JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1995, p. 2118–2123 Vol. 33, No. 8
0095-1137/95/$04.00+0

Comparison of Culture Methods for Monitoring *Legionella* Species in Hospital Potable Water Systems and Recommendations for Standardization of Such Methods

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Received 9 December 1994/Returned for modification 27 January 1995/Accepted 18 May 1995

A lack of standardization of environmental monitoring techniques for *Legionella* spp. complicates the interpretation of results and comparisons of results from different institutions. A comparative assessment of techniques recommended by the Centers for Disease Control and Prevention, the Hygiene Institute (Graz, Austria), and our laboratory was performed. Variables investigated were sampling method (swabbing and collection of water samples [250 ml] before and after swabbing), method of concentration (none, filtration, and centrifugation), acid buffer treatment (no acid treatment, treatment for 3 min, and treatment for 15 min), and choice of medium (five formulations of buffered charcoal yeast extract agar with glycine, vancomycin, polymyxin B, anisomycin, or cycloheximide). Thirty-three sites in seven hospital buildings were studied. Recovery by swab correlated with recovery from water after swabbing ($P < 0.05$). However, the quantity of *Legionella* spp. recovered from swab specimens (mean, 3.0×10^4 CFU per swab) was greater than that recovered from water (mean, 4.7×10^3 CFU/250 ml). Filtration resulted in recovery rates (mean, 5.2×10^3 CFU/250 ml) higher than those by centrifugation (mean, 2.3×10^3 CFU/250 ml). Three minutes of acid buffer treatment to reduce overgrowth by commensal flora did not improve selectivity or sensitivity for *Legionella* spp. if glycine-containing selective media were used. Fifteen minutes of acid buffer treatment reduced recovery compared with that after a 3-min treatment. All glycine-containing media tested effectively inhibited background flora, but one selective medium containing dyes, glycine, vancomycin, and polymyxin B (DGVP) resulted in the greatest quantitative recovery of *Legionella pneumophila*. Use of buffered charcoal yeast extract agar and the acid buffer treatment gave the greatest recovery of non-*pneumophila* species. A standardized protocol with an emphasis on the culturing of swab samples is presented.

Potable water is a source of nosocomial and community-acquired Legionnaires' disease (3, 14). Environmental culturing of water systems for *Legionella* spp. has been recommended for hospitals, especially those encountering cases of Legionnaires' disease (1, 6, 17). However, interpretation of the results of environmental cultures is difficult because of the current lack of standardized sampling and culturing methods.

We have performed a comparative assessment of three monitoring methods for *Legionella* spp. recommended by the Veterans Affairs Medical Center (VAMC; Pittsburgh, Pa.), Centers for Disease Control and Prevention (CDC; Atlanta, Ga.), and Hygiene Institute (Graz, Austria) to evaluate their feasibility and sensitivity in identifying the presence of *Legionella* strains. Variables that were evaluated included the collection of water versus swab samples, the concentration method, the length of acid treatment, and the choice of selective medium (Fig. 1). These variables were also assessed independently to determine the advantages and disadvantages of each technique. Our ultimate objective was to recommend an optimal sampling method for the monitoring of hospital potable water systems.

MATERIALS AND METHODS

A total of 33 selected water faucets from seven hospital buildings in Pittsburgh were sampled for *Legionella* spp.

The sampling protocol included the recommended methods from the CDC, the Hygiene Institute, and the Pittsburgh VAMC (Table 1). The CDC protocol for environmental sampling recommends that water and swab samples be collected from sample sites and that swab samples should be taken before water samples at these sites (7). Samples are decontaminated by a 15-min exposure to acid buffer and plated onto buffered charcoal yeast extract (BCYE), PAV (7), and GPAV (7) agar media. The Hygiene Institute method recommends that water alone be collected from sample sites and that the water be collected immediately upon opening the valve (zero flow time) (12). Samples are also decontaminated for 15 min and then plated onto BCYE and GVPC (12). Both the CDC and Hygiene Institute recommend that the water undergo concentration by filtration prior to culturing. The VAMC recommends that a swab sample alone be collected from sample sites (15). If a water sample is collected, centrifugation is the method recommended for concentration. Decontamination by treatment with acid buffer is for 3 min, and then the sample is plated onto BCYE and DGVP (15).

