *Candida albicans* culture, glucose.
b) Two ml of each serum/plasma is put into the tube, add by 0.5 ml of glucose 5% solution (except for CPDA plasma that previously had glucose in it) into the tube, Then with the inoculum, apply a smear of the colony into the liquid medium contained in the tube aseptically. Homogenize it well, then put it in an incubator at 37oC for the first 1 hour to be further examined under a microscope. If the germ tube has not been found yet, further incubate and then a microscopic investigation is performed every five minutes until a germ tube that is three times the length of a yeast cell is found.

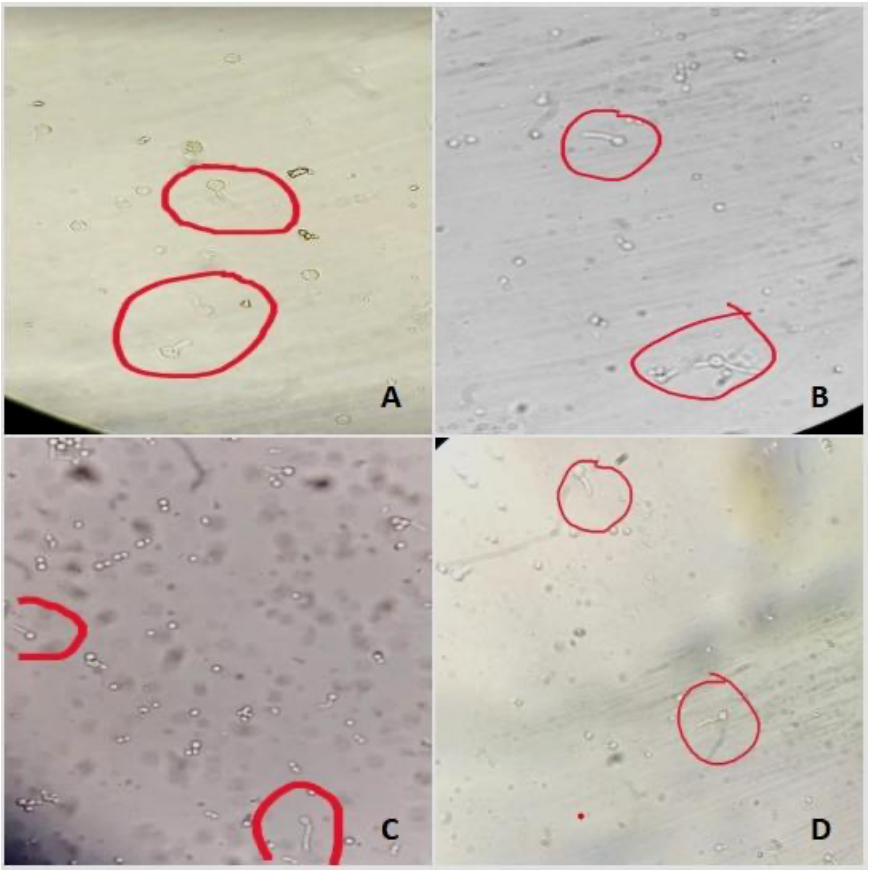


**FIGURE 1. Microscopic images of germ tube of *Candida albicans* on various media: A) serum; B) CPDA plasma; C) Citrate plasma; D) EDTA plasma (1000 X)**

c) Microscope observation: prepare a clean object glass and deck glass, then the tube that has been incubated is removed from the incubator, take the solution with a Pasteur pipette, place it on the object glass and cover it with a deck glass, examine under a microscope with a magnification of 10x10 and 45x10.

### *B. DATA ANALYSIS*

Analysis of the data in this study used Medcalc ver. 19.0.7 for Windows where the data were tested using a one-way repeated measures ANOVA test to evaluate the effectiveness of CPDA plasma, EDTA plasma, and Citrate plasma as the alternative medium in the germ tube test. The data were obtained from observing the germ tube formation time (in minutes) from each of the tested plasma media and serum as a comparison.

