Research Article

Evaluation of an alternative slide culture technique for the morphological identification of fungal species

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K**ey words:** fungi, Morphological identification, Microscopy, Slide culture

Abstract

Morphological identification is still the most commonly used method for the identification of pathogenic fungi, with slide culture being the most widely used method. However, slide culture is associated with several problems. An alternative method using cavity slides to perform slide cultures was developed and the methodology and preliminary validation results are described.

Introduction

Morphological identification of fungal species is still the most commonly used method for the identification of pathogenic fungi despite the advent of molecular techniques, due to the expenses associated with the latter. Slide culture remains the mainstay of morphological identification of fungal species in the clinical laboratory. This method preserves the morphological features relatively undisturbed compared with tease mounts and cello-tape mounts.

However, slide cultures are also associated with problems. Removal of the cover slip and agar block results in changes in the morphological features, such as the angle of branching and attachment of macro and micro spores to sporangiophores, which leads to difficulty in speciation of organisms. As the cover slip needs to be removed for staining, repeated visualization of the same slide over a period of time to observe different growth stages is not possible. Examination of structures embedded in agar is also not possible. In addition, the technique takes time to

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master and a relatively long time is needed for the spores to develop. Examination of the agar block itself could be attempted as an alternative to the conventional slide culture technique to overcome the difficulties experienced in examination of agar embedded structures. The technique described in the current study was developed with the objective of overcoming these problems.

Materials and Methods

Preparation of Cavity Slides Cultures (CSC): Packs of Petri dishes, each containing a cavity slide, cover slip and a filter paper slip were sterilized. Sabouraud agar (SA) was prepared according to manufacturer's guidelines, aliquoted in to small containers and sterilized. An aliquot of the SA was melted by heating and pipetted into the cavity of the cavity slide with a sterile pasture pipette and allowed to set for 5 minutes. Each fungal isolate to be identified was inoculated onto the SA using a sterile straight wire and a sterile cover slip was placed on top of it. The filter paper was moistened with sterile distilled water to humidify the culture environment and the Petri dishes were incubated at room temperature.

Identified cultures of *Aspergillus niger*, *Fusarium spp*, *Microsporum audouinii*, *Trichophyton mentagrophytes* and *Trichophyton tonsurans* were inoculated as described and fungal growth observed using a light microscope (x10 and x40 magnification) on a daily basis.

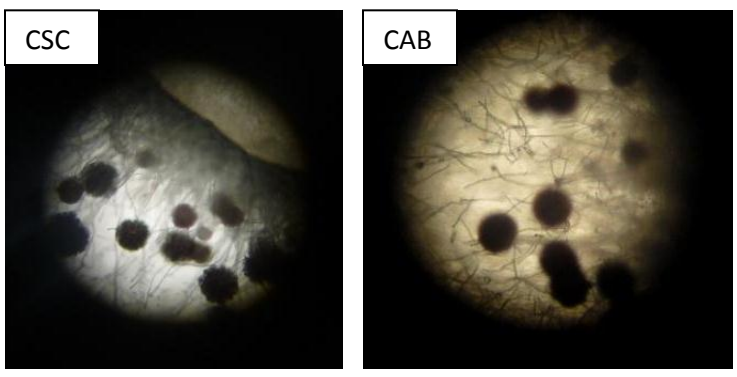
Multiple conventional slide cultures were also performed[1] using a modified method[2] which allowed examination of structures embedded in agar. For comparison, the slides were examined daily without removal of the cover slips using an x10 magnification.

When structures were noted in the CSC, the coverslip was slightly raised and lacto phenol cotton blue was applied using a Pasteur pipette. The stain would diffuse through the medium in the cavity of the slide to stain the fungal filaments within the agar. The cover slip of the conventional slide culture was removed and stained with lacto phenol cotton blue for comparison with the CSC findings of the same day.

Results

Figure 1 demonstrates the appearance in both culture systems.

Figure 1. Day 1 cavity slide culture (CSC) and conventional agar block (CAB) without staining



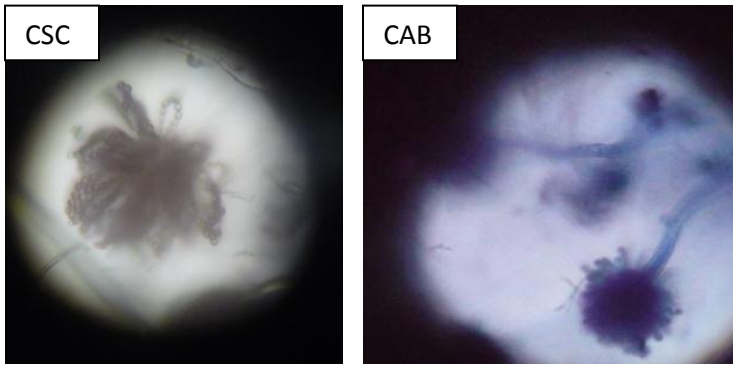
Earlier visualization of structures related to identification was possible with the cavity slide method for all 3 species as shown in Table 1. Figures 2-7 illustrate the differences in visualization between the cavity slide cultures and conventional slide cultures.