Specimens were collected as follows. Three samples were collected from each site: one water sample (250 ml), a swab sample, and then another water sample (250 ml) after swabbing. These water samples are referred to as before-swab and after-swab water. Initial water samples were taken from the hot tap outlet immediately after the valve was opened. The swab sample was then taken by inserting a sterile cotton or Dacron swab into the opening of the faucet and rotating it four times while moving the swab upward into the opening (15). After the swab was lightly streaked directly onto selective and nonselective media, it was immersed in 2.5 ml of acid buffer (0.2 M HCl-KCl [pH 2.2]) and shaken vigorously (15). After 3 and 15 min, 0.1 ml of the sample was spread with a sterile glass rod onto duplicate plates of the different media to be tested. Acid buffer treatment was used as a selective method to reduce the numbers of non-*Legionella* bacteria.

Each water sample was similarly plated. A 0.1-ml sample was plated without concentration and without acid buffer treatment. Another volume of the sample (100 ml) was concentrated by filtration through a 0.2-μm-pore-size polycarbonate membrane filter (Nuclepore Corp., Pleasanton, Calif.) by resuspending the filter in 10 ml of the original sample and vortexing for 30 s (7, 12). Another 100 ml of the sample was concentrated by centrifugation at $1,000 \times g$ for 10 min, removal of all but 10 ml of the supernatant, and vortexing (15). A 0.1-ml volume of the concentrated sample was plated directly, while 9.0-ml volumes were each treated with equivalent volumes of acid buffer for 3 and 15 min.

The nonselective medium was BCYE (15). The selective media are described in Table 1 and were DGVP (15), PAV and GPAV (PAV plus glycine) (7), and GVPC (12) (Table 1). All media were prepared and controlled for quality in our laboratory.

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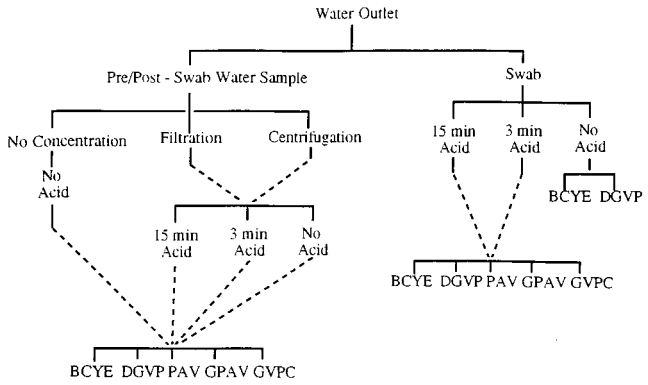

FIG. 1. Flow diagram of sample collection and processing.

The inoculated culture media were incubated at 37°C in a humidified atmosphere with no CO_2 and read at 5 to 6 days. Suspected *Legionella* colonies were subcultured onto blood agar and BCYE for verification. The species and/or serogroups were determined by the slide agglutination test and direct fluorescent-antibody staining. Identification of non-*Legionella pneumophila* species was performed courtesy of Robert F. Benson at the Centers for Disease Control, Respiratory Branch, Atlanta, Ga.

Statistical analysis was done with the Prophet system (BBN System & Technology Corp., Cambridge, Mass.). The Wilcoxon signed-rank test was used to compare two groups (i.e., the filtration and centrifugation groups and the 3-min and 15-min acid treatment groups) paired by site. The Friedman test, with all possible pairwise comparisons, was used to evaluate more than two groups (i.e., types of media and recommended monitoring methods). Percent positivity was evaluated by the chi-square test. The correlation between swab and water samples was evaluated by a linear regression model. The data were transformed prior to regression analysis to account for dilution and concentration factors.

RESULTS

Legionella spp. were isolated from all 33 sites sampled. The same species and/or serogroups detected with the swab were also found in the water samples. *Legionella bozemanii*, *Legionella rubrilucens*, *Legionella anisa*, *Legionella erythra*, and *L. pneumophila* serogroups 1, 3, and 6 were isolated.