## III. RESULTS

From the observations made on the growth experiment of the germ tube test, it was found that all plasma can be used as a medium in the germ tube test, this is indicated by the presence of elongation of short hyphae (filamentous) arising laterally from yeast cells, without point of origin. The germ tube is half the width a 3 to 4 times the length of the yeast cell and there is no nucleus.

**TABLE 1**
**Germ tube formation time (minutes)**

| Repetition | Media | | | |
|---|---|---|---|---|
| | CPDA plasma | EDTA plasma | Citrate plasma | Serum |
| 1 | 120 | 150 | 135 | 60 |
| 2 | 140 | 170 | 160 | 75 |
| 3 | 130 | 155 | 140 | 65 |
| 4 | 135 | 170 | 150 | 80 |
| 5 | 120 | 165 | 145 | 70 |
| Mean | 129 | 162 | 146 | 70 |

The microscopic observation that has been carried out during the formation of the germ tube on each medium. Each treatment and repetition in this study was carried out separately with an observation period of five minutes for each assessment of germ tube formation. From the

microscopic observation, there was no difference in the morphology of the germ tube between the tested mediums (FIGURE 1). The details of the observed formation time are presented in TABLE 1. From TABLE 1 above, it can be seen that the formation time of the germ tube in the serum is faster than the three plasmas where the germ tube was formed in under 100 minutes with an average time was 70 minutes. While in the three plasmas, the germ tube was formed with an average of 120 minutes. Then a statistical test was carried out by performing a parametric test to find out the difference between the treatments with the one-way repeated measures ANOVA test. Obtained F-ratio value is 516.13 ($P < 0.0001$). The statistical results were significant at $P < 0.05$ which means that there was a significant difference in the time of germ tube formation between serum, CPDA plasma, Citrate plasma, and EDTA plasma. Even so, it was also found that the germ tube formation time of all three plasma was still in the range of 2 to 3 hours according to standard testing protocols or procedures found in previous studies. The shortest germ tube formation time was obtained in the treatment using CPDA plasma as a media of germination compared to other treatments using EDTA plasma and Citrate plasma as observed materials in this study (FIGURE 2).

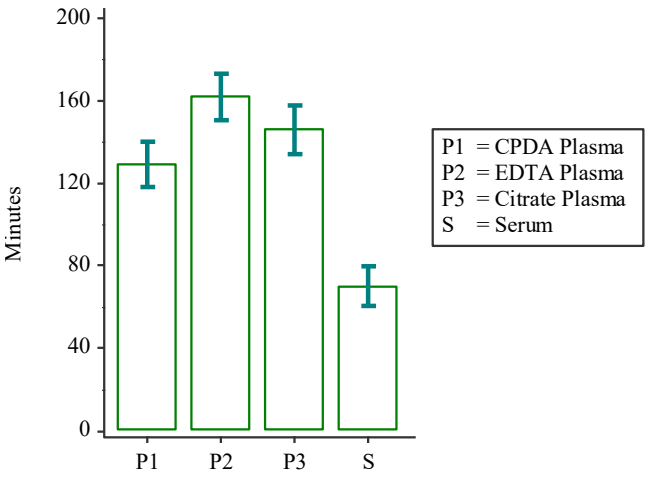


**FIGURE 2. The length of time for germ tube formation from the observed materials**

## IV. DISCUSSION

Based on the results of the research that has been done, it can be concluded that the germ tube test can be performed on serum and plasma media. This was shown by the microscopic observation of the shape of the germ tube, in which the production is defined as a filamentous outgrowth from a blastoconidium with a length at least three times as long as the parent cell without constriction at the junction.

This study shows that CPDA plasma has a germ tube formation time close to the germ tube formation time in serum from an average germ tube formation was 70 minutes. CPDA plasma was 129 minutes, citrate plasma was 146 minutes and EDTA plasma was 162 minutes, which means that of the three plasma (CPDA plasma, citrate plasma, and EDTA plasma) that have been tested were provided longer time of the formation. CPDA plasma is the best plasma as an alternative medium for serum substitution in the germ tube test for the identification of *Candida albicans.*

According to the UK Standards for Microbiology Investigations [13], an assessment of germ tube formation should be performed within 2 - 3 hours. Likewise, as stated in the standard operating procedure [14] that the observation must be carried out two hours after incubation within 2 hours. This is to avoid false positives that can be generated by other species. In this study, all the tested plasma was shown to produce germ tubes for about the recommended time. So it can be concluded that even though they are not as good as serum, they can be used as an alternative medium for the germ tube test.

