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SHORT COMMUNICATION

Modified Slide Culture Method for Faster and Easier Identification of Dermatophytes

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Basic slide culture as a morphological identification was known as the most common method for the identification of pathogenic mold fungi. This method preserved the morphological features relatively undisturbed compared with adhesive tape preparations. However, it was necessary to modify method of basic slide culture to improve its usability and shorten the time it needed to identify mold fungi. There were four kinds of method carried out in this study; two kinds of modified slide culture, one kind of direct culture on slant agar plate, and a basic slide culture for identifying mold fungi, which result would be compared with each other. These four methods were tested to 4 species of dermatophytes which were known as mold fungi that could infect skin, hair, and nails in human; those were *Trichophyton mentagrophytes*, *Microsporum canis*, *Microsporum gypseum*, and *Epidermophyton floccosum*. Result of this study showed that both modified slide culture and direct culture on slant agar plate could visualize the structure of dermatophytes faster than basic slide culture method. These methods were also easier to prepare compared to basic culture method. Conclusion of this study showed that basic slide culture method needed to be modified for better identification of mold fungi.

Key words: Identification, Dermatophytes , Modified Slide Culture

Slide culture konvensional sebagai suatu identifikasi morfologi dikenal sebagai metode yang paling umum untuk identifikasi jamur kapang patogen. Metode ini relatif lebih tahan lama untuk menyimpan gambaran morfologi dibandingkan dengan menggunakan metode selotip. Walaupun demikian, perlu dilakukan modifikasi metode *slide culture* konvensional untuk meningkatkan kegunaannya dan mempersingkat waktu yang diperlukan untuk mengidentifikasi jamur kapang. Pada penelitian ini dilakukan empat metode; dua metode *slide culture* yang dimodifikasi, satu metode *slide culture* pada plat agar miring, dan satu metode *slide culture* konvensional untuk jamur kapang, yang hasilnya akan dibandingkan satu sama lain. Keempat metode ini diujikan pada 4 spesies dermatofita yang dikenal sebagai jamur kapang yang bisa menginfeksi kulit, rambut, dan kuku pada manusia; yaitu *Trichophyton mentagrophytes*, *Microsporum canis*, *Microsporum gypseum*, dan *Epidermophyton floccosum*. Hasil penelitian menunjukkan bahwa kedua metode *slide culture* yang dimodifikasi dan *slide culture* langsung pada plat agar miring dapat memvisualisasikan struktur dermatofita lebih cepat dibandingkan metode *slide culture* konvensional. Metode ini juga lebih mudah pada tahap persiapannya dibandingkan *slide culture* konvensional. Kesimpulan dari penelitian ini menunjukkan bahwa metode *slide culture* konvensional perlu dimodifikasi untuk identifikasi jamur kapang yang lebih baik.

Kata kunci : Jamur Dermatofita, Identifikasi, Modifikasi *Slide Culture*

Superficial fungi was known to live on the dead horny layer of the skin and elaborate an enzyme that enabled them to digest keratin, causing the superficial skin to scale and disintegrate, nails to crumble, and hairs to break off. Superficial fungi were also capable of eliciting an allergic or id reaction. Superficial fungal infections were common skin diseases, affecting millions of people worldwide. These infections occurred in both healthy and immunocompromised patients, and their etiologic agents consisted of dermatophytes, yeasts and nondermatophyte molds (Mims *et al*. 2008; Richardson *et al*. 2000).

Dermatophytes were responsible for most superficial fungal infections. Dermatophytes could be classified according to their natural habitats into three categories: (1) geophilic, which normally live in the soil, (2) zoophilic, which primarily parasitize the body surfaces of animal, (3) anthropophilic, which generally infect human (Hiruma *et al.* 2003). There were three

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genera of dermatophytes could infect the human, including *Trichophyton*, *Microsporum* and *Epidermophyton*. Only *Microsporum* and *Trichophyton* invaded the hair, whereas *Epidermophyton* and *Trichophyton* caused nail infections. These species of dermatophytes were correlated with the clinical diseases, which are classified according to the location of the infection; the clinical lesion, the species of fungi, and therapy varied for different sites (Pakshir *et al*. 2009). Identification of this species was very important because of their chronic nature had a risk for significant morbidity.

Microscopic examination using KOH preparation was a simple laboratory method for detection of fungal organisms present in skin and nails or hair. It was accomplished by scraping the skin, nail lesion or hair and examining the material with the microscope. Using this method we could show the two structural elements of fungi such as the spore and hyphae, but it could not identify the species of dermatophytes (Larone 2011).

