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# Quality Control Slide for Potassium Hydroxide and Cellufluor Fungal Preparations

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**An opaque, water-insoluble quality control material with a skinlike microscopic appearance was prepared by inoculating melted xanthine (0.4%) agar with filamentous fungi and dispensing drops onto glass slides. After solidification of the agar, the material was rapidly cleared by 10% KOH, revealing fungal elements stained by Cellufluor reagent.**

Direct microscopic examination of clinical specimens submitted for mycology laboratory processing can provide a tentative diagnosis, suggest special or selective media not routinely inoculated, and confirm a diagnosis of a causative agent not easily grown in vitro. There has been a dramatic increase in fungal infections detected in patients with the acquired immunodeficiency syndrome. This has resulted in personnel from other laboratories, such as cytology and hematology, encountering fungal elements more often during routine screening procedures without the familiarization that frequent contact promotes (3).

The 10% potassium hydroxide preparation is frequently used for direct examination because it digests proteinaceous debris, bleaches many pigments, and separates keratinized cells. The chitinous walls of fungi are somewhat resistant to this action, permitting detection of fungal elements present (2, 5). Cellufluor, an optical brightener, can be added to the KOH solution. It binds to cellulose and chitin and fluoresces when excited by long-wavelength UV light, enabling microscopic detection even when fungi are present in small numbers or are obscured by debris and cells (1, 4).

Quality control testing of KOH and Cellufluor has generally been disregarded. Although it is impractical to stockpile skin scrapings or other tissue from infected patients for quality assurance testing, certain certifying agencies regard stain quality control as mandatory in mycology laboratories.

A simple, inexpensive quality control material has now been developed to permit reagent evaluation and to provide controlled, positive test results for teaching and familiarization purposes. Xanthine agar (Remel, Lenexa, Kans.), used for differentiating aerobic actinomycetes, was used as the suspending medium, containing 23 g of nutrient agar and 4 g of xanthine per liter of distilled water (pH 6.8). For preparation of quality control slides, a 25-ml tube of xanthine agar was melted and then cooled to 50°C, and phenol was added to achieve a final concentration of 0.5%. Melted agar was dispensed in 5-ml portions and refrigerated until use. For use, medium was melted, cooled to 45°C, and inoculated with mycelium from a stock culture of *Epidermophyton floccosum* (any common filamentous fungus may be used). The concentration of fungal elements was determined empirically by preparing a suspension in 5 ml of water roughly equal to a 0.5 McFarland standard and then adding a similar amount to 5 ml of the opaque melted medium. *Candida albicans* (0.2 ml of a culture at $1 \times 10^8$ CFU/ml) was added to some preparations when both yeast cells and filamentous forms were desired. Approximately 0.25 ml of inoculated liquefied agar was placed onto a series of prelabeled microscope slides with a sterile plastic transfer pipette (St. Amand Manufacturing Co., San Fernando, Calif.). A similar volume of uninoculated material was used to prepare negative control slides. Dispensed agar was manipulated with the pipette tip to ensure uniform thickness and coverage over a 2- by 3-cm area. The material dried to a hard, thin, opaque film that resisted mechanical removal. Preparations were stored in boxes at room temperature and at 4°C. No deterioration was observed after 6 months under either storage condition.

Upon addition of water to a slide, the microscopic appearance mimicked that of a skin scraping, i.e., opaque material with no clearly defined fungal elements (Fig. 1A). Addition of 10% KOH (Difco Laboratories, Detroit, Mich.) produced rapid clearing, revealing well-defined fungal elements (Fig. 1B). Treatment of a slide with equal amounts of Cellufluor reagent (Difco Laboratories) and KOH followed by excitation with long-wavelength UV light (Zeiss transmitted light microscope fitted with a 50-W mercury lamp, an exciter filter with a peak at 440 nm, and a barrier filter with a peak at 500 to 520 nm) resulted in brightly fluorescing fungal elements (Fig. 1C). Slide clearing was limited to an area equivalent to the size of the drop when 1 or 2% KOH was added. This feature permits detection of suboptimal concentrations of KOH reagent not conveniently tested by other methods.

This inexpensive, simple quality control material is useful in demonstrating the action of KOH by mimicking clearing of skin and other cellular material and allows a safe, easily stored means of testing Cellufluor and KOH reagents. Negative control slides provide a means to detect contamination of reagents. The slide promotes compliance with inspection checklist recommendations and permits a form of quality assurance testing previously not possible and neglected.

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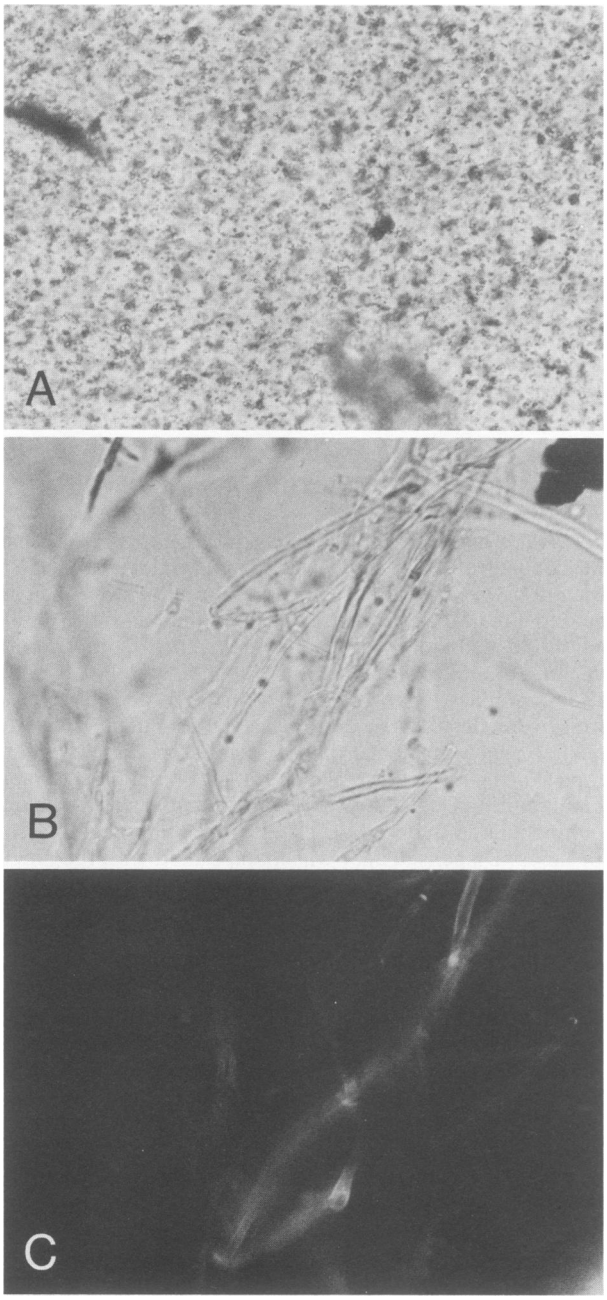


FIG. 1. Microscopic appearance of quality control slides with a drop of water (A), a drop of 10% KOH (B), and a drop of 10% KOH plus a drop of Cellufluor reagent (C). Magnification, ×400.

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