Table 1 : Comparison of Cavity Slide Cultures and Conventional slide cultures

Species		Day 1	Day 2	Day 3
Aspergillus niger	CSC	Septate hyphae seen. Biseriate conidia seen to be radiating from the conidial head.		
	CBA	Same. However, the architecture of the conidial arrangement was disturbed in many conidial heads		
Fusarium spp (Macrospores not detected due to repeated subculturing.	CSC	Septate hyphae seen. Simple conidiophores with one or 2 celled conidia arranged singly or in clusters.		
	CBA	Septate hyphae seen. Occasional 1 or 2 celled conidia seen. However, as the conidia were not well developed, arrangement was very sparse.	Septate hyphae and pointed terminal chlamydo-conidia seen.	
Microsposum audouinii	CSC	Septate hyphae seen.		
	CBA	Nil observed	Septate hyphae seen. chlamydo-conidia not seen	
Trichophyton tonsurans	CSC			Variable shaped microconidia seen. Macroconidia not seen.
	CBA			Microconidia seen. Very dispersed. Macroconidia not seen.
Trichophyton mentagrophytes	CSC			Spiral hyphae, cigar shaped macroconidia and microconidia seen.
	CBA			Spiral hyphae and macroconidia not seen. Microconidia seen.

CSC - Cavity slide cultures CAB - Agar block slide culture after removal of cover slip

Figure 2. Close up of an *Aspergillus* head on day 1.



(Note preservation of the intact spore chains in CSC)

Figure 3 -Day 2 growth of *Fusarium spp*.

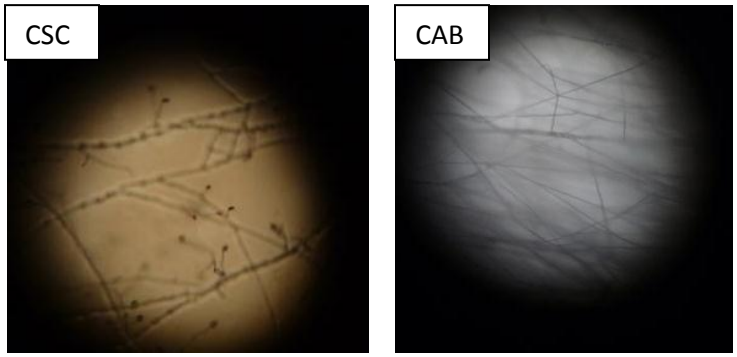


Figure 4 CSC of *Fusarium spp*. on day 2 and 4, showing the different stages of development x40 magnification

Figure 5. Terminal chlamydoconidia of *M audouinii* as seen by CSC

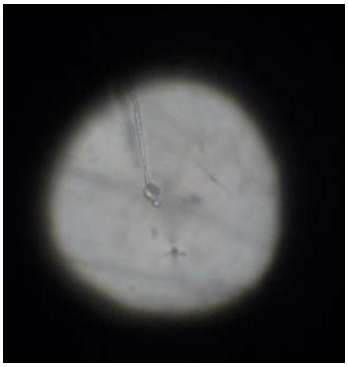
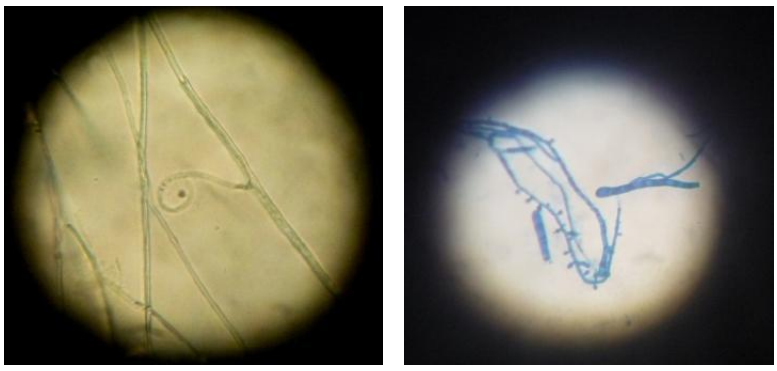


Figure 6. *Microsporum audouinii* on CSC– A & B are prior to staining and C is after staining

Figure 7. *Trichophyton tonsurans* microconidia as seen by the CSC prior to staining

Figure 8. *Trichophyton mentagrophytes on CSC* - macro and microconidia and spiral hyphae



Discussion

The conventional agar block slide culture method does not allow staining to visualize structures in the agar block using lactophenol cotton blue. Only the imprinted material on the cover slide could be stained and visualized. The cavity slide cultures allowed the visualization of structure within the agar block after staining as shown in Figures 2-8. In addition, as the sides of the agar block were exposed to the environment during the observation, contamination was always a possibility with the agar block of the slide culture. Similarly, due to the thickness of the agar block, focusing of the slide culture agar block under x 40 was not possible in many instances.

The cavity slide method was compared with conventional slide culture method for visualizing fungal structures, which is a requirement for identification. This preliminary study demonstrates that these structures can be visualized earlier than that in the conventional method. This method needs to be further validated for timeliness, accuracy and reliability with more fungal species.

References

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