EVALUATION OF DIFFERENT MEDIA FOR GERM TUBE PRODUCTION OF CANDIDA ALBICANS AND CANDIDA DUBLINIENSIS

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Abstract
The germ tube production in serum is a rapid method for identification of Candida. Because of the time required to prepare human serum and inherent safety problems concerned with its use, many laboratories have started using non human serum germ tube media. The objective of this study was to evaluate different media for germ tube production of C. albicans and C. dubliniensis. 132 C. albicans and 30 C. dubliniensis isolates were tested for germ tube production in various media like horse serum, pooled human serum, human plasma, trypticase soy broth, egg white and peptone water. Out of 132 C. albicans, germ tube production was seen in 130 isolates in trypticase soy broth, 124 isolates produced germ tube in pooled human serum and horse serum. In case of C. dubliniensis (n=30), 27(90%) isolates produced germ tube in trypticase soy broth and pooled human serum. In horse serum 26 isolates showed germ tube test positive. Our study shows that trypticase soy broth is the best medium for testing germ tube production of C. albicans and C. dubliniensis. It is more stable, effective, less expensive and safe than other media.

Keywords: Candida albicans, Candida dubliniensis, germ tube test, trypticase soy broth

1. Introduction
Fungal pathogens are becoming increasingly important cause of both community acquired and nosocomial infections and yeast species of the genus Candida are the most pathogenic fungi. While most Candida species are found in the environment, approximately a dozen or so are associated with colonization and infection in man. Candida species are commensals of the oral cavity, intestinal tract and vagina, with newborns being colonized soon after birth. Under certain circumstances they can opportunistically overgrow and can cause variety of diseases. The spectrum of candidiasis ranges from superficial infections of the vaginal and oral mucosae, to life-threatening systemic infections that spread via the bloodstream to organs throughout the body. The individuals at risk include intensive care and postsurgical patients; human immunodeficiency virus (HIV) infected hosts, patients with hematological malignancies, elderly patients and premature infants. Candida albicans is widely recognized as being the most pathogenic yeast species and in the majority of epidemiological studies has been found to be the most common cause of superficial and systemic infections. In 1995, a new Candida species was identified in HIV infected individuals with oropharyngeal candidiasis in Dublin, Ireland. This species, which was subsequently named Candida dubliniensis, is very closely related to C. albicans with which it shares many phenotypic properties, including the ability to produce germ tube and chlamydospores. Although various morphological, biochemical and molecular methods are available for identification of Candida isolates, the work up for yeast identification starts with the germ tube test in diagnostic mycology. The presumptive clinical identification of C. albicans and C. dubliniensis is usually based on its ability to produce germ tube when incubated at 37°C for 2 hours in pooled human serum. Germ tube formation was first reported by Reynold and Braude in 1956 and hence, the germ tube test is also known as a “Reynolds-Braude Phenomenon”. Taschdjian CL et al in 1960 first described a rapid method for identifying C. albicans by its ability to produce short, slender, tube like structures called the germ tubes when it is incubated in serum. In addition to human serum, a number of other mixtures induce germ tube formation, including plasma, egg white, saliva, tissue culture medium 199 (Difco laboratories, Detroit, Mich.), sheep serum, trypticase soy broth, and various peptone media. Because of the time required to prepare human serum and
inherent safety problems concerned with its use, many clinical microbiological laboratories have to use non-human serum germ tube media. Most of the studies on evaluation of media for germ tube production are focused on \textit{C. albicans}.\textsuperscript{8, 9, 10} Information on efficient media for germ tube testing of \textit{C. dubliniensis} is limited.

The present study was conducted with an aim to evaluate different media like pooled human serum, horse serum, human plasma, egg white, peptone water and trypticase soy broth for germ tube production of \textit{C. albicans} and \textit{C. dubliniensis}.

2. Materials and methods

A total of 132 \textit{C. albicans} and 30 \textit{C. dubliniensis} isolates obtained from various clinical specimens were included in study. \textit{C. albicans} and \textit{C. dubliniensis} were identified by conventional methods and growth on Hichrom Candida agar (Himedia laboratories Pvt. Ltd Mumbai).\textsuperscript{4} All isolates were sub-cultured onto Sabouraud’s dextrose agar and were incubated at 37°C for 18 to 24 hours before performing the germ tube test. For the production of germ tube different media like horse serum, pooled human serum, human plasma, trypticase soy broth, egg white and peptone water was evaluated. 0.5 ml of all the media were dispensed into 12x75 mm test tubes. The colony was lightly touched with a straight wire and then inoculated in the test tubes containing different media. A positive control (\textit{C. albicans} ATCC 10231) and a negative control (\textit{C. krusei}) were used with each batch of yeasts tested. The test tubes were incubated for 2 hours at 37°C.

