ORIGINAL ARTICLE

Can serums be replaced by Mueller-Hinton agar in germ tube test?

MA Atalay, AN Koc, OM Parkan, G Aydemir, F Elmali[1], H Sav[2]

Departments of Clinical Microbiology and [1]Biostatistics, Faculty of Medicine, Erciyes University, Kayseri, [2]Department of Clinical Microbiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey

Abstract

Background: The germ tube test (GTT) is inexpensive, easy, and well-defined test that differentiates *Candida albicans* (excluding *Candida dubliniensis* and *Candida africana)* from other species. The aim of this study was to evaluate various serums (i.e., human, rabbit, horse, and fetal bovine serum) used in the GTT and Mueller-Hinton agar (MHA).
Materials and Methods: Fifty species isolated from various clinical samples that were defined as *C. albicans* by both conventional and DNA sequence analysis methods were included in the study. One to two colonies of *C. albicans* were mixed into 0.5–1 ml of fetal bovine serum, horse serum, rabbit serum, and human serum. Serums and MHA were incubated at 37°C for GTT. They were removed from the incubator and evaluated after 30 min, 1 h, 2 h, and 3 h of incubation. The GTT was accepted to be positive only if germ tube was 1/2 the width and 3 times the length of the parent yeast cell and with no constriction at the point of origin.
Results: When the use of serums and MHA for GTT was statistically evaluated, according to the positive scoring, the best results were obtained with MHA and with rabbit, horse, and fetal bovine serum, respectively. The best definition over time statistically was the third hour.
Conclusion: It is suggested that inexpensive MHA is a fast, appropriate, and reliable medium for the probable diagnosis of GTT and *C. albicans*; however, additional studies are still needed to define other *Candida* species.

Key words: *Candida albicans,* germ tube test, Mueller-Hinton agar, sequencing

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Introduction

The increased survival of cancer patients and Intensive Care Unit patients in recent years, widespread use of bone marrow and organ transplants, use of broad-spectrum antibiotics, corticosteroids, antineoplastic and immune suppressive agents, and indwelling catheters have led to an increase in the prevalence of fungal infections and mainly the infections caused by *Candida* species.[1-3] *Candida albicans* is defined as the most common causative agent, although there is a trend of increasing prevalence of species other than *C. albicans*.[4] Many rapid assays have been developed to identify yeasts, and most of these methods are extremely expensive and labor-intense and may not be available routinely in all laboratories.[5] The germ tube test (GTT) is a well-established, inexpensive, and easy-to-administer test used to identify *C. albicans* from other species (except *Candida dubliniensis* and *Candida africana*). Reynold and Braude described the germ tube formation for the 1st time in

Address for correspondence:
Dr. MA Atalay,
Department of Medical Microbiology, Erciyes University, Faculty of Medicine, 38039 Melikgazi, Kayseri, Turkey.
E-mail: altayatalay@gmail.com

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1956, and it labeled the "Reynold Braude Phenomenon."[5] The GTT is performed to induce the formation of hyphae at 37°C and neutral pH in the presence of serum. These conditions mimic the host environment.[6] In this test, one or two colonies of yeast are mixed with test substrate (fetal bovine serum) and incubated at 37°C for 3 h. The incubation period should not exceed 4 h, because other hyphae-producing yeasts start germinating beyond this time frame.[7]

The aim of this study was to evaluate various serums (human, rabbit, horse, and fetal bovine) used in GTT in the laboratories and Mueller-Hinton agar (MHA) used in antibiotic susceptibility tests.

Materials and Methods

Ethical approval was granted for this study by the Erciyes University Ethical Committee. A total of fifty strains identified as *C. albicans* (using both conventional methods and DNA-sequencing) from various clinical samples (52% from blood, 32% from bronchoalveolar lavage fluid, 16% from other samples) of 50 inpatients were included in the study. DNA sequencing was performed using an automated sequencer (3130 Genetic Analyzer; Applied Biosystems, USA). For GTTs; one or two colonies of *C. albicans* were mixed into 0.5–1 mL fetal bovine serum (Argene-Parc Technologique, France), horse serum (E and O Laboratories, Scotland), rabbit serum (Serotec, UK), and human serum. MHA (Merck, Germany) was cut in 1 cm × 1 cm dimensions and prepared as a slide culture. An inoculum from *C. albicans* colony was streaked onto the plate. For GTT, sera and MHA were kept at 37°C. The plates were evaluated for growth at 30 min, 1 h, 2 h, and 3 h. One drop of the serum was placed on the slides with a cover glass, and MHA prepared as slide culture was directly observed under light microscope (×20). The test was considered positive if a short hyphal extension was seen arising laterally from a parent yeast cell with no constriction at the point of origin and if it was half the width and 3 times the length of the parent yeast cell with no presence of nucleus. The evaluation was performed as follows: 1–9/10 field 1^+, 1–9/1 field 2^+, 10–90/1 field 3^+, >90/1 field 4^+. *C. albicans* ATCC 64546 was used as the standard strain. The data were analyzed using the two-way repeated measures analysis of variance.

