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Comparison of Membrane Filters for Recovery of Legionellae from Water Samples

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The procedures currently used for isolating legionellae from environmental samples recommend filtration through a 0.2-μm-pore-size polycarbonate filter. In this study we evaluated the performance of 23 other filters composed of various materials and having various pore sizes. We prefer the 0.2-μm-pore-size Gelman Supor filter because of its high level of recovery, faster filtration rate, and ease of handling.

Legionellae may be concentrated in water samples by using centrifugation or filtration. In 1987, Brindle et al. (2) compared filtration with centrifugation. The results of these authors were based on the use of a 0.45-μm-pore-size cellulose nitrate membrane filter. Brindle et al. concluded that centrifugation was the preferred method because of ease of use and a slightly higher level of recovery. In a previous study (7), we found that cellulose nitrate membrane filters yielded 12 to 24% (depending on the concentration) of the initial legionella concentration following filtration. Wolford et al. (8) compared five types of membrane filters and found that membrane filters composed of polyvinylidene fluoride (Millipore Durapore HVLP filters) were far superior to the other membrane filters evaluated for recovery of legionellae. In this study we evaluated other types of membrane filters and other pore sizes in an attempt to further improve the percentage of recovery.

Several factors may affect the accuracy of recovery of bacteria from water samples when filtration is used. Many of these factors have been evaluated previously by Brenner and Rankin (1). These investigators evaluated 142 lots of 0.45-μm-pore-size membrane filters from 13 manufacturers for acceptability by using stock coliform cultures and five different media. Only 30% of the membrane filter lots were acceptable. The defects observed included wrinkles, brittleness of the filters, and hydrophobic or nonwetting areas of the filters. Some filters yielded decreased levels of recovery that may have been related to the composition of the filter material or to inhibitory compounds on the filters. In addition, these authors hypothesized that blocked pores, abnormal pore structure, or electrostatic interactions may also inhibit recovery.

In 1979, Zierdt (9) evaluated pore diameters and membrane filter materials in a study of filtration of bacteria. He successfully recovered certain bacteria when he used filters with large pore sizes.

Few studies have been performed to evaluate the factors described above for the recovery of legionellae from water samples. In 1981, Orrison et al. (5) described filtration of cooling tower water through 0.45- or 0.65-μm-pore-size Millipore membrane filters. These authors concluded that the use of 0.65-μm-pore-size membrane filters resulted in an acceptable loss of legionellae while allowing for more rapid filtration. Finally, Payment et al. (6) recovered 55% of their initial concentration of legionellae by using sequential filtration through 3.0- and 1.0-μm-pore-size electronegative fiberglass cartridge filters.

The Centers for Disease Control (4) currently recommends using 0.2-μm-pore-size polycarbonate membrane filters for recovery of legionellae from water. We compared this type of filter and cellulose nitrate membrane filters with eight other commercially available membrane filter materials with pore sizes of 0.2, 0.4, and 0.8 μm. The same lot number for each filter type was used throughout the study. The types of material and the pore sizes of the membrane filters evaluated in our study are shown in Table 1.

Samples (1 liter) of glass-distilled, heat-sterilized water were seeded with a mixture of three strains belonging to *Legionella pneumophilia* serogroup 1 (one environmental strain and two clinical strains) to achieve a final concentration of approximately 10^3 CFU/ml. This was done by diluting a preparation at a 0.5 MacFarland standard, making appropriate dilutions, and plating the dilutions onto BCYEα medium in duplicate to obtain the initial concentration of the suspension. The resulting samples were filtered through membrane filters by using a magnetic, vacuum filter apparatus that is commonly used in water microbiology. The time required for each membrane filter to filter its 1-liter sample of water was determined. Special glass membrane filter holders were required for the Anotec ceramic membrane filter to prevent breakage. Either these same holders or gaskets were required for the polycarbonate membrane filters to prevent leakage.

After filtration, the membrane filters were removed and placed in sterile 150-ml cups (diameter, 55 mm). A 5-ml portion of sterile water was added to each cup. The organisms were eluted by either mixing the preparation with a vortex mixer for 2 min at close to the maximum speed or sonicating the preparation for 10 min in a Bransonic ultrasonic cleaner (Bransonic, Inc.). Afterward, the filtrate was diluted, and 100 μl of each dilution was plated in duplicate onto BCYEα media. The colonies were counted after 4 days of incubation at 37°C in the presence of 3 to 5% CO_2, and the data were compared with the initial concentration. The percentage of recovery was then determined. This process was repeated four to eight times for each membrane filter type and method used.