Sampling methods. The results for specimens collected, processed, and cultured as specified by the CDC, Hygiene Institute, and VAMC are presented in Table 2. The means were calculated only for each specific method and one medium formulation. The VAMC swab sampling method recovered significantly more *Legionella* CFU per plate than any of the water sampling techniques for 22 of 33 sites, resulting in the greatest overall mean CFU-per-plate value ($P < 0.001$, Friedman's test) (Table 2). There was no significant difference in the sensitivities (percentage of positive sites) observed for the different methods ($P > 0.3$, chi-square test) (Table 2). Among the three recommended water sampling methods, concentrating by filtration as recommended by the CDC and Hygiene Institute gave a significantly higher yield of *Legionella* CFU than those of the other methods ($P < 0.01$, Wilcoxon signed-rank test) (Table 2).

An evaluation of the sample methods that included but was not limited to the specific recommended protocols also showed that the swab method recovered more *Legionella* CFU from the same site than did the method of collecting 250 ml of water ($P < 0.001$, Wilcoxon signed-rank test) (Table 3). The mean total of *Legionella* CFU recovered from the swab was 30.2×10^3 CFU, that from the before-swab water sample that was centrifuged was 2.0×10^3 CFU, and that from the filtered 250-ml sample of before-swab water was 5.0×10^3 CFU (Table 3). Means were calculated from 330 plates inoculated with samples from 33 sites. There was no significant difference in the quantitations of *Legionella* CFU in water samples taken before and after swabbing (Table 3). However, the yield from the swab correlated directly with the yield from the water after swabbing ($P < 0.05$, linear regression analysis). For example, in sites where recovery of *Legionella* organisms from the swab was great, recovery from water after swabbing was also great. For a given site, there was no quantitative correlation between the number of *Legionella* organisms recovered from water before swabbing and the number recovered from the swab.

Method of water concentration. Concentration greatly improved the detection of *Legionella* spp. in water samples (Table 3 and Fig. 2). Filtration was significantly more effective than centrifugation, with filtration recovering on average more than twice the number of *Legionella* organisms (CFU per plate) than centrifugation ($P < 0.001$, Wilcoxon signed-rank test). Although the chance of recovering *Legionella* spp., measured in terms of CFU per 0.1 ml, on a culture plate increased with the concentration of the sample, calculations to estimate the total CFU in the original sample indicate that some *Legionella* organisms are lost in the process. An estimate of the total CFU per 250 ml of water collected before or after swabbing (Table 3) showed that filtration recovered 76 to 77% of the expected yield (compared with a sample without concentration) while centrifugation recovered only 31 to 36% (Table 3).

Acid treatment and media used. All the selective media except PAV yielded essentially pure *Legionella* cultures (Table 4). Contaminating non-*Legionella* bacteria were frequently recovered on the PAV medium. An examination of the numbers

TABLE 1. Three currently recommended methods for monitoring *L. pneumophila* in potable water

Institution and sampling method	Concn method	Time of acid treatment (min)	Selective medium	Concn of additive to selective medium[a]					
				Gly	Vanco	PB	Aniso	Cyclo	Dyes
VAMC									
Swab	NA[b]	3	DGVP	3.0	1.0	50	0	0	10 (each)
Water	Centrifugation	3	DGVP	3.0	1.0	50	0	0	10 (each)
CDC									
Water	Filtration	15	PAV	0	5.0	100	80	0	0
			GPAV	3.0	5.0	100	80	0	0
Swab	NA	15	PAV	0	5.0	100	80	0	0
			GPAV	3.0	5.0	100	80	0	0
Hygiene Institute									
Water	Filtration	15	GVPC	3.0	1.0	80	0	80	0

[a] Gly, glycine (mg/ml); Vanco, vancomycin (μg/ml); PB, polymyxin B (U/ml); Aniso, anisomycin (μg/ml); Cyclo, cycloheximide (μg/ml); Dyes, bromocresol purple and bromothymol blue (μg/ml).
[b] NA, not applicable.

