APPLIED MICROBIOLOGY, Sept. 1973, p. 332-336
Vol. 26, No. 3
Copyright © 1973 American Society for Microbiology
Printed in U.S.A.

Comparison of Gelman and Millipore Membrane Filters for Enumerating Fecal Coliform Bacteria

WILLIAM G. PRESSWOOD AND LYNN R. BROWN
Division of Environmental Planning, Tennessee Valley Authority, Chattanooga, Tennessee 37401

Received for publication 4 May 1973

Tests of two leading brands of membrane filters used for enumerating fecal coliform bacteria showed that Gelman GN-6 filters recovered statistically more colonies of bacteria than did Millipore HAWG 047SO filters from pure cultures incubated at either 35 C (the optimal growth temperature) or 44.5 C (the standard temperature for the fecal coliform test). Standard membrane filter procedures with M-FC broth base were used to enumerate the organisms. Densities of colonies incubated on Gelman filters at 44.5 C averaged 2.3 times greater than those on Millipore filters. Plate counts of the bacteria at both temperatures indicated that incubation at 44.5 C did not inhibit propagation of fecal coliform bacteria. For the pour plates, M-FC broth base plus 1.5% agar was used. This modified medium compared favorably to plate count agar for enumerating *Escherichia coli*. At 35 and 44.5 C, colony counts on Gelman filters agreed closely with plate counts prepared concurrently, but Millipore counts were consistently lower than plate counts, especially at 44.5 C. Comparative analyses of river water for fecal coliform bacteria by the membrane filter technique gave results comparable to those for the pure cultures.

The pour plate procedure is perhaps considered by most bacteriologists the best method for determining the densities of viable bacteria. The most probable number procedure produces a wide range of 95% confidence limits, and results from this technique are often erratic and overestimate the true density of bacteria. Results from the membrane filter procedure approach the results of the pour plate procedure more nearly than do any of the other methods suggested for determining coliform bacteria.

The membrane filter technique for enumerating fecal coliform bacteria was entered in *Standard Methods for the Examination of Water and Wastewater* (1) as a standard procedure in 1971. This technique requires that cultures be incubated at 44.5 ± 0.2 C for 24 h in a constant-temperature water bath. The standard method does not, however, specify a particular commercial brand of membrane filter (1). The user may choose from the products of several different manufacturers.

However, when we began using a second commercial brand of membrane filter (Gelman GN-6) interchangeably with the brand previously used in routine analyses (Millipore HAWG 047SO), we noticed that although colonies grown on Millipore filters appeared larger, smoother, and more glistening than those grown on Gelman filters, which were generally smaller, unevenly blue, and often dull, there nevertheless was a pronounced increase in colony counts on the Gelman filters.

Later, we noticed that the colony counts on the Gelman filters agreed more closely with colony counts on pour plates than did those on the Millipore filters.

Colony counts on membrane filters have been compared with those on pour plates by several investigators. Henderson (3) compared membrane filter colony counts of coliform bacteria incubated on m-Endo MF broth at 35 C with plate counts and found excellent agreement between the two methods. Geldreich et al. (2) reported an average 88% recovery of fecal coliform bacteria on the membrane filter with fecal-coliform-organism medium (M-FC) at 44.5 C based on counts of pour plates incubated at 35 C with tryptose-glucose-extract agar.

However, Levin et al. (4) found no agreement between counts on membrane filters and pour plates with *Escherichia coli* ATCC strain 8739. For the membrane filter medium they used m-enrichment broth followed by brilliant green fuchsin broth, or m-Endo broth for some analyses, and nutrient agar for the pour plates.

In an attempt to account more completely for the differences we had observed in our analyses, we made an extensive comparative study of the recovery of pure cultures of 25 strains of *E. coli* incubated on Millipore and Gelman membrane filters and on pour plates at 35 C, the temperature for optimal growth of fecal coliform bacteria, and 44.5 C, the standard temperature for the fecal coliform test. This study was designed to investigate the possible effect of the higher temperature on bacterial growth and to provide a large number of data on enough different strains of *E. coli* to reveal any trend in the comparative performances of the two filters or in their agreement with the results of the pour plate procedure.

MATERIALS AND METHODS

Membrane filters. This investigation was limited to a comparison of only two commercial brands of 0.45-μm porosity membrane filters, chosen because they are perhaps the most widely used brands sold in the United States: Millipore HAWG 047SO (Millipore Corp., Bedford, Mass.), sterilized with ethylene oxide; and Gelman GN-6, 63077 (Gelman Instrument Co., Ann Arbor, Mich.), sterilized in an autoclave. Both brands of filters were sterilized by the manufacturer. Three different lot control numbers of Millipore and two of Gelman were used.

