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Influence of Coliform Source on Evaluation of Membrane Filters

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Four brands of membrane filters were examined for total and fecal coliform recovery performance by two experimental approaches. Using diluted EC broth cultures of water samples, Johns-Manville filters were superior to Sartorius filters for fecal coliform but equivalent for total coliform recovery. Using river water samples, Johns-Manville filters were superior to Sartorius filters for total coliform but equivalent for fecal coliform recovery. No differences were observed between Johns-Manville and Millipore or Millipore and Sartorius filters for total or fecal coliform recoveries using either approach, nor was any difference observed between Millipore and Gelman filters for fecal coliform recovery from river water samples. These results indicate that the source of the coliform bacteria has an important influence on the conclusions of membrane filter evaluation studies.

The membrane filter technique for enumeration of coliform bacteria in water is an accepted and widely used procedure (1). The method is simple and more reproducible, provides quicker results, and permits the analysis of larger volumes of water than the traditional multiple-tube technique. Only recently have reports appeared which suggest that results of studies based on the membrane filter technique can be influenced by differences in recovery performance between commercial brands of membrane filters and sterilization procedures (Table 1) (2, 4-7). Some of these reports (5, 7) caution that the conclusions of these investigations can be influenced by the experimental design and statistical analyses used.

A preliminary evaluation of three brands of membrane filters by five laboratories within the Ministry of Health laboratory system suggested differences in recovery performance (unpublished data). We subsequently undertook an investigation of four commercial brands of filters (Millipore, Sartorius, Johns-Manville, and Gelman) through two different experimental approaches. Positive EC broth cultures of routine water samples received in our laboratory were used in phase one, and samples from a surface source, the Humber River, were used in phase two.

MATERIALS AND METHODS

Membrane filters. Millipore (catalogue no. HAWG047SO) and Sartorius (catalogue no. 11456) filters used in this study were obtained from laboratory stocks; Johns-Manville (catalogue no. 045M047SG and 045M047LG) and Gelman (catalogue no. GN-6, 64194) filters were supplied by the companies for this particular investigation. Phase one of the study included three lots of ethylene oxide-sterilized filters of each brand: Millipore (lot no. 34309-14, 34433-9, and 34309-27); Sartorius (lot no. 300963219, 301073204, and 306793500); and Johns-Manville (lot. no. 409J257, 408K132, and 406K258). The same lots of Millipore and Sartorius filters were used in phase two of the study, but a different lot (no. 438R1354) of Johns-Manville filters was used, which was sterilized in our laboratory by autoclaving at 121 C for 10 min. Autoclaved Gelman filters (lot no. 80988, 80993, and 81012) were compared only against Millipore filters (lot no. 34309-15, 34309-22, and 37498-13) in phase two of the study.

Source of coliforms. Routine water samples received for bacteriological analysis were used as the source of coliforms in phase one of this study. Ten milliliters of the sample was added to 10 ml of double-strength MacConkey broth, which was incubated for 24 or 48 h at 35 C. Transfers from positive tubes were made to EC broth, which was incubated at 44.5 C for 22 to 24 h. A dilution of the positive EC broth was prepared with sterile phosphate buffer (pH 7.2) and standardized by optical density to give final colony counts on membrane filters of 20 to 80 for total coliform and 20 to 60 for fecal coliform determinations. Using EC-positive cultures of 20 water samples, five replicate filtrations were completed for each of the three brands of filters for total and for fecal coliform determinations.

In the second phase of the study, water samples were collected from a surface source, the Humber River. Eight samples were used for total coliform and seven for fecal coliform recoveries in comparisons of Johns-Manville, Millipore, and Sartorius filters; five samples were used for comparison of Gelman with Millipore filters. Based on a preliminary membrane filter screening to determine the approximate coliform density, an appropriate dilution of the