TABLE 2. VAMC swab method gave significantly greater yields of *L. pneumophila* than those of other methods

Method and institution	CFU/plate		% Positive	
	Mean[a]	SEM	Plates (n = 66)	Sites (n = 33)
Swab				
VAMC (swab, 3-min acid, DGVP)	137.6	24.9	73	76
Water[b]				
VAMC (after-swab, centrifuge, 3-min acid, DGVP)	5.6	2.1	71	85
CDC (after-swab, filter, 15-min acid, PAV)	12.4	4.3	73	82
CDC (after-swab, filter, 15-min acid, GPAV)	10.9	4.2	70	79
Hygiene Institute (before-swab, filter, 15-min acid, GVPC)	10.5	4.3	59	76

[a] Means were calculated from a total of 66 plates (duplicate plates of one medium formulation) inoculated with samples from 33 sites.
[b] The CDC and Hygiene Institute methods of water processing yielded significantly greater amounts of *Legionella* spp. than those of the VAMC method ($P < 0.01$, Wilcoxon signed-rank test).

of *Legionella* bacteria recovered without an acid buffer treatment and with a 3-min acid buffer treatment of the samples showed that the highest mean number (in CFU per milliliter) of *L. pneumophila* was recovered on DGVP, followed by GVPC, GPAV, and PAV ($P < 0.05$, Friedman's test) (Table 4). No significant differences in the recovery of *L. pneumophila* after a 15-min acid buffer treatment of the samples were observed for any of the media ($P > 0.05$, Friedman's test). In fact, extending the period of the acid buffer treatment actually reduced the yield of *Legionella* organisms, although this reduction did not reach statistical significance (Table 4). The nonselective medium (BCYE) also yielded *Legionella* spp. after an acid buffer treatment of the samples, but the recovery was sometimes difficult to interpret because of contaminating background flora (Table 4). BCYE and an acid buffer treatment were necessary for the detection of non-*pneumophila* species which grew poorly on all selective media (Table 4 and Fig. 3). No significant improvement in the recovery of *L. pneumophila* or *Legionella* spp. was observed on any medium if the acid buffer treatment was prolonged from 3 to 15 min ($P > 0.05$, Wilcoxon signed-rank test).

DISCUSSION

The degree of *Legionella* colonization of the water supply in a hospital has been shown to correlate with the incidence of nosocomial Legionnaires' disease (3, 11). Therefore, in selecting the appropriate culture method, maximal sensitivity for the detection of *Legionella* spp. is desirable, given the public health implications of the results. Furthermore, guidelines for remedial action based on quantitative *Legionella* data from environmental culturing have been proposed (13). Before any guidelines can be applied, a standardized protocol for the monitoring of *Legionella* colonization should be implemented, since different culture methods will yield different results. This issue is particularly important if environmental culturing is used to determine the necessity for specialized laboratory tests for *Legionella* infection in patients with pneumonia or to provide the basis for a decision for water system disinfection (1, 13).

Several different approaches to performing environmental monitoring for *Legionella* spp. have been recommended (7, 12,

TABLE 3. Recovery of *Legionella* spp. from swabs was greater than that from 250-ml water samples from the same site

Sample	CFU/plate		Estimated total CFU (10^3) in sample[a]
	Mean[b]	SEM	
Swab[c]	140.6	11.2	30.2
Water before swabbing			
No concn	2.6	0.5	6.5
Centrifugation	7.9	1.5	2.0
Filtration	22.0	4.1	5.0
Water after swabbing			
No concn	2.8	0.5	7.0
Centrifugation	9.9	1.6	2.5
Filtration	23.5	4.3	5.3

[a] Estimates of the total CFU in the original samples (per swab and per 250 ml of water) were calculated by converting CFU per 0.1 ml to CFU per milliliter and multiplying these figures by the dilution factor (swab) or concentration factor (water) and those figures by the total volume of the original sample.
[b] Means were calculated from 330 plates (five different medium formulations in duplicate) inoculated with samples from 33 sites in 7 buildings.
[c] Swab samples were suspended in 2.5 ml of acid buffer for 3 min; water samples received no acid buffer treatment.