Cultures. The fecal coliform group is predominantly a conglomerate of many strains of *Escherichia* with a smaller number of *Enterobacter* and *Klebsiella*. Individual strains of the fecal coliform group respond differently to different substrates and temperatures of incubation. For our comparison of filters, we judged it better to test many strains of *Escherichia* individually and to draw conclusions from the results of the composite of microorganisms rather than use one strain of *E. coli*.

To obtain such a diverse population, we isolated 20 strains of fecal coliform bacteria from river water at different time periods from various sources within the Tennessee Valley, and five strains directly from domestic sewage by selecting typical blue colonies cultured at 44.5 C on membrane filters with M-FC broth base medium. The microorganisms were streaked onto eosin methylene blue agar to check for purity, and a characteristic *E. coli* colony was picked and grown in elevated coliform (EC) medium at 44.5 C to confirm gas production. Stock cultures were maintained at 35 C in EC medium. IMViC classifications were done on each culture by standard procedures (1). Only recently isolated bacteria were used in the investigation, and all were identified as *E. coli* type I (IMViC ++--).

Culture media. For the membrane filter tests, M-FC broth base plus 0.01% rosolic acid was used as the recovery medium. To provide a similar growth medium for the pour plate comparisons, M-FC broth base plus 1.5% agar was autoclaved, 0.01% rosolic acid was added, and the medium was cooled to about 50 C before use. This modified medium was compared with plate count agar for its ability to recover *E. coli* by doing five replicate plate counts at 35 C with each medium for three of the pure cultures. The means were not significantly different at the 5% level.

Cell suspensions. Cell suspensions of *E. coli* were made from cultures incubated 24, 48, or 72 h in EC medium at 35 C. For most of this work, we added 0.01 ml of stock culture to a 99-ml phosphate buffer water blank and then diluted this 1:1,000 in sterile phosphate buffer solution. To keep all cell suspensions within a workable range of bacterial concentrations, however, cultures of strains that grew exceptionally well in EC broth and reached very high densities required greater dilution. Similarly, cultures incubated for 24 h were usually at the peak of the growth cycle and had to be diluted 1:10,000 to reduce the bacterial concentration to the countable range.

Procedure. For each of the 25 pure strains of *E. coli*, we prepared 20 replicate cultures on Millipore filters, on Gelman filters, and on pour plates. For the membrane filter cultures, 1 ml of the cell suspension was filtered through each filter. A Millipore filtering apparatus was used for all filtrations. Pour plates were prepared on the agar-modified medium according to standard procedures (1). The filter cultures and pour plates were prepared alternately to prevent bias. Of the 20 replicate samples for each lot, 10 were incubated in an environmental incubator at 35 $\pm$ 1 C and 10 were incubated at 44.5 $\pm$ 0.2 C in a water bath. Colonies were counted under a stereomicroscope at a magnification of 7$\times$.

RESULTS

The resulting data were analyzed statistically by linear regression analysis, by the *t* test, and by the coefficient of variation. These data were used as the basis for three comparisons. To determine the effects of the higher incubation temperature on enumeration of bacteria, the densities of bacteria incubated at 35 and 44.5 C were compared within each lot of replicates (Millipore compared with Millipore, Gelman with Gelman, pour plates with pour plates) (Fig. 1). To determine differences between the membrane filters, colonial densities on Millipore filters were compared with those on Gelman filters incubated at corresponding temperatures (Fig. 2). To determine the relative accuracy of results on the different membrane filters, densities on Millipore and Gelman filters were compared with those on pour plates incubated at corresponding temperatures (Fig. 3 and 4).

