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SLIDE CULTURE TECHNIQUE FOR MICROSCOPIC EXAMINATION OF FILAMENTOUS FUNGI AND GERM TEST TUBE TECHNIQUE FOR MICROSCOPIC EXAMINATION OF YEAST FUNGI.

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Abstract

The slide culture technique aids in the study of undisturbed microscopic morphology details of filamentous fungi.

Germ tube test, Candida albicans is the most prevalent yeast isolated from clinical samples. Differentiation of Candida albicans from non-candida albicans, without the extensive time required for the preparation and testing of pooled human serum.

Method: Germ test tube, and Slide culture**,**

Result: Microscopy,

Keywords: Slide culture technique, filamentous fungi, Germ tube test, Candida albicans, Microscopy,

INTRODUCTION

Conventional slide culture technique for the detailed examination of undisturbed microscopic detail of filamentous fungi remains one of the mainstay procedures for morphology identification of fungi in diagnostic mycology laboratories across the world. An accurate identification based on visualisation of microscopic details like shape, delicate arrangement of

conidia and attachment f conidia with conidiogenous cells is of paramount importance and has a bearing on clinical management of the mycoses. **(1)** Many micro fungi form very fragile reproductive structures which are at least partially disrupted by even the most careful manipulation. This well-known characteristic has resulted in the development of a variety slide culture techniques. **(2)** The usual procedure is to examine teased wet-mount preparation. These have a number of disadvantages, however. The structures depended upon for the recognition of such fungi as blastomycoces dermatitidis or Sporotrichum schenkii, for example, are often so distorted or disarranged in these teased mounts that identification is made difficult or impossible, prepared, ,make possible the definite recognition of these genera, and also help materially with the classification of other varieties of fungi**.(3)** This method preserved the morphological features relatively undisturbed compared with adhesive tape preparation**.(4)**

GERM TEST TUBE TEST

Candida albicans is the most prevalent yeast isolated from clinical samples. **(5)** However an increase in the emergence of nonalbicans Candida spp. has been ported in last decade. The presumptive identification of C. albicans is usually based on its ability to produce germ tube when incubated at 37 c for 2 hours in pooled human serum. But C. dubliniensis also produces true germ tubes germ tube and C.tropicalis may produce false germ tubes which basically are pseudohyphae with constriction at its origin in human serum in similar condition. **(6)** So a simple, fast safe and practical method, which can be routinely performed in average mycology laboratory, is needed to differentiate C. albicans from other species of candida more accurately. In addition to human serum, a number of other media induce germ tube formation, including plasma , saliva , sheep serum, fetal bovine serum, rabbit serum, and hours serum. Newer techniques using serum-free media like egg white, tissue culture media. **(7, 8)**

METHOD

All C. albicans isolates were sub-cultured onto Sabouraud's dextrose agar and were incubated at 37c for 24-48 hours before performing the germ tube test. **(11**) For the germ tube test, a light inoculum was made, of 2-3 colonies of each isolate from fresh culture in 0.5 ml of all the

above media which were dispensed in 12×75 mm test tube. A positive control C. albicans ATCC 10231) and a negative control in (C. krusei) were used with each batch of yeasts tested. Then the inoculated test tubes were incubated at 37°C in a water bath for 3 hours. Evaluation of germ tube formation was done by placing a drop of incubated suspension placed on a glass slide and covered with coverslip. Microscopic examination was done under magnification of 40X for the presence of germ tube. 8 Of typical C. albicans reveals thin germ tube. 3 to 4 mm in diameter and up to 20 mm long; unlike pseudo hyphae that is not constricted at their point of origin. 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 10X high power fields for the presence of germ tubes. (10)