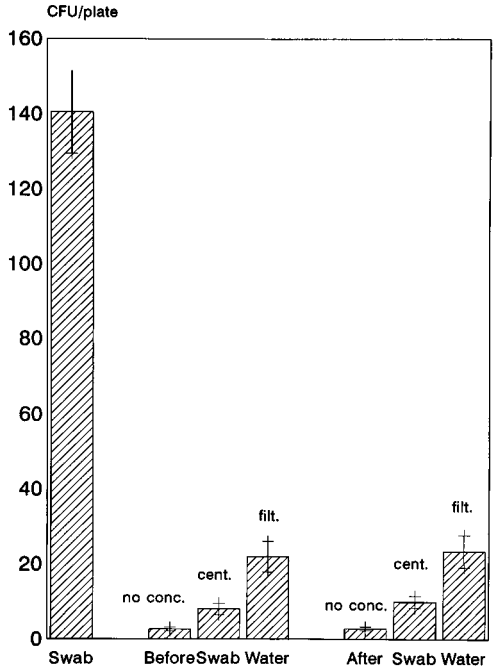

FIG. 2. Recovery of *L. pneumophila* from swab and water samples. The swab samples yielded significantly more CFU per plate than any water sample ($P < 0.001$, Wilcoxon signed-rank test). The quantitative recovery of *L. pneumophila* from water was greatest following filtration ($P < 0.001$, Wilcoxon signed-rank test). Values given are means calculated from 330 plates inoculated with samples from 33 sites. Error bars indicate standard deviations. no conc., no concentration; cent., centrifugation; filt., filtration.

TABLE 4. Effects of acid buffer treatment and culture medium on recovery of *Legionella* spp.

Organism(s)	Time of acid treatment (min)	Recovery (mean CFU/ml)[a]				
		Nonselective medium (BCYE)	Selective medium			
			No glycine (PAV)	With glycine		
				DGVP	GPAV	GVPC
L. pneumophila	0	12.1	13.9	17.2	15.2	16.4
	3	16.4	14.8	16.6	14.8	16.0
	15	15.8	13.8	15.8	14.2	15.8
Legionella species	0	1.5	4.1	0.7	0.6	1.3
	3	10.0	4.5	0.5	0.7	2.2
	15	9.7	4.3	0.6	0.7	1.7
Other microflora[b]	0	Heavy	Moderate	Rare	0	0
	3	Light	Light	0	0	0
	15	Light	Rare	0	0	0

[a] Means were calculated from a subgroup of 148 plates for *L. pneumophila* and non-*Legionella* organisms and from 84 plates for *Legionella* species. Standard errors of the mean: *L. pneumophila* and *Legionella* species (BCYE, 3- and 15-min acid), 3.0 CFU/ml; *Legionella* species (BCYE, no acid), 0.8 CFU/ml; *Legionella* species (PAV), 11.5 CFU/ml; *Legionella* species (DGVP, GPAV, GVPC), 0.3 CFU/ml. Heavy, >100 CFU per plate; moderate, 50 to 100 CFU per plate; light, 5 to 50 CFU per plate; rare, 1 to 5 CFU per plate; 0, <1 CFU per plate.

[b] Other microflora, non-*Legionella* organisms.

15). The objective of this study was to compare three of these recommended methods in an attempt to assess the advantages and disadvantages of each method. Ultimately, a standardized method for the recovery of *Legionella* spp. from hospital water distribution systems would be advantageous.

Our data demonstrate that the method of sample collection is critical to determining the level of *Legionella* colonization in a hospital water system. The swab method of sample collection and processing used at the Pittsburgh VAMC detected the same number of *Legionella*-positive sites but revealed higher concentrations of *Legionella* organisms than two other recommended techniques that sampled water (Table 2). The swab method also recovered more than 300 CFU per plate from 14 of 33 sites (data not shown). In such cases, we have found that dilutions of 1:10 and 1:100 were required to quantitatively assess the level of contamination. The increased yield of *Legionella* CFU from the swab was likely due to its direct sampling of the biofilm to which *Legionella* organisms can adhere. Also of note is that an increase (from a mean of 15 CFU per plate to a mean of 300+ CFU per plate) in the recovery of *Legionella* organisms with the swab was observed after faucet aerators were removed at two sample sites (data not shown).

