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Germ Tube Formation in Candida Albicans: Evaluation of Human and Animal Sera and Incubation Atmosphere.

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Abstract:
Using the method of Elmer et al (11), the germ tube test was performed for 10 previously confirmed germ-tube positive Candida albicans isolates, using sera obtained from human, rabbit, sheep, cow and goat blood. The possibility of germ tube formation within 30 minutes has been further confirmed in this study. Comparism of mean time of germ tube formation in the sera used showed a significant difference for cow and goat sera ($P<0.05$), with human serum inducing germ tubes at a faster rate. There was no significant differences ($P>0.05$) for both rabbit and sheep sera. Using Student t-test analysis, there was significant difference ($p<0.05$) when different atmospheric conditions of incubation were compared, with a faster rate of formation of germ tubes in 10% CO2. The preferential use of rabbit or sheep sera for demonstrating germ tube formation is suggested, to forestall possible transmission of infectious pathogens through human blood.

Key Words: Candida albicans, Germ-Tube Formation; Human and Animal Sera, Incubation Atmosphere.

Introduction:

The genus Candida contains more than 150 species, but only 10 are regarded as important pathogens for humans (1). The most pathogenic of these species is Candida albicans. C. albicans. is a dimorphic fungus, which characteristically develops both as yeast cells and as pseudohyphae. The organism has been recovered from soil, hospital environments, inanimate objects, and food. The normal residence is the vagina, bowel, mouth and skin of man, the organism assuming a pathogenic role whenever the normal defense mechanisms are interrupted.

C. albicans causes a wide variety of disorders, which include thrush (2), candidal enteritis (3), vulvovaginitis and urinary tract candidiasis (4), mucocutaneous candidiasis (5), and invasive candidiasis (6, 7, 8,). The importance of C. albicans in causing human diseases requires that the organism be identified from clinical specimens early enough. Because germ tubes develop quickly, they are used as a rapid presumptive diagnostic identification of C. albicans, usually within 90 minutes. (9). Germ tube formation is believed to contribute to pathogenicity of C. albicans (10).

The objective of this study was to evaluate the performance of other animal sera alongside human serum usually used in the germ tube test for diagnosis of C. albicans infection. The effect of carbon dioxide (CO2) in the production of germ tube was also investigated.

Materials and Methods:

Ten(10) young isolates of C. albicans grown on sabouraud agar medium and previously confirmed for ability to form germ tubes were used for the study. The test sera were aseptically collected from human, sheep, rabbit, cow and goat's blood. The method of germ tube test as described by Elmer et al (11) was used for this study.

Germ Tube test: Using a sterile loop, a small portion of a pure colony of C. albicans was inoculation in to sterile test tubes containing 0.5ml of each of the test sera. The resulting mixture was incubated aerobically at 37oC for not more than 2 hrs. To 0.5ml of human serum in a separate sterile test tube was inoculated a small portion of a pure colony of yeast and incubated in a 10% CO2 jar for not more than 2hrs. At 10minutes intervals, a drop of the yeast-serum mixture was placed on a clean microscope slide, covered with a cover slip and examined microscopically, using the x10 and x40 objective lenses. The appearance of small filaments projecting from the cell surface confirmed formation of germ tubes. The earliest time of such germ tubes production was noted for each test serum.

Results:

Table 1. shows the time of germ tube formation by 10 candidal isolates tested using human and other animal sera. It is evident from these results that the earliest time of germ tube formation varied from 30minutes to 60 minutes.

Table 2. shows a statistical comparism of the various animal sera with human serum. There is significant difference ($P<0.05$) in the time of production of germ tubes in human serum from that recorded for goat and cow's sera. However, the P-values for rabbit and sheep were found to be greater than 0.05 ($P>0.05$) indicating that there was no significant difference in the time for germ tube formation using rabbit, sheep or human serum.

The comparism of mean time of germ tube formation using human serum incubated aerobically and in 10% CO2, is shown in Table 3. Using t-test statistical analysis at 95% confidence limit, there was significant difference in atmosphere of incubation, with faster production of germ tube in CO2 atmosphere.

Table 1. Time (minutes) for germ tube formation using human and different animal sera.