Microscopy

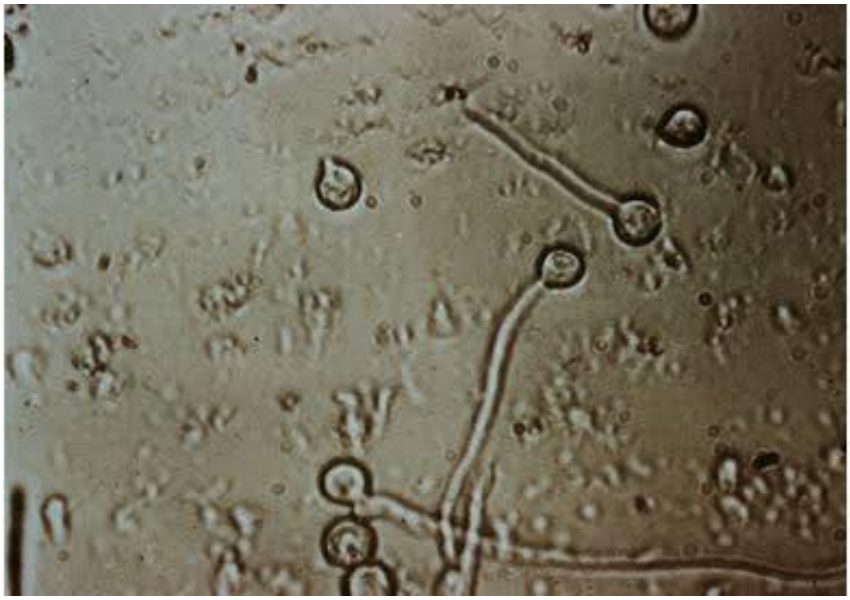

Figure 1: Positive germ tube test

Materials Required

Culture

7-10 day old fungal culture

Media

Sabouraud Dextrose agar

Preparation of Sabouraud Dextrose agar (ph-5.6)

Contents

Peptone -10g/litter

Dextrose-40g/litter

Agar-15g/litter

Equipment's

- Sterile glass petri dish
- Filter paper (9 cm diameter)
- V-shaped glass rod
- Microscope slides (26×76) and coverslips (sterile)
- Sabourauds dextrose agar plate with mixed culture of fungi
- Sabourauds dextrose agar plate
- Lactophenol cotton blue stain
- Glass capillary tube
- Scalpel
- Inoculating needle
- Sterile distilled water
- 95% ethanol
- Forceps

Basic Slide Culture a bent glass rod was placed in sterile petri plate side, and a sterile glass slide was put on the glass rod. A 1-by 1-cm block SD agar cut with a sterile scalpel was then transferred to the glass slide ff. Using sterile wire needle, the fungus would then be inoculated from culture plate to the four sides of the agar block. Sterile coverslip was put over the block with slight pressure to ensure adherence. Approximately 2 mL of sterile water was put on the bottom of petri plate, and then the plate cover was replaced. **(1)**

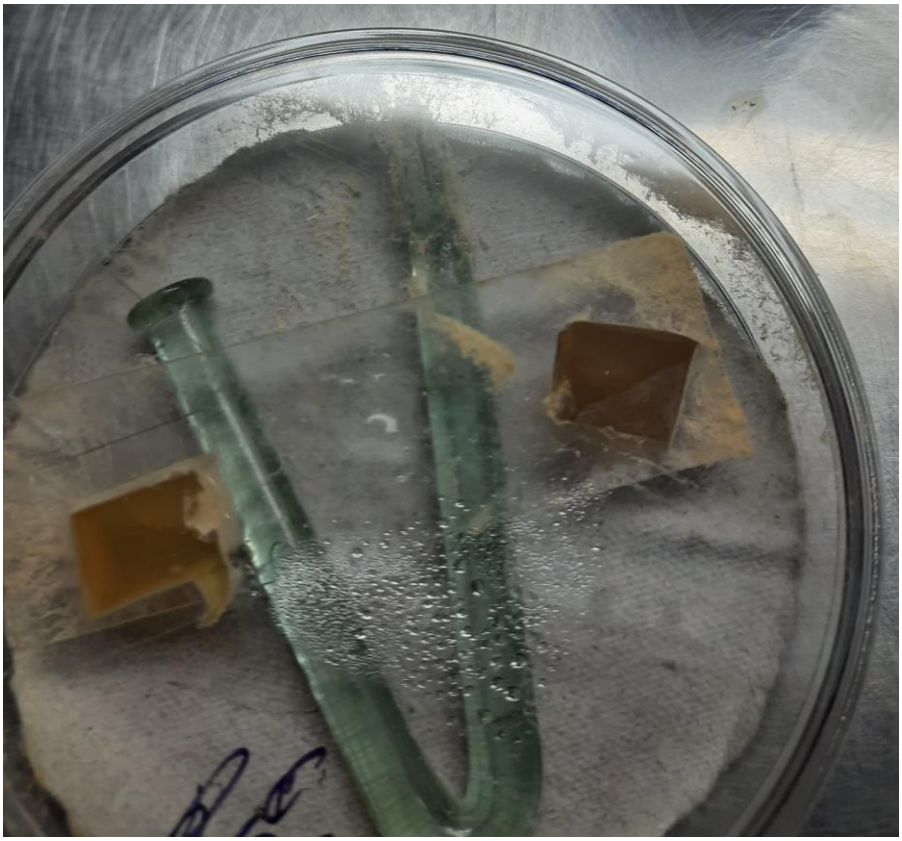

Figure 2: Slide culture test.