TABLE 1. *Summary of reported studies on membrane filter performance*

Membrane filters compared	Source of coliforms	Conclusions	Reference
Gelman and Millipore	*E. coli* type I isolated by m-FC method	Gelman superior to Millipore for fecal coliform recovery	6
Gelman and Millipore	ATCC strains of *E. coli* and *E. aerogenes*, and m-FC isolate IMViC type I	Gelman equivalent to Millipore for total coliform but superior for fecal coliform recovery	5
Gelman and Millipore	Unchlorinated water	Gelman superior to Millipore for fecal coliform recovery	4
Gelman, Millipore, and Sartorius	River water	March: Gelman superior to Millipore and Sartorius for total coliform recovery; Gelman superior to Sartorius but equivalent to Millipore for fecal coliform recovery. June: Gelman superior to Millipore but equivalent to Sartorius (autoclaved) for total coliform recovery; all filters equivalent for fecal coliform recovery	2
Gelman and Millipore	Natural water	Gelman superior to Millipore for total coliform but equivalent for fecal coliform recovery	7

refrigerated sample was prepared the following day to provide colony counts on membrane filters of 20 to 80 for total coliform and 20 to 60 for fecal coliform determinations. Ten replicate filtrations were completed for each brand of filter on each water sample for total and for fecal coliforms in comparisons of Johns-Manville, Millipore, and Sartorius filters. Fifteen replicates for fecal coliforms only were used in comparing Gelman with Millipore filters.

Cultural techniques. For total coliform determinations, m-Endo MF broth (Difco) with 0.8% agar (Oxoid) was used and was incubated at 35 C for 22 to 24 h under high-humidity conditions. Fecal coliform recoveries utilized m-FC broth base (Difco) with 0.8% agar (Oxoid) added. Petri plates were sealed in plastic bags and immersed in a water bath at 44.5 C for 18 to 22 h.

Statistical analyses. Analysis of variance was used for statistical evaluation of the results. When the analysis indicated a significant difference among the means at a level of 0.05, the Tukey test for multiple comparison of means was applied (3).

RESULTS

Total coliform recoveries on m-Endo medium from EC-positive cultures were not significantly different among the three brands of filters (Table 2). Recoveries of fecal coliforms on m-FC medium at 44.5 C showed Johns-Manville filters to be superior to Sartorius but not different from Millipore, nor was Millipore significantly different from Sartorius (Table 3). Using river water samples, Johns-Manville filters showed superior recovery to Sartorius for total coliforms (Table 4), whereas no significant difference among the three brands was observed for fecal coliforms (Table 5). No difference between Gelman and Millipore filters was observed for fecal coliform recovery from river water samples (Table 6). In summary, the only difference in recovery performance was between Johns-Manville and Sartorius filters; however, the superiority of Johns-Manville filters for either total or fecal coliform recovery varied with the source of the organisms (Table 7).

TABLE 2. *Recovery of EC-positive cultures on m-Endo medium with Johns-Manville, Sartorius, and Millipore membrane filters*

Determination	Johns-Manville	Sartorius	Millipore
No. of comparisons	100	100	100
Mean	52.0	44.5	43.7
Standard deviation	30.70	30.87	32.28

Analysis of variance[a]

Source of variation	SS	df	MS	F ratio[b]
Within	290,885.70	297	979.31	
Between	4,194.06	2	2,097.03	2.14

[a] SS, Sum of squares; df, degrees of freedom; MS, mean square.

[b] $F_{0.05(2,\infty)} = 3.00$.

TABLE 3. *Recovery of EC-positive cultures on m-FC medium with Johns-Manville, Sartorius, and Millipore membrane filters*

Determination	Johns-Manville	Sartorius	Millipore
No. of comparisons	100	100	100
Mean	40.5	30.3	33.1
Standard deviation	32.47	26.75	28.19

Analysis of variance[a]

Source of variation	SS	df	MS	F ratio[b]
Within	25,387.02	297	854.78	
Between	5,496.17	2	2,748.08	3.22

Multimean comparison (Tukey test)

Determination	Johns-Manville vs. Millipore	Johns-Manville vs. Sartorius	Millipore vs. Sartorius
$(\bar{x}_1 - \bar{x}_2) \pm T^c$	+17.03 to −2.33	+19.83 to +0.47	+13.48 to −6.88
Conclusion (α = 0.05)	Not significant	Significant	Not significant

[a,b] See Table 1.
[c] T = Tukey statistic.