Research by Mattei et al (2013) which analyzed the determination of germ tube, phospholipase, and proteinase production by bloodstream isolates of *Candida albicans* showed germ tube formation in almost all blood isolates from the patients studied, and succeeded in proving the relationship between germ tube as one of the virulence factors that determine the ability of *Candida albicans* to cause disease under certain conditions [15].

A recent study (Hemaid et al., 2021) conducted on patients of various diseases with reduced immunity succeeded in finding the development of germ tubes indicating *Candida albicans* infection in 48 of the 86 subjects studied. In this study, serum was used as germ tube medium with an incubation time of 2-3 hours [16].

CPDA plasma is known as the plasma that is obtained from blood transfusion units, blood plasma is obtained by separating whole blood and its cellular elements. Consists of the stable factor fibrinogen, albumin, and globulin. From 250 cc of whole blood, 125 cc of plasma is obtained and can last for 2 months at 4$^{o}$C. CPDA plasma had gone through infectious disease screening, so it is very safe to use by health workers, especially medical laboratory technology as a medium in germ tube tests, besides that CPDA plasma is very easy to obtain in large quantities when compared to serum, and is also not widely used, CPDA plasma is used only in certain cases. So the availability of CPDA plasma is very much in the blood transfusion unit [17–19].

A previous study by Mulyati et al (2019) observed the formation of germ tube on egg white media. The result was similar to this study, where egg white media was also able to produce germ tubes in 96.4% of the observed isolates within 2-3 hours. However, the presence of such media must be provided in particular, because it is not routinely available in healthcare services [20]. Unlike plasma, such as CPDA, Citrate or EDTA plasma in this study which can be found easier in laboratories or blood bank units. Researches on several commercial media that is particularly available in the laboratory also showed that the average times of germ tube

formation on those media were longer compared to serum [21,22].

Another research succeeded in proving that the germ tube test had the same effectiveness as phenotypic methods, such as PCR. In conclusion, this study recommended the germ tube test which was considered to have a lower cost and a faster turn-around time than PCR as the gold standard method [23]. This research used YEPD (Yeast extract Peptone Dextrose) broth as medium. The use of serum was avoided due to the consideration of the presence of the risk that the blood might be contaminated by viruses, such as HIV and Hepatitis [24–26].

We conducted this research for the same reasons as described above regarding the time and cost of testing. However, we recommend CPDA plasma as a safer media to use for germ tube test in order to avoid the risk of viral infection. This is considering that CPDA plasma has pass through the screening examinations for those viruses.

There are no previously reported studies comparing serum as a standard germ tube test material with plasma which is commonly found in healthcare services in Indonesia. This study shows the potential of plasma that can be utilized in the test. Moreover, plasma is obtained from aliquots of the Blood Bank Service at the most hospital, where it has pass through a series of tests for possible pathogens in it, such as bacteria, viruses, protozoa, etc.

## IV. CONCLUSIONS

This study found that there was a difference in the time of germ tube formation of the three plasmas tested against serum. However, all three plasma (CPDA, EDTA and Citrate plasma) still managed to show their ability to germinate even though it took longer (over 120 minutes) than serum (70 minutes). CPDA plasma even exhibits abilities that are very close to the protocol of germ tube test (2 to 3 hours) and appears to give a faster formation time than others. Therefore, CPDA plasma can be recommended as an alternative medium to substitute serum. CPDA plasma is obtained from a blood transfusion unit that has passed various screenings to ensure that plasma is safe to donate so that CPDA plasma is very safe to be used by laboratory personnel for media in the germ tube test to identify *Candida albicans*.

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