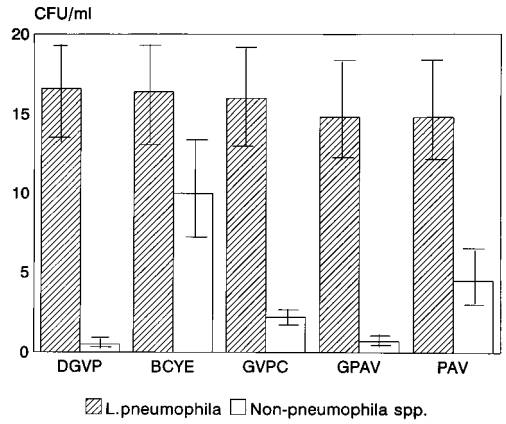

FIG. 3. Comparison of culture yields by medium for samples subjected to 3 min of acid buffer treatment. DGVP yielded the greatest mean amount of *L. pneumophila*, but BCYE was able to recover more than twice the amount of non-*pneumophila* species than any selective medium ($P < 0.001$, Wilcoxon signed-rank test). Error bars indicate standard deviations.

Biofilm research has shown that in virtually every habitat, bacteria grow preferentially on surfaces and not in the bulk aqueous phase (8). Since our data agree with those from other investigations that have shown that the numbers of *Legionella* organisms sampled from the biofilm were greater than those of *Legionella* organisms sampled from water (16), it appears that *Legionella* spp. are members of the biofilm consortium.

The concentration of water samples by filtration yielded greater numbers of *Legionella* organisms and produced more positive cultures than those produced by centrifugation (Table 3). There was, however, some loss of *Legionella* numbers if the concentration methods were used. The yield following concentration by filtration was 77% of the yield from the original water samples (Table 3). A similar reduction in yield (76 to 78% recovery) was observed in another study by Barbaree et al. (2). The recovery of *Legionella* organisms by centrifugation can be improved by increasing the spin speed to achieve a relative centrifugal force of $6{,}100 \times g$ (4).

Unlike many other bacteria, *Legionella* spp. are relatively resistant to acidic pH. Therefore, acid buffer treatment can be used to select for *Legionella* spp. However, in this study, acid buffer treatment did not significantly improve the recovery of *L. pneumophila* from the samples tested. This is likely due to the fact that any one of the selective media containing glycine was able to inhibit the growth of competing microorganisms from potable water samples. In fact, increasing the time of the acid exposure from 3 to 15 min actually reduced the recovery of *Legionella* spp. (Table 4). It is important to note, however, that on occasion, we (13a) and others (10, 12) have encountered samples from water systems which required acid treatment to inhibit competing microorganisms. Therefore, samples should not be discarded so that a culture can be repeated if overgrowth of competing organisms occurs.

Acid treatment was required, however, for a maximal yield of the non-*pneumophila* species (Fig. 3). These species were generally recovered on BCYE or PAV only. BCYE and PAV media do not contain glycine (Table 4), which suggests that glycine inhibits the growth of non-*pneumophila* species. Calderon and Dufour have also reported the inhibition of *Legionella* species (especially *L. gormanii*) by media containing glycine (5).

Several different medium formulations are used for the recovery of *Legionella* spp. from environmental samples. We found that the quantitative recovery of *L. pneumophila* was greatest from DGVP. DGVP medium was also as effective as the other selective media in inhibiting background flora (Table 4). DGVP has the advantage of being the most cost-effective to use since it contains fewer antibiotics at lower concentrations than the other media tested. The use of selective media with high concentrations of antibiotics (e.g., GPAV) and extended acid buffer treatment did not result in the increased recovery of *Legionella* spp. from the potable water samples in this study. These methods may be more appropriate for cooling tower water, which is more heavily contaminated with competing flora. Culturing on BCYE after acid treatment (3 or 15 min) was similar to that on DGVP with respect to *Legionella* quantitation. This result is in agreement with those of previous reports (10). However, culturing on BCYE after acid treatment was not as effective as that on DGVP in inhibiting competing microflora (Table 4).

Since the level of competing microorganisms may vary by geographic location, different selective media may be optimal in different regions. For example, Edelstein (9) reported that MWY (selective supplements, DGVP plus anisomycin [80 μg/ml]) (9) performed better than BMPA-alpha (selective supplements, polymyxin B [80 μg/ml], anisomycin [80 μg/ml], (9), cefamandole [4 μg/ml]) (9), while Reinthaler et al. (12) reported no difference between MWY and BMPA-alpha and preferred GVPC.