TABLE 4. *Total coliform recovery from Humber River water samples with Johns-Manville, Sartorius, and Millipore membrane filters*

Determination	Johns-Manville	Sartorius	Millipore
No. of comparisons	80	80	80
Mean	49.1	34.7	42.7
Standard deviation	25.81	22.01	27.12

Analysis of variance[a]

Source of variation	SS	df	MS	F ratio[b]
Within	149,039.18	237	628.86	
Between	8,327.48	2	4,163.74	6.62

Multimean comparison (Tukey test)

Determination	Johns-Manville vs. Millipore	Johns-Manville vs. Sartorius	Millipore vs. Sartorius
$(\bar{x}_1 - \bar{x}_2) \pm T^c$	+15.6 to −2.8	+23.6 to +5.2	+17.2 to −1.2
Conclusion (α = 0.05)	Not significant	Significant	Not significant

[a-c] See Table 3.

We observed certain undesirable qualitative characteristics of the filters during the course of this investigation. Irregular hydrophobic areas reported by Dutka et al. (2) on Sartorius filters were evident on wetting and showed a slower rate of dye uptake when placed on m-Endo medium. Both Johns-Manville and Millpore filters showed an inhibition of growth by grid markings, more pronounced with total coliform then fecal coliform colonies. Colonies growing near grid lines developed flat edges, conforming to the restrictions imposed by the lines.

Ethylene oxide-sterilized but not autoclaved Johns-Manville filters showed a spreading of the coliform colonies which frequently filled an entire grid square. Despite this "plaquing" feature, which would seem to reduce colony counts, Johns-Manville was the only brand of filter showing significantly greater recovery in any of the comparisons made.

TABLE 5. *Fecal coliform recovery from Humber River water samples with Johns-Manville, Sartorius, and Millipore membrane filters*

Determination	Johns-Manville	Sartorius	Millipore
No. of comparisons	70	70	70
Mean	30.1	26.7	26.0
Standard deviation	12.39	10.15	10.40

Analysis of variance[a]

Source of variation	SS	df	MS	F ratio[b]
Within	25,150.87	207	121.50	
Between	668.41	2	334.20	2.75

[a-b] See Table 1.

TABLE 6. *Fecal coliform recovery from Humber River water samples with Millipore and Gelman membrane filters*

Determination	Millipore	Gelman
No. of comparisons	75	75
Mean	25.6	26.9
Standard deviation	10.16	10.36

DISCUSSION

Although there seem to be real differences in recovery performance between different brands of membrane filters, one can come to quite different conclusions regarding the performance of a particular brand of membrane filter depending upon the experimental design used, particularly with variations in the source of coliforms (Table 1). Presswood and Brown (6) and Harris (4) concluded that Gelman membrane filters were superior to Millipore for fecal coliform recovery. Schaeffer et al. (7), disagreeing with the statistical methods of Presswood

TABLE 7. *Summary of statistical comparisons between Johns-Manville, Sartorius, and Millipore membrane filters for total coliform and fecal coliform recovery*

Source of coliforms	Johns-Manville vs. Millipore		Johns-Manville vs. Sartorius		Millipore vs. Sartorius	
	Total coliform	Fecal coliform	Total coliform	Fecal coliform	Total coliform	Fecal coliform
EC-positive cultures of water samples	Equivalent	Equivalent	Equivalent	Johns-Manville superior	Equivalent	Equivalent
Humber River water samples	Equivalent	Equivalent	Johns-Manville superior	Equivalent	Equivalent	Equivalent

and Brown, found that Gelman and Millipore were equivalent for recovery of fecal coliforms but that Gelman was superior for total coliform recovery. Schaeffer et al. used natural samples as the source of coliforms, Presswood and Brown used pure cultures of *Escherichia coli*, and Harris used unchlorinated water samples. Dutka et al. (2) reported conflicting results with Gelman, Millipore, and Sartorius filters in two studies with river water samples completed at different times of the year but using the same experimental design.

In phase one of our study we used fecal coliforms from EC-positive broths to ensure that we were working with heterogeneous rather then pure strains of coliforms. Believing that the truest test of membrane filter performance can only come from the use of coliforms as they exist in nature, we wanted to avoid the laboratory manipulations necessary to obtain isolates meeting the definition of "total coliform" (1). In addition, we wanted to utilize the same organisms at both test temperatures, which would not have been possible with nonfecal coliforms. In phase two of our study we did use natural water samples and came to a different conclusion regarding membrane filter performance.

Our results demonstrate that the source of the coliforms has an influence on the conclusions of membrane filter evaluation studies. We will, no doubt, continue to see conflicting reports on the superiority of one brand of filter over another until such time as standardized procedures for filter evaluations, including source of test organism and statistical analysis, are established and accepted.

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