Simple Procedure for Detection of *Mycobacterium gordonae* in Water Causing False-Positive Acid-Fast Smears

DELIA DIZON, CORNELIA MIHAILESCU, AND H. C. BAE*

Bacteriology Laboratory, Department of Laboratories, The Long Island College Hospital, Brooklyn, New York 11201

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A simple procedure for detecting a few cells of *Mycobacterium gordonae* in laboratory water that yielded spurious smear results is described.

In November 1973, a sudden increase in the number of positive acid-fast smears was noted in the Bacteriology Laboratory of the Long Island College Hospital. A review of the laboratory records revealed that the majority of the concentrated smears (digestion and decontamination in sterile 3% NaOH followed by washing in 0.067 M phosphate buffer, pH 6.8) were positive, whereas nearly all of the cultures and direct smears were negative. Initial examinations of the Kinyoun acid-fast smears (2) of the centrifuged laboratory tap and deionized waters, 3% NaOH, phosphate buffer, and washings from the centrifuge tubes (50-ml size, Falcon) and specimen containers, as well as stains, did not reveal any clue as to the source of contamination. Since the laboratory deionized water was a common denominator in 3% NaOH and phosphate buffer, it was strongly suspected of contributing the acid-fast contaminant. Without adequate facilities, such as membrane filters (Millipore Corp.) or a high-speed centrifuge, to effectively concentrate the cells of the contaminant, it was difficult to demonstrate the presence of acid-fast bacilli in the deionized water. When 50-ml portions of laboratory tap and deionized waters were centrifuged at $1,830 \times g$ for 20 min in an International Equipment Company model UV centrifuge (head type 279), no visible pellet was obtained. It appeared that these water-borne, acid-fast organisms were not sedimented during centrifugation. It seemed necessary to suspend a medium in the water, which could facilitate settling of the cells during centrifugation and help form a compact sediment at the bottom of the tube that could be retained while the supernatant was being decanted.

When cells of a *Candida* species were suspended in the laboratory water samples at a concentration of approximately 10^6 /ml, cell pellets resulted after centrifugation as described above. Examination of the concentrated smears of the cell pellets revealed rare acid-fast bacilli. Culture of the cell pellets yielded two to six smooth, yellow colonies on Lowenstein-Jensen medium after 2 weeks of incubation at 37 C.

The isolate was identified as *Mycobacterium gordonae* and was confirmed by the Mycobacteriology Laboratory of the New York City Department of Health. After digestion and decontamination, all *Candida* cells lost their structural integrity and neither interfered with reading of the smears nor yielded yeast colonies or other contaminants on culture media. (Other particulate materials may replace the *Candida* cells for this purpose.)

It was concluded that the laboratory tap water was contaminated with acid-fast bacilli that, in turn, colonized the deionizer filter, and the resultant contaminated, deionized water was responsible for the false-positive smears of the apparently negative specimens. The use of sterile water (Travenol) for injection instead of the deionized water in preparing 3% NaOH and phosphate buffer resolved the problem of contamination.

Acid-fast smear is still a major screening test for tuberculosis, although its diagnostic effectiveness decreases in a population with a low prevalence of the disease (1, 3, 4). Therefore, stringent quality control is of great importance for the reliability of acid-fast smear.

The procedure described above may be employed as an additional method of detecting a few microbial cells in water and/or other fluid specimens during an episode of contamination.

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LITERATURE CITED

1. Boyd, J. C., and J. J. Marr. 1975. Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. *Ann. Intern. Med.* 82:489-492.
2. Lennette, E. H., E. H. Spaulding, and J. P. Truant (ed). 1974. *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Marraro, R. V., E. M. Rodgers, and T. H. Roberts. 1975. The acid-fast smear: fact or fiction? *J. Am. Med. Technol.* 37:277-279.
4. Weinstein, R. A., W. E. Stamm, and R. L. Anderson. 1975. Early detection of false-positive acid-fast smears. An epidemiological approach. *Lancet* 2:173-174.