Results

When the use of sera and MHA in GTT was evaluated according to positive scoring, the best results were obtained using MHA followed by rabbit, human, horse, and fetal bovine serum. The evaluation at 3 h offers the best detection time followed by 2 h, 1 h, and 30 min [Figure 1]. There were also significant differences between rabbit-fetal bovine and human fetal bovine serum at 2 h, and rabbit-fetal bovine, human-fetal bovine, and horse-fetal bovine serum at 3 h ($P < 0.05$).

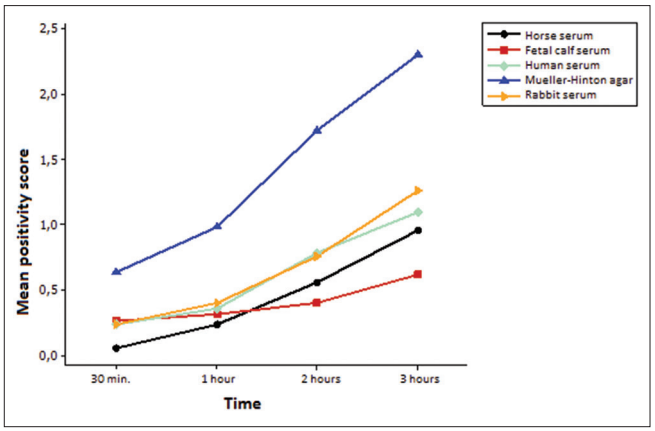

Figure 1: Mean positivity scores of serums and Mueller-Hinton agar according to time in germ tube test

Discussion

C. albicans, a component of the normal flora of the gastrointestinal tract, vagina, and oral cavity is the leading cause of opportunistic fungal infections reported in epidemiological studies.[5,8] Although various morphological, biochemical, and molecular methods are available for the identification of *C. albicans*, GTT is a simple, rapid, and highly reliable test that has been used for many years.[9,10]

Studies conducted with human serum reported a sensitivity of 91–100% and a specificity of 95–100% for GTT, and the studies using fetal bovine serum, rabbit serum, and horse serum reported a sensitivity of 92.3%, 90%, and 35%, respectively.[11-14] In the study by Hilmioglu *et al*.,[14] comparing 12 fluids, the best results in GTT were obtained with human serum, followed by rabbit and heart-brain infusion agar with the worst results obtained from horse serum. For GTT, human serum was found to be superior in the study by Arora *et al*.,[15] and horse serum was found to be superior in the study by Makwana *et al*.,[16] In studies that evaluated various broths, rice cream agar, 2% oxgall broth, and rice infusion-oxgall-Tween 80 agar yielded a sensitivity ranging from 98% to 100%.[17,18] Kim *et al*.,[19] reported that incubation in serum-free YEPD (1% yeast extract, 2% peptone, 2% dextrose) at 39°C for 1 h provided a rapid and reliable test protocol for germ tube formation in the detection of *C. albicans*. On the other hand, Rimek *et al*.,[11] reported that such broths (like serum-free YEPD) are not available commercially and are produced specifically for GTT, and they reported 91.5% and 60.0% sensitivity rate using commercially available MHA in the identification of *C. albicans* and *C. dubliniensis*, respectively. In our study, *C. albicans* statistically turned out to be the best to produce germ tube in MHA followed by rabbit, human, horse, and fetal bovine serum.

Many laboratories use human serum for GTT. However, the use of human serum has some disadvantages. Serum sample must be fresh or stored frozen. The yeast inoculum must contain $<10^7$ cells/mL. Otherwise, germ tube formation is inhibited, and the use of pooled human serum poses some risks such as transmission of HIV or hepatitis virus infections.[5,11] Mackenzie,[20] reported 50% reduction in germ tube formation when human serum is kept at +4°C for more than 15 days. According to our calculations, the cost of fetal bovine serum, horse serum and rabbit serum for GTT were approximately 130 USD, 20 USD, and 42 USD for 100 tests, respectively. However, when MHA is used (1 × 1 dimensions) for GTT, the cost was approximately 7 USD for 100 test. Hence, MHA is an inexpensive medium widely used in the microbiology laboratories and offering a long shelf life, avoids such risks.

Conclusion

MHA is an appropriate and reliable medium for the GTT and the presumptive identification of *C. albicans*, and additional studies are required to determine the identification of other *Candida* species.

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Conflicts of interest
There are no conflicts of interest.

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