For the statistical evaluation, the following two assumptions were made: (i) all of the recovery data were normally distributed and independent of one another; and (ii) the values of the recovery variances were equal and unknown.

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TABLE 1. List of membrane filters evaluated in this study

Material	Filter type	Pore size(s) (μm)
Polysulfone	Gelman Supor[a]	0.2, 0.4, 0.8
	Gelman Tuffryn[a]	0.2, 0.45
Polycarbonate	Nuclepore[b]	0.2, 0.4
	Poretics[c]	0.2, 0.4
Mixed cellulose esters	Gelman GN Metricel[a]	0.45, 0.8
	Gelman GN Metricel grid[a]	0.45, 0.8
	Schleicher & Schuell ME[d]	0.2
	Millipore MF[e]	0.2, 0.45
	Millipore MF[e] grid	0.45
Cellulose acetate	Schleicher & Schuell OE[d]	0.2, 0.45
Cellulose nitrate	Micro Filtration Systems grid[f]	0.45
Polyvinylidene difluoride	Millipore Durapore HVLP[e]	0.2, 0.45
Nylon	Schleicher & Schuell[d]	0.2, 0.45
Ceramic	Anotec[g]	0.2

[a] Gelman Corp., Ann Arbor, Mich.
[b] Nuclepore Corp., Pleasanton, Calif.
[c] Poretics Corp., Livermore, Calif.
[d] Schleicher & Schuell, Keene, N.H.
[e] Millipore Corp., Bedford, Mass.
[f] Micro Filtration Systems, Dublin, Calif.
[g] Anotec Separations, Oxon, England.

Statistical differences were calculated by using the Student t-test (3).

Tables 2 and 3 show the rates of filtration for the various membrane filters and pore sizes tested. The filtration rates for the 0.2-μm-pore-size membrane filters ranged from 21 min/liter for the Gelman Supor and the Anotec ceramic filters to 71 min/liter for the Millipore Durapore filter. The rates for the 0.4-μm-pore-size membrane filters ranged from 6 min/liter for the Gelman GN grid filter to 28 min/liter for the nylon filter marketed by Schleicher & Schuell.

Table 2 shows the percentages of recovery for the 0.2-μm-pore-size membrane filters. The Nuclepore polycarbonate membrane filter yielded a mean recovery rate of 66%, followed by the Gelman Supor filter (56%) and the Gelman Tuffryn filter (55%). The Millipore Durapore HVLP filter (53%) and the cellulose filters had lower rates of recovery, and the nylon and ceramic membrane filters yielded the lowest rates of recovery. A statistical analysis was performed in which the rate of recovery for each filter was compared with the rate of recovery for the standard Nuclepore polycarbonate filter currently recommended. There was no statistical difference ($P > 0.20$) when the recovery rates for the Gelman Supor and Gelman Tuffryn filters were compared with the polycarbonate filter recovery rates. The recovery rates with the Nuclepore filter were statistically superior to recovery rates for the other membrane filters tested. These P values are also shown in Table 2.

Table 3 shows the percentages of recovery obtained with the 0.4-μm-pore-size membrane filters. The Nuclepore, Gelman Supor, Millipore cellulose ester, and Gelman GN filters all gave comparable results. As with the 0.2-μm-pore-size membrane filters, the 0.4-μm-pore-size nylon, cellulose acetate, and cellulose nitrate membrane filters did not perform as well. Our analysis of water samples in which the various pore sizes were used showed that while the 0.8-μm-pore-size membrane filters permitted rapid filtration, they yielded less than 12% of the initial concentration of legionellae.

In general, for each filter type, elution by using sonication resulted in better recovery rates than elution by using a vortex mixer. Only three of the membrane filters (the Millipore Durapore, Schleicher & Schuell cellulose acetate, and nylon filters) did better when elution was performed by mixing with a vortex mixer.