Finally, to determine whether the results from the studies of pure cultures were characteristic of results that might be expected during routine use of these membrane filters for typical water quality analyses, Millipore filters were compared with Gelman filters for their efficiency in recovering fecal coliform bacteria from

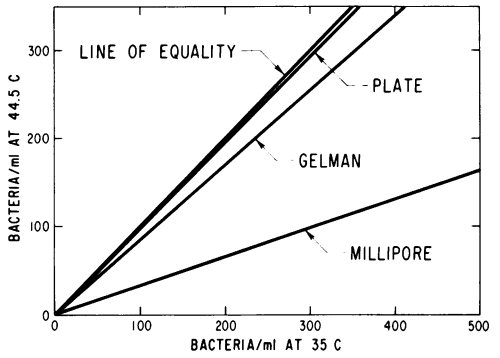

FIG. 1. *Membrane filter and plate counts of fecal coliform bacteria at 35 and 44.5 C. Regression coefficients: plate counts = 0.976, Gelman counts = 0.855, and Millipore counts = 0.319.*

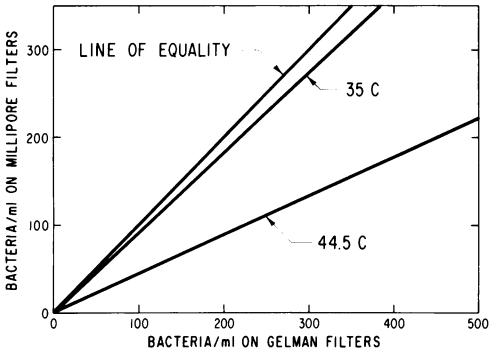

FIG. 2. *Comparison of fecal coliform bacterial densities on Millipore and Gelman membrane filters at 35 and 44.5 C. Regression coefficients: Millipore counts = 0.905 at 35 C and 0.441 at 44.5 C.*

samples of river water (see Table 3). The two brands of filters were alternated during filtrations.

The data used for calculating the regression lines in Fig. 1 through 4 are the mean counts of 10 replicates at 35 C and 10 replicates at 44.5 C for Gelman and Millipore membrane filters and pour plates. The mean values are not shown on the figures but are listed in Table 1.

The regression lines with zero intercept were calculated by the method of least squares. This method fits data to an optimized line by the equation Y = bX, where b is the regression coefficient or slope, Y is the dependent variable, and X is the independent variable.

The results of the enumerations of fecal coliform bacteria at 35 and 44.5 C on Gelman and Millipore membrane filters and by the pour plate procedure are compared in Fig. 1. Each regression line is compared with the equality line, which has a regression coefficient or slope of 1.0 and which would result if, for either membrane filters or the plate counts, the means at 35 and 44.5 C were the same. The extent to which each regression line falls below the line of equality shows the decrease in colonial densities at the higher temperature. Comparison of the regression coefficient for the plate count line (0.976) and the regression coefficient of the equality line by the *t* test shows no statistical difference at the 5% significance level in counts on pour plates incubated at 35 and 44.5 C. The regression lines for bacteria cultured on membrane filters were statistically different from equality at the 5% significance level. However, this difference was markedly greater for cultures on Millipore filters than for those on Gelman filters. The slope of the regression line for Gelman filters (0.855) is approximately 2.7 times greater than that for Millipore filters (0.319).

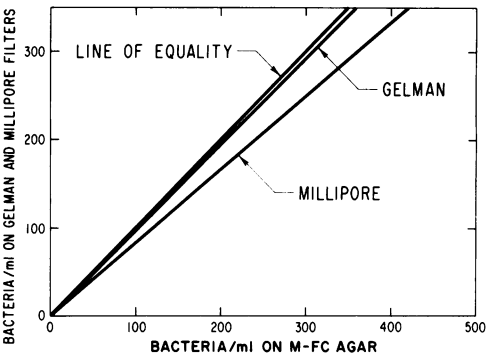

FIG. 3. *Comparison of fecal coliform bacterial densities on Gelman and Millipore membrane filters with those on plate counts at 35 C. Regression coefficients: Gelman counts = 0.967, and Millipore counts = 0.826.*

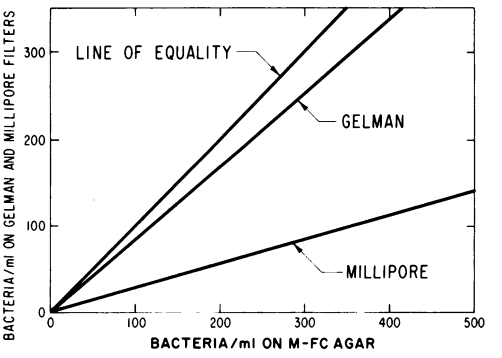

FIG. 4. *Comparison of fecal coliform bacterial densities on Gelman and Millipore membrane filters with those on plate counts at 44.5 C. Regression coefficients: Gelman counts = 0.845, and Millipore counts = 0.280.*

TABLE 1. *Mean counts of 10 replicates of 25 strains of fecal coliform bacteria on membrane filters and by plate counts*

