Use of a Disposable Water Filter for Prevention of False-Positive Results due to Nontuberculosis Mycobacteria in a Clinical Laboratory Performing Routine Acid-Fast Staining for Tuberculosis[▽]

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A point-of-use 0.2-μm filter was evaluated for elimination of nontuberculosis mycobacteria in laboratory water to reduce false-positive acid-fast bacillus staining results. Use of the point-of-use filter can significantly reduce the false-positive rate to 1.2% compared to samples treated with tap water (10.7%) and deionized water (8.7%).

Microscopic examination of respiratory specimens for acid-fast bacilli (AFB) is the standard procedure for the initial diagnosis of tuberculosis (TB), monitoring of treatment, and determination for patient release from isolation (1). Although several nucleic acid amplification tests are available for rapid detection of TB, acid-fast staining still offers the advantages of ease, rapidity, and low cost for preliminary TB diagnosis. The current Taiwan Center for Disease Control guideline for TB suggests that symptomatic patients should immediately receive anti-TB therapy for 14 days in an isolation ward if one or more specimens are acid-fast staining positive.

However, pseudo-outbreaks of TB have occurred in Taiwan when a high number of positive results were found by acid-fast staining of patients' sputum specimens. These pseudo-outbreaks caused management problems and unwanted public attention for the hospitals (4, 12, 14). Many patients may undergo intensive antimicrobial therapy and isolation. One reason for pseudo-outbreaks of TB may be the presence of non-tuberculosis mycobacteria (NTM) in the laboratory tap water that is used to process the specimens and subsequently causes false-positive acid-fast stain results. Removal of NTM from laboratory processing water may minimize false-positive results for TB diagnosis. Thus, the objective of our study is to evaluate a point-of-use 0.2-μm water filter for elimination of NTM in laboratory water and reduction of false-positive results for the acid-fast staining procedure.

During the study period of 21 December 2005 to 17 March 2006, the first 10 sputum specimens for TB smear/culture analysis were enrolled. The specimens were decontaminated and concentrated for smear and culture by following standardized procedures (5, 16). The sputum specimens were inoculated onto three glass slides (A, B, and C) for acid-fast staining. The concentrated sputum specimens also were inoculated into BACTEC MGIT 960 liquid medium and Lowenstein-Jensen solid medium (Becton Dickinson, Franklin Lakes, NJ) for culture (7, 13).

A disposable, point-of-use 0.2-μm water filter (Pall-Aquasafe filter; Pall Corporation, East Hills, NY) was installed at one faucet in the microbiology laboratory (Fig. 1). A bypass valve and a faucet were installed to withdraw tap water without water flowing through the filter for regular use. Three liters of tap water was cultured for *Mycobacterium* species by filtration every workday for 6 weeks from 6 February to 17 March 2006. The modified Kinyoun acid-fast stain was used throughout the study (8). The slides were then rinsed with water according to the following protocol: water treated with a filter ("filter water") for slide A, tap water for slide B, and deionized water for slide C. The slides were read under a microscope at a magnification of 1,000×.

Mycobacterial species were identified with nucleic acid probes by the BD ProTecET system (Becton Dickinson, Franklin Lakes, NJ) (6, 11) and conventional biochemistry tests (8). The specimen was defined as "culture positive" if either MGIT or Lowenstein-Jensen solid medium yielded a positive result. Culture positivity was used as the endpoint for evaluation of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The analysis of variance test was used to determine the statistical difference between each processed water type.