Microscopy examination after slide culture test

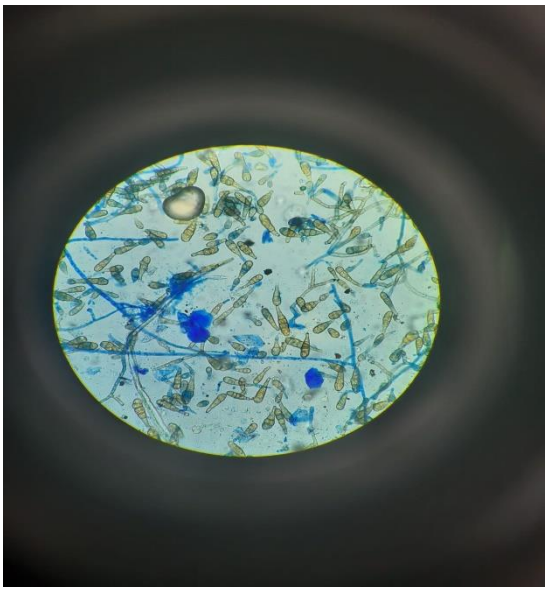

Figure 3: Alterneria species

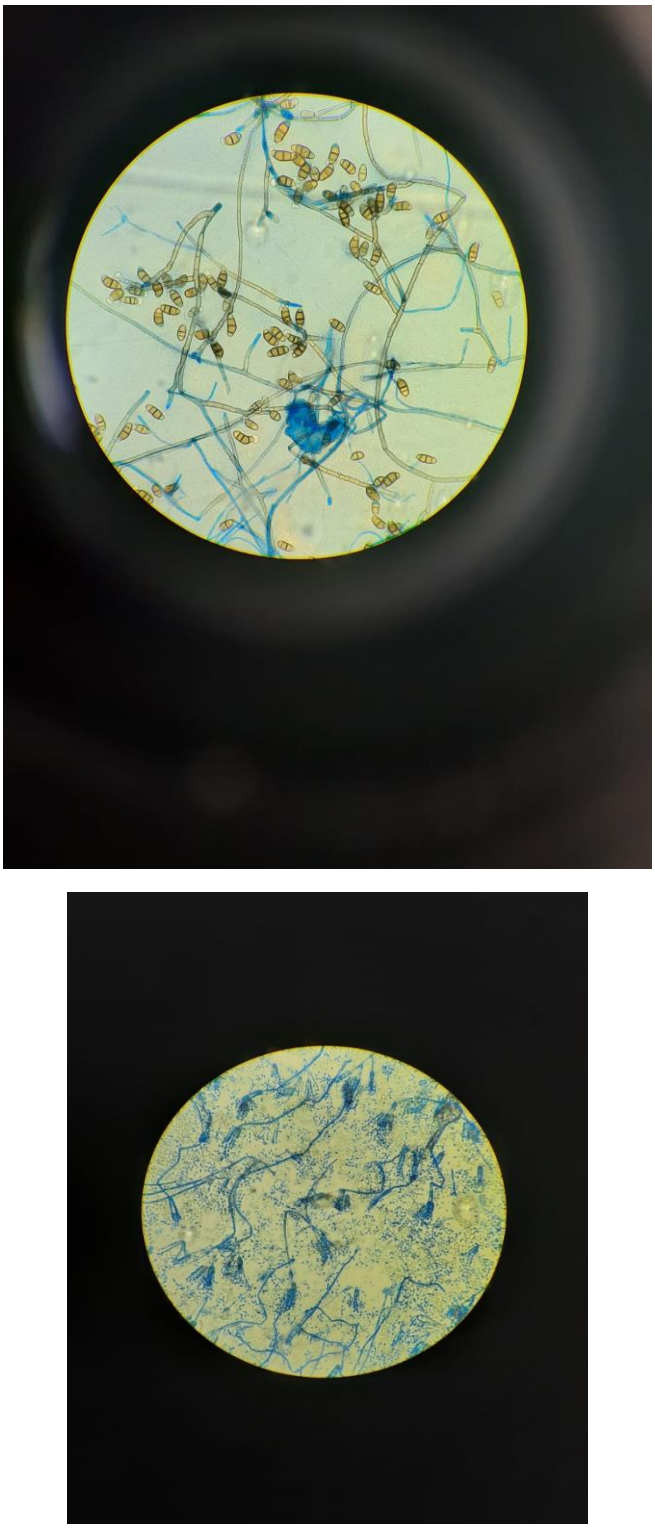

Figure 4: Penicillium species

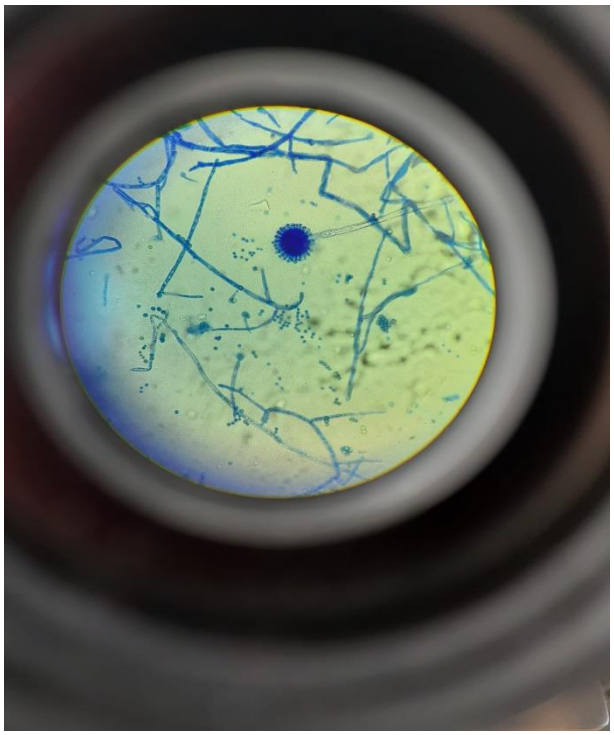

Figure 5: Aspergillus species

REFERENCES

1.YegneswaranPrakash, Kanika Bhargava, A modified micro chamber agar spot slide culture technique for microscopic examination of filamentous fungi,https://doi.org/10.1016/j.mimet.2016.02.015Get rinhtsand content

2.JAMES L. HARRIS, modified method for fungal slide culture. Vol . 24, No.3 sept. 1986,p.460-460.

3.EDWIN A. JOHNSON. An improved slide culture technique for the study and identification of pathogenic fungi, February 2, 1956)

4.Yeva Rosana, Tetsuhiro Matsuzama, Tohru Gonoi, Anis Karuniwati, Modified slide cultre method for faster and easier identification of dermatophytes. DIO: 10.5454/mi.8.3.7.

5.Deorukhkar SC, Saini S, and ladhav PA. evaluation of different media for germ tube production of candida albicans and candida dubliniensis international journal of biomedical and advance research 2012;03;704-07.