For reading the test 1 or 2 drops of content from each test tube was withdrawn with a Pasteur pipette, placed on clean microscopic slide, and examined under magnification of X400 for the presence of germ tube. In order to investigate the germ tube structure, the elongated daughter cells from the round mother cell without constriction at their origin were referred to as germ tubes, and constriction hyphae at the round mother cell were referred to as pseudohyphae.\textsuperscript{8} A criterion for germ tube positivity was observation of minimum five germ tubes in entire wet mount preparation. Negative results were confirmed by examining at least 10 high power fields for the presence of germ tubes.

3. Results and Discussion

In the present study, as shown in figure No. 1, out of 132 \textit{C. albicans} isolates, germ tube production was seen in 124 (94%) isolates in trypticase soy broth, 124 (94%) isolates produced germ tube in pooled human serum and horse serum. In peptone water only 91 (69%) isolates showed germ tube test positive. In case of \textit{C. dubliniensis} (n=30), 27(90%) of isolates produced germ tube in trypticase soy broth and pooled human serum. In horse serum 26 (86.6%) isolates showed germ tube test positive. In egg white and peptone water, germ tube production was seen in only 15 (50%) isolates (Figure No.2).

Rapid identification of \textit{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. Several other rapid methods for the identification of yeasts have been developed. Most of these techniques, however, require expensive and labor-intensive technologies that are not commonly available in routine microbiology laboratory services. The germ tube test has been a long well-established routine procedure for identification of medically important yeasts.\textsuperscript{9}

Although \textit{C. albicans} cells reproduce normally by budding, giving rise to the formation of yeast cells, they frequently produce germ tubes under unfavorable conditions. The production of germ tubes results in conversion to filamentous growth or mycelial form. These morphological transitions often represent a response of the fungus to changing environmental conditions and may permit the fungus to adapt to different biological niches.\textsuperscript{8}

In the present study, 130 (98.4%) of total \textit{C. albicans} (132) and 27(90%) of total \textit{C. dubliniensis} (30) isolates showed germ tube production in trypticase soy broth. In pooled human serum and horse serum, the positivity for germ tube test was noted in 124 (94%) \textit{C. albicans}. 27 (90%) \textit{C. dubliniensis} produced germ tubes in pooled human serum whereas 26 (86.6%) showed germ tube production in horse serum. In a study by Joshi KR et al \textsuperscript{11} 100% strains of \textit{C. albicans} were positive for germ tube in trypticase soy broth. Berardinelli S and Opheim DJ \textsuperscript{12} reported a germ tube induction medium, composed of three parts rabbit coagulase plasma with EDTA and two parts trypticase soya broth for better production of germ tube in \textit{C. albicans}. On the other hand, Arora DR et al \textsuperscript{7} and Makwana G E \textsuperscript{16} et al reported trypticase soy broth to be
less effective for germ tube test. Arora DR et al suggested human serum to be best for the germ tube test, whereas Makwana GE et al found horse serum to be more effective for germ tube production. Pooled human sera which is routinely used in diagnostic laboratory has effect of biological inhibitors present in it, so there may be chances of false negative result. Mackenzie DWR reported that when human serum was stored at 4°C for 15 days there was a 50% decrease in germ tube formation. Taschdjian CL et al suggested that only freshly prepared or frozen human serum be used in germ tube test. Pooled human serum requires more time for preparation and may have inherent safety problems for the laboratory personnel. Trypticase soy broth is easily available in all clinical microbiological laboratories. It can be sterilized and stored at 4°C for at least 30 days without loss of germ tube production. This difference between the stability of pooled human serum and trypticase soy broth at 4°C is important since the use of trypticase soy broth would eliminate the time required to prepare, freeze, and subsequently thaw the individual vials required for the use with human serum.

Both C. albicans and C. dubliniensis showed poor rate of germ tube production in peptone water and egg white. Auger et al reported 67.6% Candida isolates and Arora DR et al reported 61.8% positivity in bactopeptone which is in agreement with our study. In contrast to our observation, Joshi KR et al reported, 100% of C. albicans to be positive for germ tube test in 1% bactopeptone whereas only 74% were positive in trypticase soy broth.

Conclusion:
Trypticase soy broth is found to be more effective than pooled human serum for germ tube test and subsequently for the presumptive differentiation of C. albicans and C. dubliniensis from other clinically significant species of Candida, without the extensively time required for the preparation and testing of pooled human serum. Furthermore, this medium is more stable, effective and less expensive than the major media used for germ tube test.

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**Figure No. 1** Number of Candida albicans showing germ tube production in different media.

**Figure No. 2** Number of *Candida dubliniensis* showing germ tube production in different media.