Culture no.	No. of bacteria per ml					
	35 C			44.5 C		
	Plate	Millipore	Gelman	Plate	Millipore	Gelman
1	33.0	29.3	31.2	32.9	3.9	28.3
2	135.4	146.2	183.6	129.5	13.7	157.1
3	272.0	247.1	247.3	261.5	81.5	224.2
4	142.9	154.7	167.6	152.8	28.1	146.0
5	97.1	97.7	100.4	81.2	7.8	22.4
6	71.7	70.6	77.8	75.4	30.2	75.1
7	256.2	257.9	276.1	270.5	78.7	244.2
8	359.2	378.3	396.8	357.1	68.7	344.7
9	136.4	125.7	140.6	122.5	10.3	125.0
10	141.7	127.2	138.7	49.5	35.8	115.2
11	45.7	48.1	47.2	49.2	21.6	38.4
12	139.9	123.0	136.4	138.2	51.7	125.6
13	145.0	130.4	146.2	147.5	77.1	130.0
14	101.2	91.6	104.7	99.4	23.5	90.1
15	171.4	76.2	150.4	163.7	39.6	114.5
16	256.6	259.9	248.3	258.2	153.1	251.1
17	69.7	69.5	68.9	74.5	19.5	62.3
18	44.4	43.3	51.2	50.0	9.6	36.8
19	216.3	127.5	206.1	229.2	145.0	210.6
20	198.4	202.9	200.0	207.5	69.8	182.7
21	150.3	113.2	160.2	97.6	0.2	35.9
22	158.3	146.1	159.4	140.6	8.7	93.3
23	245.1	228.8	245.4	238.1	101.8	220.5
24	111.6	15.3	58.7	105.1	0.5	36.9
25	356.0	114.8	245.8	366.0	20.8	212.4

When Gelman cultures were compared with Millipore cultures at 35 and 44.5 C, results showed that larger numbers of colonies were recovered on Gelman filters (Fig. 2). If the bacterial populations on the two filters were not different, the regression lines would coincide with the line of equality. Since the regression lines are below the equality line at both temperatures, the indication is that colonial densities on Millipore filters were consistently lower than those on Gelman filters. This difference occurred at both temperatures, but the disparity between the filters was greater by a factor of 2.3 at 44.5 C. The differences were statistically significant at the 5% level.

Bacterial colony counts on Gelman and Millipore filters were compared with colony counts on pour plates at 35 C (Fig. 3) and at 44.5 C (Fig. 4). The regression lines derived from the comparison of counts on Gelman filters and pour plates at both 35 and 44.5 C are nearer to the line of equality than are the corresponding regression lines for Millipore filters and pour plates. A t test comparison of the regression coefficients for cultures on Gelman and Millipore filters to the regression coefficient for the line of equality showed that colony counts on Gelman filters were not statistically different from counts on pour plates at 35 C. Colony counts on Millipore filters were statistically different from the line of equality at this temperature. Colony counts on Gelman and Millipore filters were statistically different from counts on pour plates at 44.5 C. On the average, however, colony counts on pour plates were 3.6 times greater than those on corresponding Millipore filters but only 1.2 times greater than those on Gelman filters.

The coefficient of variation shows the degree of precision in the method of bacterial enumeration. The results with the lowest coefficient of variation are the most precise. Table 2 lists the average coefficients of variation for the 25 cultures at 35 and 44.5 C. Counts on pour plates and Gelman filters at 35 C had the lowest average coefficient of variation (0.09). Counts on pour plates at 44.5 C had an average coefficient of variation of 0.10. Cultures incubated on Gelman filters at 44.5 C had an average coefficient of variation of 0.14, whereas those incubated on Millipore filters at this temperature had the largest coefficient of variation of 0.48.

Gelman and Millipore membrane filters were compared in a typical application for efficiency in recovering fecal coliform bacteria by analyzing samples of river water collected near the laboratory at Tennessee River mile 464.0. The elevated temperature fecal coliform test was used to determine the fecal coliform density in the water. Fecal coliform bacterial densities on Millipore filters were only 53.1% of those on the Gelman filters (mean of 3 runs) (Table 3). These results compare favorably with the results of the analyses of pure cultures.