6.Mattei AS, Alves SH, Severo CB , Guazzelli LS, Oliveira FM. And Severo LC. Use of Mueller-Hinton broth and agar in the germ tube test. Rev. inst. Med. Trop. Sao Paulo 2014; 56:483-5.

7.Abiroo Jan, Gulnaz Bashir, Rubhana Qadir, Bashir A Fomda, Sofia, Aamir yoqoob Haka, modified germ tube test: A rapid test for differentiation of candida albicans from candida dubliniesis.volum 5/ issue 3/march 2018:2454-7379.

8.Atalay MA, Koc AN, Parkan OM, Aydemir G, Elmali F, Sav H. can serum be replaced by muller-Hinton agar in germ tube test?. Niger L Clin Pract 2017;20:61-3.

9. Yeva Rosana, Tetsuhiro Matsuzawa, Tohru Gonoi, et al. modified slide culture method for faster and easierv identification of dermatophytes. Vol.8, no.3, September 2014, p 135-139 doi: 10.5454/mi.8.3.7

10.Kim D, Shin WS, Lee K H, Park J K, Koh CM. Rapid differentiation of candida albicans from other candia species using its unique germ tube formation at 39 dgeree C. yeats 2002;19(11):957-62.

11. Upasana Bhumbla1, Amit gupta2. Acomparative study for production of germ tube in candida albicans of various pulmonary samples, by different methods in atertiary care hospital of south west Rajasthan. DOI: https://dx.doi.ORG/10.18203/2394-6040.IJCMPH20214598.