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Modified Method for Fungal Slide Culture

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A modified slide culture method which combines advantages found in several slide culture methods is described. A block of inoculated nutrient agar sandwiched between two sterile cover glasses is placed in a plastic petri dish containing water agar. After adequate growth has occurred, the slide culture is disassembled and mounted in a conventional manner.

Many microfungi 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 of slide culture techniques (1–3, 7, 8, 10–13). Each of these was devised to meet the special needs of the investigator or the requirements of the fungi under study.

There was a need in this laboratory for an economical, space-saving, easy-to-prepare-and-maintain system in which sporulating cultures could be grown for semipermanent slide mounts. A modified method adapted from Roberts (9) and Koneman and Roberts (5) and described here fulfills those requirements.

Sterile 1.5% water agar (7 to 8 ml) was poured into sterile 60-mm plastic petri dishes and allowed to solidify. A sterile 22-mm^2 cover glass was centered on the agar. The desired nutrient agar medium (10 ml) was poured into a second 60-mm petri dish, allowed to solidify, and cut with a sterile stainless steel spatula into blocks approximately 5 to 8 mm^2. One block was aseptically removed and placed on the cover glass. Inoculation of the agar block on one or more sides with fungal hyphae or conidia was followed by placement of a second sterile cover glass on top of it. After the petri dish lid was replaced, the completed modified slide culture (Fig. 1) was incubated at the desired temperature until adequate growth and conidiogenesis had occurred. Each cover glass was used to prepare a semipermanent mount on a standard microscope slide (3 by 1 in. [7.62 by 2.54 cm]). The top cover glass was lifted off with forceps and wetted on the specimen side with a drop of ethanol (70 to 90%). One drop of fungus mounting medium (e.g., lactophenol cotton blue) was applied to the specimen, and the cover glass was lowered gently onto the slide, specimen side down. The bottom cover glass was lifted from the water agar and similarly mounted on a second slide. The nutrient agar block adhered to one of the cover glasses during the cover glass mounting procedure and was carefully lifted off with a sterile dissecting needle before the application of alcohol and mounting medium. Heat fixation of the cover glasses before mounting as described by McGinnis (6) may improve the stability of the conidium-bearing structures.

Zygomycetous fungi and other organisms with particularly tenacious hyphae required a different mounting strategy. Following incubation and growth of the slide culture, the top cover glass was removed from the nutrient agar block. A second clean cover glass was lowered in a knifelike fashion perpendicularly onto the remaining slide culture cover glass near the agar block, thereby separating some of the fungal growth from the nutrient agar medium. While the vertical cover glass was held in place, a dissecting needle was used to score between it and the nutrient agar medium at the intersection of the cover glasses. The agar block was then pushed off the slide culture cover glass, and the vertical cover glass was gently removed and discarded. The slide culture cover glass, with adequate fungal growth remaining attached, was lifted off the water agar, wetted with alcohol, and mounted as described above.

The modified slide culture combines features that make it most suitable for this laboratory. The only sterile glassware required is a supply of dry-heat-sterilized cover glasses. The use of disposable, sterile plastic dishes eliminates glass petri dish sterilization and washing. The small dish of agar is a space-saving moist chamber which eliminates the need for bent glass slide support rods and filter papers, as in the traditional Riddell procedure (8). A supply of water agar dishes may be refrigerated for at least 3 weeks without excessive moisture loss. The culture may be tilted at any angle for examination without danger of liquid splashing on the specimen or displacement of the cover glass and agar sandwich.

Most bacteriological-grade agars will yield a water agar layer which is clear enough to permit inspection of an intact slide culture through the agar by using a low-power objective lens. Periodic checks of the fungal growth may also be made by removing the petri dish lid and lowering a low- or high-power objective lens over the top cover glass or by leaving the lid in place and using a dissecting microscope. Fogging of the cover glass with moisture does occur in some of the slide cultures, but this moisture evaporates completely during the mounting of the cover glasses on slides for microscopic study. The fogging which has been observed does not interfere significantly with periodic checks of the intact slide culture through the bottom of the dish. In situations in which fogging presents a problem, as in a time course study of a developing conidial structure, the addition of glycerol to the water agar has been reported to reduce or eliminate this condensation (1).

The more complex preparation involved in the slide culture methods of Riddell (8), Reiss (7), and Cole and Kendrick (2) is avoided with the modified slide culture. For some purposes, more elaborate specimen chambers are necessary. The Cole-Kendrick culture chamber is still one of the best chambers for time-lapse studies of conidiogenesis (2).

For studies of geotropism in fungi, the modified slide culture chamber may be conveniently placed on edge and taped to the incubator wall, providing a simpler alternative to the method of Reiss (7). It also may be an alternative to the nutrient shift method of Ellis and Ajello (3) used to stimulate sporulation of zygomycetous fungi. If larger plastic petri dishes are used, the culture may be set up between

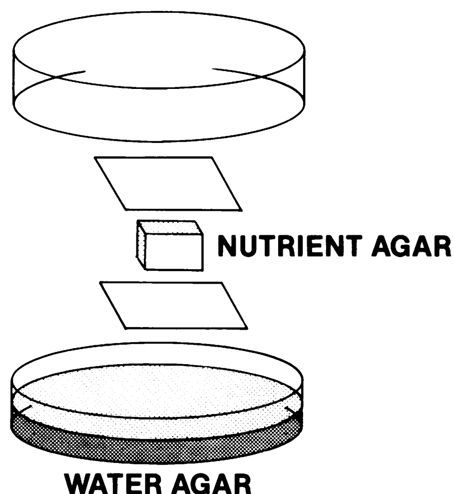

FIG. 1. Expanded view of the modified slide culture.

cover glasses positioned on the water agar surface near the edge of the dish. When the shift of the nutrient agar block to the water agar surface is desired, the dish may be opened and the nutrient agar block may be pushed off the lower cover glass onto the water agar.

Smaller cover glasses could be used in the modified slide culture to prepare the more durable and permanent mounts described by Kohlmeyer and Kohlmeyer (4).

Although some persons find "dipping small cover glasses in molten agar to be simpler than cutting and handling small squares from a film of agar (1)," the method described here has become the preferred technique in this laboratory.

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