For the comparison of degrees of colonization at different institutions, it would be ideal if the units were standardized. The results for sampling and processing methods in this study were given in CFU per milliliter, CFU per plate, CFU per swab, and CFU per 250 ml in order to demonstrate each technique's ability to recover *Legionella* spp. (Table 3). The swab culture should ideally be reported as CFU per swab rather than CFU per plate to account for possible differences in dilution. This can be calculated from the original volume of acid or water in which the swab was suspended. In addition, if water samples are concentrated, the units should be adjusted to reflect the CFU-per-milliliter value of the original sample.

The methods that we have evaluated for the monitoring of *Legionella* spp. in potable water were equal in sensitivity, i.e., in their abilities to detect *Legionella* spp. if *Legionella* spp. were present. However, these methods differed significantly in the quantities of *Legionella* spp. detected. This is extremely important, given that outbreaks of Legionnaires' disease have been linked to exposure to amplified levels of *Legionella* organisms (3, 13) and that criteria for remedial action and disinfection have been suggested on the basis of the levels of *Legionella* spp. recovered from water samples (13). Shelton et al. (13) have recommended that remedial action be taken if *Legionella* spp. are isolated from potable water samples in quantities of >10 CFU/ml. On the basis of our experience with the swab method, quantities of >1,000 CFU per swab would warrant remedial action or disinfection (13a). If we used these criteria as a basis for the evaluation of the results from the sites we sampled, water samples collected before the swabbing of the site would have identified only 21% (7 of 33) of the sites in need of intervention while swab samples would have identified 42% (14 of 33) of the sites. In addition, for 3 of 14 samples with greater than 2,000 CFU per swab, the before-swab water was completely negative for *Legionella* spp., even after filtration. These results suggest that if hospitals rely solely on water samples for the monitoring of distal outlets for *Legionella* contamination, false-negative results with an accompanying false sense of security may occur.

A correlation of the degree of *Legionella* colonization with the subsequent risk of hospital-acquired Legionnaires' disease necessitates comparisons of data from different hospitals. This is difficult today because of the variation in environmental sampling methods. Standardizing the culturing protocol would be an important step in forming a scientific foundation for decision-making with regard to the prevention of Legionnaires' disease. Allegheny County of Pennsylvania now mandates routine environmental cultures for *Legionella* spp. in all hospitals in Allegheny County as a preventive measure against hospital-acquired legionellosis (1). The Environmental Protection Agency and Occupational Safety and Health Administration have traditionally used minimal levels of toxins and microbes in assessing the safety and quality of the water supply. A standard culturing protocol is necessary before mandated guidelines can be implemented.

On the basis of our findings, we recommend that swab samples be collected as part of any *Legionella* sampling protocol. Sampling for *Legionella* spp. with swabs was equivalent in sensitivity to and resulted in a greater yield than sampling of water, and the swab was easy to use and transport and required less processing time since no concentration step was necessary. This aspect is especially important, because many sites in a hospital building need to be sampled. We suggest the following approach for consideration. Sampling by swabbing the inner walls of a faucet is preferred to the sampling of large volumes of water from distal outlets of water distribution systems. Because faucet aerators may prevent the adequate sampling of the biofilm for *L. pneumophila*, they should be removed before swabbing. If a water sample is taken, filtration is the concentration method of choice. The swab or water sample should be treated with acid for 3 min to maximize the recovery of *Legionella* species and minimize the growth of competing microorganisms. The sample should then be inoculated onto BCYE and DGVP media.

Although we have not addressed a method for the sampling of hospital hot water storage tanks in this study, we have previously recommended that 10 to 50 ml of water be collected immediately after the drain valve is opened, that the water be allowed to flow for 30 s to flush the pipe, and that a second 50-ml sample be collected. The water collected immediately after the opening of the valve often represents stagnant water in the supply pipe, while the second sample reflects the tank contents. Direct plating of 0.1 ml of these samples onto selective media is usually adequate to detect *Legionella* spp. (1).

Because these recommendations were based on data extrapolated from a relatively small sample size in one geographic area, our findings should be confirmed by others before a standardized method is implemented.

ACKNOWLEDGMENTS

We thank Franz F. Reinthaler, Paul Edelstein, Barry Fields, and Bryan Shelton for reviewing the manuscript; Linda Szalla and Shirley Brinker for secretarial assistance; Richard M. Vickers for assistance with glassware; and Saul Weiss for producing the figures.

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