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Comparative study for identification of Candida albicans with germ tube test in human serum and plasma

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ABSTRACT:
INTRODUCTION:
Candidiasis is one of the most common opportunistic infection occurring in humans. Clinical spectrum of candidiasis includes mucocutaneous infections, GI tract infections, Blood stream infection, CNS infections, and also Hospital acquired infections.
C. albicans grows as creamy-white pasty colonies with distinct budding yeasts on Sabouraud Dextrose Agar (SDA) at 37°C. It can be differentiated from other candida species by variety of biochemical test like growth on chrom agar, cornmeal agar, growth at 37° C temperature and Germ tube formation. Germ tube test is very cost effective, rapid method for differentiation between candida albicans and other candida species. Germ tube test is simple to perform, turn around time is 2 hours and is safe to perform. Human serum is most common media used for germ tube production. In addition to human serum, number of other media induce germ tube formation including plasma, saliva, sheep serum, fetal bovine serum, rabbit serum and horse serum. Various other newer serum free media like egg white, YEPD medium, tissue culture medium is also commercially available for germ tube formation. Human plasma is one of the good alternatives of human serum for performing Germ tube test.
OBJECTIVES:
The aim of the study was to compare the use of human serum and plasma for germ tube production for the identification of Candida albicans.
METHODS:
This study was conducted in the Mycology Division of Department of Microbiology of a tertiary care hospital in Jamnagar. The study was approved by the Institute's ethics committee.
250 different types of samples such as urine, sputum, BAL & pus received at mycology section were included in the study.
Samples were inoculated on Sabouraud Dextrose Agar (SDA) containing gentamicin at 37°C for 48 hours. Creamy white colonies appearing visually as yeast were confirmed by Gram staining and microscopic examination. Isolates suspected of being Candida albicans were confirmed by the germ tube formation method using human serum and plasma. Triplicate sets of test tubes containing 0.5–1.0 mL of pooled human serum and plasma were inoculated with 2-3 colonies of each isolate. The tubes were inoculated at 37°C for 3 hours after which a drop of each suspension was placed on labelled microscope slides for examination of germ tubes.
RESULT: *250 samples were included in the study, of which 150 samples showed yeast like colonies on SDA media. Of these 150 samples,110 samples showed germ tube formation hence were labelled as candida albicans. 100 samples showed germ tube formation on both human serum and human plasma and 10 samples showed germ tube formation in human serum and not in human plasma. The sensitivity of the study turned out to be 90%. 40 samples showed no germ tube formation and hence were labelled candida non-albicans suggesting specificity of 100%.*
DISCUSSION: *Many methods can be used for germ tube formation detection, human serum being the most used and frequently employed. However, the need for serum to be used fresh, the storage conditions, the presence of various inhibitors in serum, alternate methods are tested for the germ tube formation. Human plasma offers advantages of being readily available from the blood bank, being tested against all the blood borne conditions and cost effectiveness, the results obtained from human serum and human plasma are comparable as is evidenced from the study,this is a good alternative that can be used for the diagnosis of candidiasis on the basis of germ tube formation.*
KEYWORDS: *germ tube, human serum, human plasma.*

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I. INTRODUCTION:

Candidiasis is one of the most common opportunistic infection occurring in humans. Clinical spectrum of candidiasis includes mucocutaneous infections, GI tract infections, Blood stream infection, CNS infections, and also Hospital acquired infections. Candida albicans is most prevalent yeast isolated from clinical samples. C. albicans can grow as budding yeast cells or as filamentous hyphal forms (mycelial state) depending on the growth conditions.[1,2] It is understood that the physiological conditions in an immunocompromised host can induce dimorphism in C. albicans to a hyphal state of growth. These growth conditions are simulated in the laboratory whereby C. albicans grows as yeast only or short-term germ tubes (hyphal state).[3,4,5]

C. albicans grows as creamy-white pasty colonies with distinct budding yeasts on Sabouraud Dextrose Agar (SDA) at 37°C. It can be differentiated from other candida species by variety of biochemical test like growth on chrom agar, cornmeal agar, growth at 37° C temperature and Germ tube formation. The hyphae are the predominant form with characteristic chlamydospores upon 24–48 hours incubation at 30°C on cornmeal agar or rice agar.[3,4,5] Endotrophic germ tube formation is the endogenous germination of C. albicans yeast cells. The germ tube has parallel walls and no constriction at the point of origin at the blastospore mother cell. It has been suggested to be a contributory virulence factor in the pathogenesis of C. albicans.[6,7,8] Germ tube test is very cost effective, rapid method for differentiation between candida albicans and other candida species. Germ tube test is simple to perform, turn around time is 2 hours and is safe to perform. Human serum is most common media used for germ tube production. In addition to human serum, number of other media induce germ tube formation including plasma, saliva, sheep serum, fetal bovine serum, rabbit serum and horse serum. Various other newer serum free media like egg white, YEPD medium, tissue culture medium is also commercially available for germ tube formation. Human plasma is one of the good alternatives of human serum for performing Germ tube test. It is easily available from blood banks. As it is taken from blood banks, the plasma is screened for blood borne pathogen which decreases chances of blood borne infections. Numerous studies have concluded that human plasma is as good as human serum for germ tube production.