To identify or confirm the diagnosis of superficial fungal infections, fungal culture must be done using appropriate medium (Difco 2003). It was best to examine dermatophytes microscopically when the culture had form conidia or spores. The best method for preserving and observing the actual structure of dermatophytes were the slide culture (Fujita 2013). This method preserved the morphological features relatively undisturbed compared with tease mounts and cello-tape mounts (Hughes *et al*. 2004). Slide culture was not a rapid method, but it was unsurpassed as a routine means of studying the fine points of the microscopic morphology of dermatophytes (Patrick *et al*. 2010, Wijedasa *et al*. 2012). Because basic slide culture method was quite difficult to carry out and requires a long time to get a result, it was needed to modify slide culture method to gain a better result.

In this study, there were carried out four methods plate to identify dermatophytes fungi that could infect skin, hair, and nails in human: a basic slide culture method, two kinds of modified slide culture and one kind of direct culture on slant agar. Although basic slide culture was a standard method for mold identification, but it needs space to incubate and not easy to prepare. The method of modified slide culture, agar block was put directly to a plate agar to produce of reproductive structure more rapid. It did not require bent glass rod and glass slide, and without adding sterile water on the petri plate. Moreover it could be put more than two slides. The difference between two kinds of modified slide culture were time to put agar block, the one was placed on the sterile medium while the other was put after the growth of the dermatophytes. Method of direct culture on slant agar only need half volume of standard media for culture, and can visualize structure the dermatophytes directly under the microscope. These *four* kinds method were tested to 4 species of dermatophytes, which were *Trichophyton mentagrophytes*, *Microsporum canis*, *Microsporum gypseum*, and *Epidermophyton floccosum*.

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 potato dextrose agar (PDA) cut with a sterile scalpel was then transferred to the glass slide (Fig. 1A). 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. The whole procedure was repeated for each of culture used in this study. When everything set, the plates were put on clean plastic basket and incubated at 30^0 Celcius.

Modified Slide Culture. A 1-by-1-cm block PDA cut with a sterile scalpel was transferred to a plate of PDA (Fig. 1B). Fungus was inoculated using sterile wire needle onto the four sides of the agar block. Sterile coverslip was put over the block with slight pressure to ensure adherence, and the plate cover was replaced afterwards. The whole procedure was repeated for each of culture used in this study. After it finished, the plates were put on clean plastic basket and incubated at 30^0 Celcius.

Another modified slide culture method used fungus that was grown on potato dextrose agar (Fig. 1C). A 1-by-1-cm block PDA was put on fungus culture. Sterile coverslip was put over the block with slight pressure to ensure adherence, then the plate cover was replaced. The whole procedure was repeated for each of culture used in this study. Then, the plates were put on clean plastic basket and incubated at 30^0 Celcius.

Direct Culture on Slant Agar Plate. Approximately 10 mL PDA was poured on sterile petri plate and was spread on the plate. Then the petri plate was put on its cover to gained about half of the plate covered with thin layer of PDA. After potato dextrose agar was solid, the petri plate was covered and kept in room temperature.