Five hundred sixty-two sputum specimens from 387 patients were included in this study. A total of 79 sputum specimens were culture positive for mycobacterial species. Tap water samples from the microbiology laboratory tap for acid-fast staining were found to be contaminated by *Mycobacterium gordonae* (100%, 30/30), followed by *Mycobacterium avium* complex (93%, 28/30), *Mycobacterium fortuitum* (23%, 7/30), *Mycobacterium abscessus* (13%, 4/30), and *Mycobacterium chelonae* (10%, 3/30). The modified Kinyoun acid-fast stain-positive rates for specimens processed with filter water, tap water, and deionized water were 7.5% (42/562), 18.0% (101/562), and 16.2% (91/562), respectively (Table 1). By using the mycobacterial culture results as a gold standard, the false-positive rates of specimens treated with filtered water, tap water, and deionized water were 1.2% (7/562), 10.7% (60/562), and 8.7% (49/562), respectively. It appears that the false-positive rate of

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FIG. 1. A point-of-use water filter attached to the water outlet for acid-fast staining procedures.

TABLE 1. Performance of modified Kinyoun acid-fast staining processed with filter water, tap water, and deionized water

Processing water and culture status	No. of smears		%			
	Positive	Negative	Sensitivity	Specificity	PPV[a]	NPV[b]
Filter[c]			44.3	98.6	83.3	91.5
Positive	35	44				
Negative	7	476				
Tap			51.9	87.6	40.6	91.8
Positive	41	38				
Negative	60	423				
Deionized			53.2	89.9	46.2	92.1
Positive	42	37				
Negative	49	434				

[a] Proportion of specimens with positive culture results which are correctly detected by smear.
[b] Proportion of specimens with negative culture results which are correctly detected by smear.
[c] P values for sensitivity, specificity, PPV, and NPV in comparison with tap water are 0.17, <0.001, <0.001, and 0.45, respectively, and in comparison with deionized water are 0.13, <0.001, <0.001, and 0.36, respectively.

samples treated with filtered water is significantly lower than that of samples treated with tap water and deionized water (for both, $P < 0.001$), while no significant difference was found between samples treated with tap water and those treated with deionized water (Table 1). Furthermore, slides rinsed with water treated with a filter had significantly higher specificity and PPV (98.6% and 88.3%, respectively) than did those treated with tap water (87.6% and 40.6%, respectively) and deionized water (89.9% and 46.2%, respectively) (for both, $P < 0.001$) (Table 1).

NTM are commonly found in the human-made environment including drinking water and aerosol generating systems (2, 3, 4, 9, 10, 15). The resistance of NTM to chlorine disinfection contributes to the ability of NTM to persist in potable water (1, 10). Although the presence of NTM had not caused any outbreaks or epidemic mycobacterial infections in the study hospital, the presence of NTM in tap water contaminates the specimens and compromises the ability of the clinical microbiology laboratory to detect potential TB patients.

The false-positive reading of AFB in clinical specimens can lead to overdiagnosis of TB and subsequently to initiation of unnecessary anti-TB therapy. The current Taiwan Center for Disease Control guideline recommends 14 days of anti-TB therapy in an isolation room if a patient's smear is positive for AFB. It would cost the healthcare provider approximately U.S. $2,250 (New Taiwan $72,000) for a 14-day stay in an isolation room and antimicrobial therapy for each patient excluding treatment of adverse effects, further outpatient visits, personnel, and other indirect costs. Processing the modified Kinyoun acid-fast stain sample using filtered water provided superior performance and significantly higher specificity and PPV (Table 1) compared with tap water or deionized water in detection of AFB in clinical specimens.

The application of a disposable point-of-use water filter adds an additional cost to the laboratory operation. However, this cost can be easily justified by the reduction of a large number of false-positive acid-fast stain results at U.S. $2,250 per patient in Taiwan. The retrograde contamination of the filter outlet by back-splashing water and contaminated aerosols may shorten the life of the filter. Our practice is to use an additional sterile plastic bag to cover the filter when it is not in use. The

manufacturers also provide new-generation filters in which the filter housing contains bacteriostatic additives to overcome such retrograde contamination.

In conclusion, the use of the disposable point-of-use water filter can effectively remove NTM from laboratory processing water, which can help to minimize false-positive results for TB diagnosis. The cost of the water filter can be justified by the immediate financial advantage of the hospitals and healthcare providers saving money and medical resources by avoiding unnecessary antimicrobial therapy and use of isolation rooms.

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