II. AIMS & OBJECTIVES:

The aim of the study was to compare the use of human serum and plasma for germ tube production for the identification of Candida albicans.

III. MATERIALS & METHODS:

This study was conducted in the Mycology Division of Department of Microbiology of a tertiary care hospital in Jamnagar. The study was approved by the Institute's ethics committee.

250 different types of samples such as urine, sputum, BAL & pus received at mycology section were included in the study.

Samples were inoculated on Sabouraud Dextrose Agar (SDA) containing gentamicin at 37°C for 48 hours. Creamy white colonies appearing visually as yeast were confirmed by Gram staining and microscopic examination. Isolates suspected of being Candida albicans were confirmed by the germ tube formation method using human serum and plasma. Triplicate sets of test tubes containing 0.5–1.0 mL of pooled human serum and plasma were inoculated with 2-3 colonies of each isolate. The tubes were inoculated at 37°C for 3 hours after which a drop of each suspension was placed on labelled microscope slides for examination of germ tubes. Elongated daughter cells originating from the round mother cell without any constriction at their origin were considered as real and positive germ tube structure, and the constriction of the hyphae at the round mother cell was referred to as pseudo hyphae.[9.10.11]

IV. RESULT AND DISCUSSION:

Total 250 samples were included in this study, out of these 150 showed yeast like creamy white growth on Sabouraud dextrose agar. These 150 yeasts were analysed based on colony morphology, microscopy and germ tube formation.

Elongated daughter cells from the round mother cell without constriction at their origin are referred to as true germ tubes while constricted hyphae as pseudo hyphae. 110 isolates showed germ tube production using human serum as substrate and 100 isolates gave positive result with human plasma.

Type of Sample	N	Yeast like colony on SDA	Germ tube test positive (Candida albicans)	Germ tube test negative (Candida non albicans)
Urine	60	49	36	13
Sputum	90	52	37	15
Pus	80	42	32	10
BAL	20	07	05	02
Total	250	150	110	40

Table.01 Distribution of Candida albicans and Candida non albicans using Human serum

Of 250 samples tested for candida species,150 samples showed yeast like colonies on SDA. Of these 150 samples, 49 were urine samples in which, 36 were candida albicans and 13 were candida non-albicans based on germ tube formation. 52 were samples of sputum from which,37 were candida albicans and 15 were candida non-albicans, 42 were samples of pus, from which 32 were candida albicans and 10 were candida non-albicans, 07 were samples of BAL in which,05 were candida albicans and 02 were candida non albicans. The study showed that a total of 110 samples isolated from yeast like colonies were candida albicans and 40 samples were candida non-albicans.

Germ Tube (Human Plasma)	Germ Tube Test + (Human Serum)	Germ Tube Test – (Human Serum)
+	100 (True Positive)	00 (False Positive)
-	10 (False Negative)	40 (True negative)

Table.02 Statistical analysis of Germ tube test using plasma compared to human serum

Sensitivity= TP/(TP+FN) = 90% **Positive predictive value=TP/TP+FP =100%**
Specificity= TN/(TN+FP) = 100% **Negative Predictive value=TN/TN+FN= 80%**

In this study formation of germ tube was compared in human serum and human plasma, which showed, 100 germ tube formations in both human serum and human plasma, and 10 samples showed germ tube formation only in human serum and not on human plasma, giving false negative value of 10 and thus sensitivity rate of 90%. 40 samples showed no germ tube formation, in both human serum and human plasma, there were no samples that showed germ tube formation in human serum and not in human plasma hence, the specificity of the test turned out to be 100%. The positive predictive value was 100% and the negative predictive value turned out to be 80%. 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[4.] In spite of its low cost and easiness, the use of human serum for this test may have several disadvantages for example; serum has to be fresh otherwise frozen serum at 4° C for 15 days may have 50% decrease in germ tube production, false negative result due to the effect of biological inhibitors present in it, the yeast inoculum has to contain < 107 cells mL1, different batches of serum may produce different results and most importantly the possible risk of biohazard[3]. Hence, alternate method using human plasma was employed. The sensitivity and specificity in the present study was 90% and 100% respectively (Table 2). These results are comparable with other studies for validation of commercial media, where it is reported a ~96% of sensitivity and ~98% of specificity in several media[9,10,12,13.] S. C Deourukhar et al[4] suggested human plasma usage as third best alternate means for germ tube formation.

V. CONCLUSION:

Our results demonstrated a potential utility of human plasma in germ tube testing. Human plasma could be obtained from the Blood Bank Service, where it can be tested for the human blood borne pathogens like HIV, HTLV and others. This study allowed us to demonstrate that the technique of germ tube formation in human serum as compared to human plasma which showed high performance.

In spite of being cost effective and easy procedure, the use of human serum for this test may have several disadvantages too, like serum has to be fresh or it may hamper with germ tube formation, presents a hazard for transmission of blood borne diseases and hence, human plasma can be used as an alternative for germ tube formation and diagnosis of candidiasis.

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