DISCUSSION

The lack of any significant statistical difference for the results of the comparison of pour plates incubated at 35 and 44.5 C (Fig. 1) indicates that the higher temperature is not in

TABLE 2. *Average coefficient of variation at 35 and 44.5 C for Gelman and Millipore cultures and for plate counts*[a]

Culture	Coefficient of variation	
	35 C	44.5 C
Plate	0.09	0.10
Gelman	0.09	0.14
Millipore	0.13	0.48

[a] Calculated from the sum of the coefficients of variation for each of the 25 strains of bacteria.

TABLE 3. *Comparison of Gelman and Millipore membrane filters for recovery of fecal coliform bacteria from river water*

Date of sample collection	No. of bacteria per ml[a]		Loss on Millipore (%)
	Gelman	Millipore	
25 Sept 72	88.6	43.4	51.0
26 Sept 72	134.2	63.0	53.0
28 Sept 72	60.4	27.0	55.3
Mean loss (%)			53.1

[a] Mean of five filtrations.

itself detrimental to the propagation of *E. coli*. Therefore, the statistical difference revealed for cultures incubated on the membrane filters at the two temperatures (Fig. 1) indicates that the detrimental effect of propagation of the fecal coliform bacteria is attributable to the filters rather than to the increased temperature. The greater difference between the equality line and the regression line for the results of cultures on Millipore filters, as compared with that for Gelman filters, therefore suggests an effect of some difference in the characteristics of the two brands of filters that increases with increasing temperature or that may be related to the method of sterilization. Although all cellulose brands of membrane filters are basically similar, additives such as wetting agents and inks used for grid markings vary. These or other compounds such as ethylene oxide residues contained in the filters could possibly be toxic to bacteria or become toxic at 44.5 C and account for the smaller number of colonies at this temperature. Such an effect is also suggested by the comparison of results for the two filters at each temperature (Fig. 2), since the difference occurred at both temperatures but was greater at 44.5 than at 35 C.

Gelman and Millipore filters appear to have different pH values. Gelman filters are blue whereas Millipore filters are amber when placed on M-FC medium. The acid-base indicator system in M-FC medium gives a blue color at acidic pH ranges. While it seems unlikely that the difference in pH is responsible for the disparity in the number of colonies on the two filters, this likelihood should be investigated.

Regression analysis also showed that results with Gelman filters were not statistically different from results with pour plates at 35 C, whereas those with Millipore filters were statistically different. And although results with both filters were statistically different from those with pour plates at 44.5 C, the difference was far greater for Millipore filters than for Gelman filters.

Temperature alone had no significant effect on the precision of results, for the average coefficients of variation for pour plates at 35 and 44.5 C were 0.09 and 0.10, respectively. The use of the membrane filter procedure did affect precision at the higher temperature, however, and this effect was greater for the Millipore filters than for the Gelman filters. Gelman filters produced results that equaled the precision of pour plates at 35 C (average coefficient of variation 0.09) but were less precise (0.14) at 44.5 C. The precision of results for Millipore filters at 35 C (0.13) did not approach the precision of pour plates and Gelman filters at 35 C and, in fact, was little better than that for Gelman filters at 44.5 C. The least precise results were those for Millipore filters at 44.5 C.

The results from the practical analysis of river water suggest that the results of the studies of the 25 pure cultures indicate the typical performance of these two brands of filters.

The clear indication that Gelman filters recover significantly more fecal coliform bacteria than do Millipore filters suggests the need for further investigation of these and other brands of membrane filters to determine their relative efficiencies and to identify the reason for the difference in the extent of their effects on the propagation of bacteria. Since one obvious difference in the characteristics of the Millipore and Gelman filters is the method of sterilization, this may be the next logical step in this investigation.

In any case, examination of the results in Table 1 make it clear that no such investigations should be based on results for a single strain of bacteria. The difference in results for any one strain may be great or small; therefore, generalizations about relative performance can only be based upon an investigation of a large number of different strains.

ACKNOWLEDGMENTS

We thank Don Rucker for editing the manuscript, Shirley Ray for the statistical analyses, Isaac Jones for his technical assistance, Ed Richards for the drawings, and Janice Johnson for her patience in typing the paper.

LITERATURE CITED

1. American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association Inc., New York.
2. Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best. 1965. Fecal coliform organism medium for the membrane filter technique. J. Amer. Water Works Ass. 57:208-214.
3. Henderson, W. L. 1959. Studies on the use of membrane filters for the estimation of coliform densities in sea water. Sewage Ind. Wastes 31:78-91.
4. Levin, G. W., V. L. Stauss, and W. C. Hess. 1961. Rapid coliform organism determination with C^{14}. J. Water Pollut. Contr. Fed. 33:1021-1037.