Candida albicans Isolate.	Human	Rabbit	Sheep	Cow	Goat	Saline Control
1.	30	30	30	40	40	Nil
2.	60	50	60	60	60	Nil
3.	30	30	40	40	40	Nil
4.	40	40	40	60	60	Nil
5.	40	40	40	40	40	Nil
6.	30	30	30	40	40	Nil
7.	30	30	40	40	40	Nil
8.	50	40	50	50	60	Nil
9.	60	60	60	60	60	Nil
10.	30	30	30	30	30	Nil
Total	400	380	420	460	470	
Mean± S.D	40± 12.47	38±10.32	42± 11.35	46± 10.74	47± 11.60	

Table 2. Comparism of the mean time of germ tube formation for human and other animal sera.

Animal Sera	Mean time(minutes)	Mean Difference	Standard Error of mean	T-value	P-value
Human	40	-	-	-	-
Rabbit	38	2	1.33	1.50	>0.05
Sheep	42	2	1.33	1.50	>0.05
Cow	46	6	2.21	2.71	<0.05
Goat	47	7	2.41	3.29	<0.05

Number of Candida albicans isolates = 10
<0.05 = Statistically significant.
>0.05 = Statistically insignificant.

Table 3. Mean comparism of time of germ tube formation using human serum incubated in different atmospheric conditions.

Condition of incubation	Mean ±S.D	Mean Difference	Standard Error of mean	T-Value.	P-Value.
Aerobic	40±12.47	-	-	-	-
10% Carbon dioxide	36±8.47	4	1.63	2.45	<0.05
<0.05 = Statistically Significant					

Discussion:

Results from this study have shown the possibility of germ tube formation by Candida albicans within 30 minutes as previously shown by Cruickshank et al (12) All the candidal isolates with the exception of E, I and J, showed variations in their time of germ tube formation (Table 1.). Interestingly, germ tube formation in all test sera was as rapid as 30 minutes with isolate J, while it was delayed till 60 minutes in isolate I. We, however, did not investigate these candidal strains further. Although the literature describing the formation of germ tubes in C. albicans is controversial and contradictory (13), we believe that certain essential growth nutrients required for germ tube formation may have been originally present in sufficient amount in the original growth environment in the human host from where isolate J was cultured, while isolate I lacked sufficient nutrients.

For instance, a platelet-derived factor has been found to stimulate germ tube production (14). Nutrient such as haemin (15) glutamine (16), and certain amino acids (17) in the growth medium enhance the production of germ tubes by Candida albicans. The delay in formation of germ tubes in some of the isolates could be an index of low degree of pathogenicity in the strain. This suggestion agrees with the work of Richardson and Smith (18) which reported a greater ability of the virulent strain of C. albicans to form germ tubes more rapidly than attenuated strains. The association of virulence with ability to produce germ tubes has been previously reported (10).

This present study has also confirmed that sera from rabbit and sheep can be used for demonstrating the germ tube test, as reported by earlier workers (19). As shown in Table 2, there was significant difference ($P<0.05$), using Student t-test, in the time of germ tube production when human serum was compared with that of cow and goat, with human serum inducing germ tube production at a faster rate. There was however no significant difference ($P>0.05$)

when rabbit and sheep sera were compared with human serum.

Attempts in this study to perform the germ tube test with human serum under a different atmospheric condition revealed that there was faster production of germ tubes by C. albicans in CO2 atmosphere than in aerobic condition (Table 3). This finding agrees with those of Sims (20) who found that appearance of long unbranched mycelial tubes was much more evident in Candida albicans growing in 10% CO2. According to Joshi et at (21), placing a cover slip over the inoculated streak of C. albicans on a growth medium, helps to create a local anaerobiosis which stimulates germ tube formation. Lee et al (22) however could produce germ tubes only by aerating the liquid form of their amino-acid medium, which failed to induce germ-tubes when dispensed as plates. These apparently contradictory findings were reconciled by the explanation of Sims (20), that in both cases, the crucial factor was an increased concentration of CO2. Sims concluded that it was the trapping of the CO2 under the cover slip, and not anaerobiosis, that induced germ tubes in the techniques of Joshi et al (21). Also, since CO2 is found to be more than 20 times soluble than O2 at 37oC, the results of Lee and his colleagues (22) can be explained logically by the increase in the concentration of CO2 in the aerated liquid cultures over that at the surface of an agar plate rather than by a corresponding increase in O2.

The need to perform the germ tube test in 10% CO2 atmosphere is therefore indicated in this study. We also suggest the preferential use of rabbit and sheep sera in the germ tube test, in view of the increase of deadly diseases such as HIV/AIDS and hepatitis virus transmissible in human blood.

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