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

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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 x 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 in water and/or other fluid specimens during an episode of contamination.

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LITERATURE CITED


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