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A Method to Render Unstained Mycobacterial Smears Safe for Storage or Shipment

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Sterilization and optimal staining of *Mycobacterium tuberculosis* in sputum smears was accomplished by heating at 65 C for 30 min followed by exposure for 10 min to 10% formaldehyde fumes.

A facet of mycobacteriology laboratory procedures which probably receives too little attention is provision for the safe handling of smears prior to staining. It is often desirable to retain unstained smears for future studies, for shipment to central reference laboratories, or for inclusion in laboratory evaluation surveys. One would not expect fixing smear material by rapid flaming of the slides to kill all bacilli present. Also, the efficacy of extended heating on an electric slide warmer to kill these organisms has been questioned.

Smears prepared on microscope slides are usually air-dried and then fixed by heating over a flame or by heating on an electric slide warmer for 2 hr at 65 C (1). To determine the effect of heat-fixing on viability of *Mycobacterium tuberculosis* in sputum specimens, smears on 10 sterile microscope slides were prepared from a concentrate containing numerous bacilli (>2 bacilli per high-power field). The smears were air-dried and then heated for 2 hr on a slide warmer at 65 C. The concentrate material was taken up on a sterile moistened swab and inoculated into Middlebrook 7H10 broth, and the tubes were incubated for 8 weeks at 36 C. Growth of *M. tuberculosis* occurred in cultures from 6 of 10 slides.

In another experiment, air-dried concentrates on slides were exposed for 10 min in a closed container to fumes from a 1:10 aqueous dilution of 37% formaldehyde solution (Mallinckrodt Chemical Works, St. Louis, Mo.—inactive ingredients: methanol and water 63%) and inoculated to 7H10 broth to check for viability of *M. tuberculosis*. Growth of *M. tuberculosis* was observed in cultures from 5 of 10 slides. However, exposure of air-dried smears for 10 min to fumes from 10% formaldehyde (HCHO) resulted in negative cultures from all slides.

Concentrates fixed only with HCHO lost much more material during staining than did the heat-fixed smears. This difficulty was overcome by heating the slides for 30 min at 65 C followed by exposure for 10 min to 10% HCHO fumes. The heat-HCHO procedure revealed two advantages over the 2 hr-65 C fixation: (i) less material was lost from the slide during staining than when smears were heated for 2 hr or only exposed to HCHO, and (ii) the brilliance of fluorochrome-stained bacilli appeared to be slightly increased. No differences in cell morphology were observed in comparisons of smears fixed by the heat-HCHO method with those fixed in the conventional manner.

Thus, heat fixing for 30 min at 65 C followed by exposure to 10% HCHO fumes for 10 min renders unstained microscopic slides of specimens containing viable *M. tuberculosis* safe for storage or shipment. The fluorochrome-staining reaction is unaffected or perhaps enhanced by this treatment.

LITERATURE CITED

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