Various problems arose with some of the membrane filters. The polycarbonate membrane filters were very thin, and they not only required special equipment for filtration to prevent leaking, but tended to fold and wrinkle. There was also a static charge present that made them difficult to

TABLE 3. Percentages of legionellae recovered by 0.4-μm-pore-size membrane filters and flow rates

Membrane filter	Flow rate (min)	Elution with sonicator: mean % of recovery	Elution with vortex mixer: mean % of recovery
Nuclepore polycarbonate	11	49	35
Millipore cellulose ester	15	43	20
Gelman GN Metricel	13	42	32
Millipore cellulose ester grid	8	41	41
Gelman Supor	10	40	38
Gelman GN Metricel grid	6	35	34
Gelman Tuffryn	9	33	31
Poretics polycarbonate	12	33	22
Millipore Durapore	23	25	37[a]
Schleicher & Schuell cellulose acetate	16	21	24[a]
Schleicher & Schuell nylon	28	21	22[a]

[a] The vortex mixer procedure yielded a higher recovery rate than the sonicator procedure.

TABLE 2. Percentages of legionellae recovered by 0.2-μm-pore-size membrane filters and flow rates

Membrane filter	Flow rate (min/liter)	Elution with sonicator: mean % of recovery	Elution with vortex mixer: mean % of recovery	P value in comparison with Nuclepore polycarbonate filter value
Nuclepore polycarbonate	32	66	59	
Gelman Supor	21	56	48	>0.20
Gelman Tuffryn	34	55	40	>0.20
Millipore Durapore	71	33	53[a]	<0.01
Millipore cellulose ester	44	49	40	<0.01
Schleicher & Schuell cellulose acetate	34	41	46[a]	<0.01
Poretics polycarbonate	25	45	38	<0.05
Schleicher & Schuell cellulose ester	25	39	34	<0.01
Schleicher & Schuell nylon	66	22	26[a]	<0.01
Anotec ceramic	21	7	ND[b]	<0.01

[a] The vortex mixer procedure yielded a higher recovery rate than sonication.
[b] ND, not done.

handle. Buckling occurred with some of the cellulose ester filters. The Anotec filters were very fragile and cracked easily, preventing elution by using the vortex mixer. They also required special filtration equipment. The Gelman Supor and Gelman Tuffryn filters are sturdy membranes and easy to handle.

As expected, the 0.8- and 0.4-μm-pore-size membrane filters yielded the fastest filtration rates, with the majority of these filters filtering 1 liter of water in less than 20 min. The filtration rates with the 0.2-μm-pore-size membrane filters were more variable, ranging from 21 to 71 min/liter depending on the membrane filter used. This may have been related to several factors, such as membrane material and clogging of the smaller pores with bacteria or debris.

Despite the reduced filtration time, the percentages of legionellae recovered by using the 0.8-μm-pore-size membrane filters were unacceptably low. These filters were evaluated on the basis of the findings of Zierdt (9). This author indicated that particles smaller than the pore size of the membrane are quite likely to remain firmly adherent on the filter because of an electrostatic charge. He found that this was true with *Staphylococcus aureus*, *Escherichia coli*, and *Candida* spp. Our findings with legionellae do not support this theory.

Although Orrison et al. (5) proved that the pores of 0.45-μm-pore-size membrane filters were small enough to prevent legionellae from passing through, our recovery rates for the 0.4-μm-pore-size filters were fairly low compared with the recovery rates for the 0.2-μm-pore-size filters. This may have been due to retention of the bacteria in the holes of the membranes, which prevented elution. We obtained the best recovery rates with the 0.2-μm-pore-size membrane filters.

Our results were similar to those of Wolford et al. (8), who found a range of recovery rates from environmental samples of 39 to 93% depending on the type of membrane filter used. Similarly, our rates of recovery for the 0.2-μm-pore-size filters ranged from 7% for the ceramic filters to 66% for the Nuclepore polycarbonate filters. As discussed above and by Brenner and Rankin (1), there are many variables among filters. The membrane material, pore size, electrostatic charge, and inhibitory substances in the membranes are just a few of the variables. Even with the same type of filter, lot-to-lot variation may exist (1), a factor that was not evaluated in our study.

Although the Nuclepore polycarbonate membrane filters gave the highest percentages of recovery, we found that these filters are very difficult to handle. The Gelman Supor (polysulfone) filters gave slightly lower percentages of recovery than the Nuclepore polycarbonate membrane filters, but the differences were not statistically significant ($P > 0.20$). These membranes do not require a special apparatus, nor do they fold, crease, or tear during handling. The Gelman Supor filters also had the highest filtration rate among the 0.2-μm-pore-size membrane filters when 1 liter of water was filtered. Magnetic filtration devices used in most water laboratories make the Gelman Supor filters more practical than the polycarbonate filters. We prefer this type of membrane filter because of its ease of handling and faster filtration rate.

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