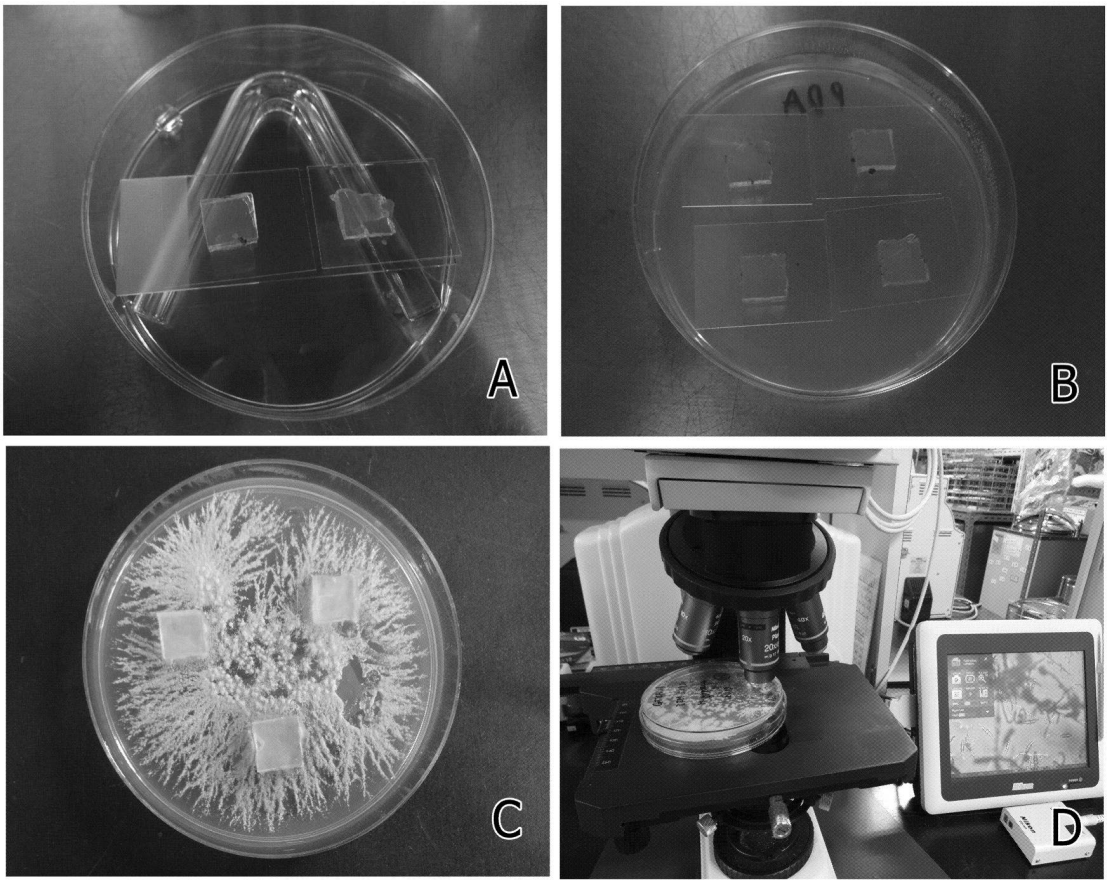

Fig 1 Basic slide culture, agar block PDA on the glass slide put on the bent glass rod (A); Modified slide culture 1, agar block PDA was put on the sterile medium (B); Modified slide culture 2, agar block PDA was put after the growth of the dermatophytes (C); *Direct culture on slant agar plate,* visualize structure the dermatophytes directly under the microscope (D).

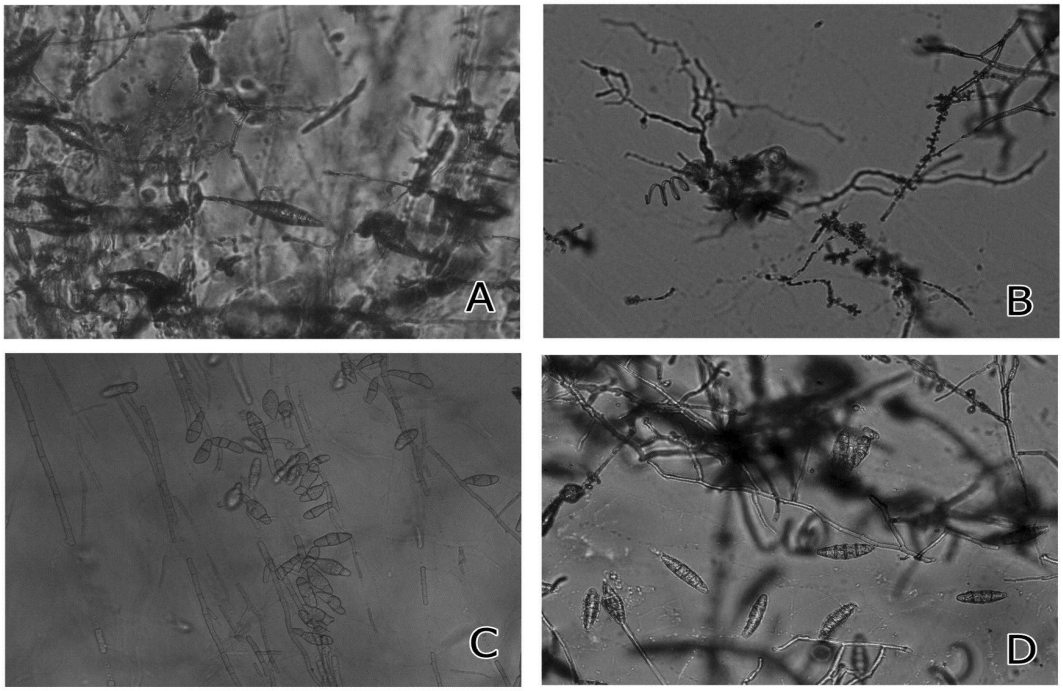

Fig 2 Macroconidia of *Microsporum canis* (A); Spiral hyphae and microconidia of *Trichophyton mentagrophytes* (B); Macroconidia of *Epidermophyton* floccosum (C), Macroconidia of *Microsporum gypseum* (D).

