Evaluation of Coli-Count Samplers for Possible Use in Standard Counting of Total and Fecal Coliforms in Recreational Waters

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Received for publication 7 July 1975

Millipore Coli-Count Samplers were used to enumerate colonies of laboratory cultures of coliform bacteria. The samplers gave significantly lower counts than standard membrane-filter procedures for both total and fecal coliforms. Although the samplers are useful for semiquantitative analysis as indicated by the manufacturer, they are not suitable for standard examinations of recreational waters.

The Coli-Count Sampler as described by the manufacturer, Millipore Corp., is used to test untreated waters for total and fecal coliform bacteria and is recommended "for determining the range of bacteria present in surface waters in order to arrive at the proper dilutions required for analysis by the standard MF technique" (3). Each unit includes an absorbent pad containing dehydrated medium (composition not reported), a membrane filter overlying the pad, and a plastic support in a plastic chamber which provides a moist atmosphere during incubation. In principle, when the sampler is immersed in water, 1 ml is absorbed through the membrane into the absorbent pad and rehydrates the medium. To enumerate total coliforms, incubation is at 35 ± 0.5 C; for fecal coliforms, it is at 44.5 ± 0.2 C. Because of queries about the possible use of the sampler for analysis of the microbiological quality of recreational waters, we compared the samplers with accepted standard procedures (1) for recovery of several laboratory strains of coliform bacteria.

Coli-Count lot no. 36607 was tested using eight strains of Escherichia coli, one strain of Enterobacter cloacae, and two strains of Klebsiella pneumoniae. Six E. coli strains were isolated from sewage, one from bathing beach water, and one from food; one K. pneumoniae was isolated from a human source; one K. pneumoniae and the E. cloacae were laboratory strains in use for many years. Eighteen- to 24-h cultures in nutrient broth were diluted either 1:10 or 1:2 x 10^5 in phosphate-buffered dilution water to obtain 20 to 80 bacteria per ml. Total coliform and fecal coliform counts were performed with five 1-ml portions of each diluted culture using both Coli-Count and standard membrane-filter procedures with LES Endo agar at 35 C and M-FC broth at 44.5 C. Gelman lot no. 80901 membranes were used for standard procedures except in one series, in which Millipore lot no. 056185 membranes were used for counts at 35 C. The Gelman lot had been tested previously and found to yield counts of E. coli (strain 743) on M-FC medium at 44.5 C and on LES Endo agar at 35 C equivalent to plate count agar colony counts at 35 C. The Millipore lot had not been evaluated. Only the E. coli strains were tested at 44.5 C, since the other coliform strains tested did not grow at this elevated temperature. The results are shown in Table 1.

Analysis of significance by Student's t test showed that 12 of the 19 standard procedure colony counts were significantly higher than corresponding counts obtained with the samplers. For five of these pairs, P < 0.05; for seven, P < 0.01. The relative efficiency of recovery by the sampler versus the standard procedure was similar for both total and fecal coliform tests.

Analysts using the Coli-Count Samplers observed that 10 of 60 units were dark blue throughout after incubation at 35 C and that colonies on these units were smaller than on other Coli-Count units in the same series. The manufacturer states that 1 to 150 colonies may be counted (3). This range is unsatisfactory, based on experience with standard membrane-filter procedures. The upper limits established for standard procedures, 60 fecal coliform colonies on M-FC medium at 44.5 C and 80 coliform colonies on M-Endo medium at 35 C, reflect the diminished growth or atypical appearance of colonies which can occur with higher counts (2). The test area on a 47-mm membrane filter is 962 mm², whereas that of a Coli-Count Sampler is 920 mm². In addition, coliform colonies were larger on the Coli-Count than on LES agar at
TABLE 1. Comparison of total and fecal coliform counts of standard membrane-filter procedure and Coli-Count Sampler

| Test culture | Colony count (per ml) | Ratio of Coli-Count:standard | t* |
|-------------|-----------------------|-----------------------------|----|
|             | Coli-Count | Standard |                   |    |
| Total coliform |           |           |                   |    |
| *Escherichia coli* 743 | 56.0 | 68.6 | 0.82 | 2.013 |
| *E. coli* 274-1 | 39.6 | 50.6 | 0.78 | 3.510 |
| *E. coli* 274-2 | 25.2 | 35.0 | 0.72 | 2.535 |
| *E. coli* 274-4 | 36.0 | 47.0 | 0.77 | 2.767 |
| *E. coli* 077 | 35.8 | 47.8 | 0.75 | 3.768 |
| *E. coli* 30-1 | 66.8 | 62.2 | 1.07 | 0.781 |
| *E. coli* 30-2 | 30.4 | 35.2 | 0.86 | 1.883 |
| *E. coli* 276 | 61.8 | 56.4 | 1.10 | 0.716 |
| *Enterobacter cloacae* GM8 | 33.4 | 43.6 | 0.77 | 3.222 |
| *Klebsiella pneumoniae* 4908 | 24.6 | 31.2 | 0.79 | 2.711 |
| *K. pneumoniae* 2343 | 21.8 | 36.0 | 0.61 | 6.979 |
| Fecal coliform |           |           |                   |    |
| *E. coli* 743 | 37.0 | 56.8 | 0.65 | 5.803 |
| *E. coli* 274-1 | 29.6 | 37.2 | 0.80 | 1.854 |
| *E. coli* 274-2 | 14.0 | 24.4 | 0.57 | 4.670 |
| *E. coli* 274-4 | 26.4 | 38.8 | 0.68 | 2.907 |
| *E. coli* 077 | 35.4 | 36.6 | 0.97 | 0.301 |
| *E. coli* 30-1 | 47.4 | 61.6 | 0.77 | 3.412 |
| *E. coli* 30-2 | 15.2 | 17.4 | 0.87 | 0.625 |
| *E. coli* 276 | 39.8 | 55.2 | 0.72 | 3.953 |

a Incubation temperatures: total coliform, 35 C; fecal coliform, 44.5 C. Cultures diluted before testing to yield 20 to 80 bacteria per ml.

b Each value is a mean of five replicates.

c Student's t test; *P < 0.05, t = 2.306; P < 0.01, t = 3.355; 8 df.

d Millipore membranes; all other standard tests were made with Gelman membranes.

The lower counts for both total and fecal coliforms could be due only to a difference between Gelman and Millipore membranes. Presswood and Brown (4) and Schaeffer et al. (5) have reported lower recoveries on Millipore membranes in comparison with Gelman membranes for fecal and total coliform determinations, respectively. Quality control procedures in our laboratory have revealed variation among lots from both manufacturers. It is possible that the lot of Coli-Count Samplers tested was prepared with an unsatisfactory lot of membranes.

Evaluation of the Coli-Count Sampler using natural samples was not undertaken because of the results of this preliminary study, since coliforms under stress and in competition with other species would be less likely to be recovered quantitatively than would freshly transplanted pure cultures.

The technical assistance of Janet Orloff and Judy Vallee is acknowledged with appreciation.

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