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NOTE

A METHOD OF CLEANING MICROSCOPE SLIDES FOR THEIR REPEATED USE IN THE EXAMINATION OF SPUTUM FOR TUBERCLE BACILLI*

The difficulty with which acid-fast bacilli are removed from glass slides has led several workers to recommend that only new slides should be used for the preparation of smears to demonstrate the presence of tubercle bacilli (Wilson & Miles, 1955; Baker, Silverton and Luckcock, 1957; Cruickshank, 1960). This practice cannot easily be followed in many countries where microscope slides of good quality are expensive and often difficult to obtain.

The experiment reported here was undertaken to determine whether a modern method of cleaning, incorporating the use of a detergent, can remove the tubercle bacilli from glass slides on which they have been heat-fixed and stained.

Methods

Preparation, staining and examination of smears

Smears were prepared by spreading a small portion of the most purulent or most mucoid part of a sputum specimen over two-thirds of the surface of 3″ × 1″ (7·5 cm. × 2·5 cm. approx.) glass slide using a wire loop. After fixing by heat, the smears were stained by the auramine-phenol method and examined by fluorescence microscopy (Holst, Mitchison & Radhakrishna, 1959). Smears were graded as 3-plus positive if they contained more than approximately 5,000 bacilli/mm.^{2}, 2-plus if they contained between approximately 300 and 5,000 bacilli/mm.^{2} and 1-plus if they contained less than approximately 300 bacilli/mm.^{2}.

Cleaning of used slides

Used slides were discarded into jars containing 5% phenol and after a few days, the slides were autoclaved at 1·5 kg./cm.^{2} for 30 minutes. The slides were then boiled in 5% 'KINRAY R'† (an alkaline detergent containing sodium carbonate, sodium metasilicate, a wetting agent and a source of phosphates) for 30 minutes and after washing in running water, left in 10% dichromate cleaning solution (Baker, Silverton & Luckcock, 1957) for 18 hours. The slides were then washed in 3 changes of running tap water, rinsed in distilled water, and dried in a hot-air oven at 100° C.

Examination of previously used slides

Smears were prepared from a sputum specimen obtained from a patient with bronchitis, which did not contain tubercle bacilli, on 223 slides which had previously been reported as positive for acid-alcohol-fast bacilli and washed by the above method in order that they could be used again for bacteriological purposes other than the examination of smears for tubercle bacilli. Of the 223 slides, 17 had been reported as 3-plus positive, 104 as 2-plus positive and 102 as 1-plus positive. These 223 test slides were mixed in a random order with 90 known positive slides (6 3-plus positive, 48 2-plus positive, and 36 1-plus positive) and stained and examined by fluorescence microscopy in batches of 25. The technician who examined the smears was neither aware of the purpose of the experiment nor able to distinguish between the 2 types of slides being examined.

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† Made by the Reddish Chemical Co. Ltd., Globe Works, Reddish, Stockport, England.

None of the 223 test slides was found to be positive for acid-alcohol-fast bacilli. On the other hand, all the 90 known positive slides were reported as positive.

It may be concluded that slides, cleaned in the manner described, may be used at least twice for the microscopic examination of sputum for tubercle bacilli.

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