Fungus was directly inoculated on slant agar plate. The whole procedure was repeated for each of culture used in this study. When done, the whole plates were put on clean plastic basket and incubated at 30^0 Celcius.

Reading and Interpretation of the Results. The growth of fungus was examined periodically. The fungus could grow on the surface of the slide and also under the surface of the coverslip. The closed petri plate could be placed on the microscope stage, and the slide culture could be examined with the low-power (10x) objective. When reproduced structures had developed well, forceps was used carefully to remove the coverslip and to put it on a second slide filled with a drop of LPCB. Agar block was removed using flamed wire needle and put into container of antifungal disinfectant. A drop of LPCB was put on the remaining slide and the slide was then covered with a new coverslip. Each of the microscopic slide culture was sealed around the edges with the nail polish and kept on room temperature.

For direct culture plates, the examination was done on the slant agar plate under a microscope (Fig. 1D). The dermatophytes structures which are key features for species identification were observed through thin layer of PDA directly.

Earlier visualization of structures related to identification was possible for all 4 species as shown in Figures 2. All of the methods allowed observation of very delicate fungal structures of diagnostic significance. This method could overcome three major drawbacks that were associated with adhesive tape preparations. First, fungal structures were be prevented from crushed and damaged during the preparation procedures, affecting the accurate identification of the fungus. Second, as a result of the smear was not dry, slide culture can be used for microscopic examination for long period from the initial preparation. Third, fungal structures embedded in the agar can be observed. Slide cultures *was sealed around the edges with the nail polish* were also suitable for long-term storage *on room temperature*.

Table 1 Comparison of basic slide cultures, modified slide sulture and direct culture on slant agar plate

Species	*	Day 5	Day 7	Day 10
Microsporum canis	**BSC**	Macroconidia (-)	Macroconidia (-)	Macroconidia (+)
	MSC1	Macroconidia (-)	Macroconidia (+)	
	MSC2	Macroconidia (-)	Macroconidia (+)	
	DSC	Macroconidia (-)	Macroconidia (+)	
Trichophyton mentagrophytes	**BSC**	Spiral hyphae (-) macroconidia (-) Microconidia (-)	Spiral hyphae (-), macroconidia (-), Microconidia (+)	Spiral hyphae (+), macroconidia (+), microconidia (+)
	MSC1	Spiral hyphae (-) macroconidia (-) Microconidia (+)	Spiral hyphae (+) macroconidia (+) microconidia (+)	
	MSC2	Spiral hyphae (-) macroconidia (-) Microconidia (+)	Spiral hyphae (+) macroconidia (+) microconidia (+)	
	DSC	Spiral hyphae (+) macroconidia (+) microconidia (+)		
Epidermophyton floccosum	**BSC**	Macroconidia (-)	Macroconidia (-)	Macroconidia (+)
	MSC1	Macroconidia (-)	Macroconidia (+)	
	MSC2	Macroconidia (-)	Macroconidia (+)	
	DSC	Macroconidia (+)		
Microsporum gypseum	**BSC**	Macroconidia (-)	Macroconidia (-)	Macroconidia (+)
	MSC1	Macroconidia (-)	Macroconidia (+)	
	MSC2	Macroconidia (-)	Macroconidia (+)	
	DSC	Macroconidia (-)	Macroconidia (+)	

* BSC (basic slide cultures); (MSC1) modified slide sulture 1; (MSC2) modified slide culture 2; DSC (direct culture on slant agar plate)

The comparison of each method could be seen in Table 1. Basic slide culture required more days to visualize morphology of dermatophytes, compared to modified slide culture 1, modified slide culture 2, and direct culture on slant agar plate. *Microsporum canis* required the longest time to visualize compared to others species in this study. In addition, as the sides of the agar block were exposed to the environment during the observation, contamination of the slide culture was always a possibility. Similarly, due to the thickness of the agar block, focusing on the slide culture agar block under x 40 was not possible in many instances.

Modified slide culture 1 and 2 was the best method for storage slide purpose. It could visualize faster than basic slide culture, but only cover slide could be stained and visualized. Direct culture on slant agar plate took the shortest time to visualize all of the dermatophytes species in this study compared to the other methods. Although the direct culture method did not allow staining to visualize structures using lactophenol cotton blue, it seems the direct culture on slant agar plate was the best method to rapid identification for diagnoses purpose, but it was not suitable for storage. Direct examination of the agar plate under a microscope was particularly useful for observation of dermatophytes structure which was the key features for species identification.

In conclusion, this study demonstrates that basic slide culture method visualized structure of dermatophytes longer than modified slide culture and direct culture on slant agar plate. Basic culture method needed to be modified for identification of dermatophytes.

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