Comparison of the Novel ColiPlate™ Kit and the Standard Membrane Filter Technique for Enumerating Total Coliforms and *Escherichia coli* Bacteria in Water

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ABSTRACT: The new ColiPlate (CP) kit was evaluated comparatively with the standard membrane filter (MF) technique for enumerating total coliforms and *Escherichia coli* in water. In testing natural water samples, good correlations were observed for enumerating total coliforms (R^2 = 0.84) and *E. coli* (R^2 = 0.95). However, counts of *E. coli* population density estimated by CP were 47% higher than counts estimated by MF. With the water samples spiked with culture-grown *E. coli* cells, the correlation between the methods was strong for both total coliforms (R^2 = 0.95) and for *E. coli* (R^2 = 0.94). *E. coli* densities were estimated to be 20% higher using CP compared with MF. Samples spiked with rehydrated freeze-dried *E. coli* cells (with high portion of injured or weakened cells) showed a strong correlation between the two methods (R^2 = 0.93, for either total coliforms or *E. coli*). However, estimated total coliforms and *E. coli* densities were higher by CP than MF counts (38 and 168%, respectively). The CP test is therefore considered a more reliable method than the traditional MF for enumerating *E. coli* in samples with high levels of injured or weakened cells. Environ. Toxicol. Water Qual. 13: 157-164, 1998.

Keywords: coliforms; *E. coli*; membrane filter; ColiPlate; MPN

INTRODUCTION

Currently, the membrane filter (MF) technique, as described in Standard Methods for Examination of Water and Wastewater [American Public Health Association (APHA), 1995] is routinely used worldwide to quantify density of coliforms and *E. coli* in water and wastewater. The popular MF 24-h test is considered highly reproducible and yields quantitative results more rapidly and less costly than the alternative multiple-tube fermentation (MTF) procedure. Recently, commercial kits, ColiPlate™ and Colilert Quanti-tray™, have been developed for quantifying total coliforms and *E. coli* in water. These kits are based on the MTF principle, but are considerably more convenient to use than either traditional MTF or MF techniques. The aim of this study was to evaluate the new ColiPlate™ test comparatively with the standard MF test using natural water samples as well as sterile water samples spiked with pure *E. coli* culture.

MATERIALS AND METHODS

Natural Samples

Seventy-three water samples were collected from water treatment plants' intakes and water pollution control plants' final effluents during August and September 1996 in the York-Durham region, Ontario, Canada. Selection of sample sites was based on previous bacteriological data to provide a wide range of counts. Samples were collected in 300 mL sterile plastic bottles containing 300 mg sodium thiosulfate and were immediately placed on ice in coolers for transport. All samples were analyzed on the same day as collected.

Preparation of Spiked Samples

Suspensions of *E. coli* strain ATCC 13706 were prepared from either culture-grown cells or from freeze-dried cells. Cultured bacteria grown in LB medium (Difco) at 35°C overnight resulted in approximately $2x10^7$ cfu/mL. Freeze-dried bacteria were prepared according to Reinhartz et al. (1987). Freeze-dried bacteria were rehydrated in 10 mL cold sterile distilled water, resulting in a final concentration of approximately $2.5x10^8$ cfu/mL. Bacterial suspensions were diluted in sterile tap water and mixed in 300 mL aliquots of sterile tap water at different concentrations for the comparative analyses.

Membrane Filter (MF) Procedure

The procedure was performed as described in the Standard Methods (APHA, 1995, Section 9222). Aliquots of 100 mL water sample or sample dilution were filtered through a 4.5 cm diameter and 0.45 μm pore size nitrocellulose membrane (Gelman, Canada). Membranes were then placed on m-ENDO-LES agar for total coliform enumeration and on m-FC-BCIG agar for *E. coli* enumeration (Ciebin et al., 1995). Growth media were obtained from Difco Laboratories (Michigan, IL). Chromogenic substrate 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (BCIG) was obtained from Diagnostic Chemicals Ltd. (Charlottetown, PEI, Canada). Coliform colonies were determined based on red color and gold metallic sheen observed after 24 h incubation at 35°C on m-ENDO-LES agar. *E. coli* were identified by a blue color after 24 h incubation at 44.5°C on m-FC-BCIG arar.

ColiPlate (CP) Procedure

ColiPlate™ kits were obtained from Environmental Biodetection Products Inc. (Brampton, Ontario, Canada) and were used according to the manufacturer's instructions. The kit is based on a standard 96-well microtiter plate, whereby each well is coated with soluble specialized medium (Ossmer, 1993). For each sample, 200 μL aliquots were dispensed to all wells using a multichannel pipettor. Plates were then covered and incubated at 35°C for 24 h. Total coliform were enumerated based on the number of wells that turned blue. *E. coli* density was determined based on the number of wells that turned blue and fluoresced under long-wave UV light (366 nm). Most-probable-number (MPN) cell density values were estimated from the number of wells giving positive reaction, based on Thomas' model (APHA, 1995; Thomas, 1942).

RESULTS

Comparison of the CP and MF Methods for Enumerating Total Coliforms and *E. coli* in Environmental Water Samples

Of the 73 natural water samples analyzed, 61 (84%) and 40 (55%) samples were found to be positive for total coliform and for *E. coli*, respectively, by either one or both test methods. Counts in these samples ranged from a few to over 1900 total coliform and 400 *E. coli* cells per 100 mL, respectively. A comparison between the two methods in enumerating total coliform is shown in Fig. 1. Linear regression analysis resulted in a correlation coefficient (R^2) value of 0.8366 ($p < 0.05$) and a slope (y) value of 1.0898. Estimated E. coli counts between the methods were even more strongly correlated ($R^2 = 0.9539$, $p < 0.01$). However, the linear regression slope value of 1.4678 (Fig. 2) indicated a significant bias of higher *E. coli* counts obtained by the CP method compared with the MF method ($p < 0.01$).

To determine possible reasons for this observed discrepancy between the two test methods, two controlled laboratory experiments were performed using sterile tap water samples inoculated with different densities of *E. coli* (strain ATCC 13076). In one experiment, sterile tap water samples were spiked with organisms grown overnight in nutrient broth, presumably with relatively few injured or weakened cells. In the other experiment, sterile tap water samples were spiked with rehydrated freeze-dried cells, presumably with relatively high levels of injured or weakened cells. All samples were split and analyzed comparatively by the CP and MF methods for total coliform and *E. coli.*

Comparison of the CP and MF Methods for Enumerating Total Coliform and *E. coli* in Water Spiked with *E. coli* Culture

Counts of total coliform and *E. coli* in 25 samples ranged from nondetectable to 1200 cells per 100 mL (Figs. 3 and 4). Correlation coefficients between the

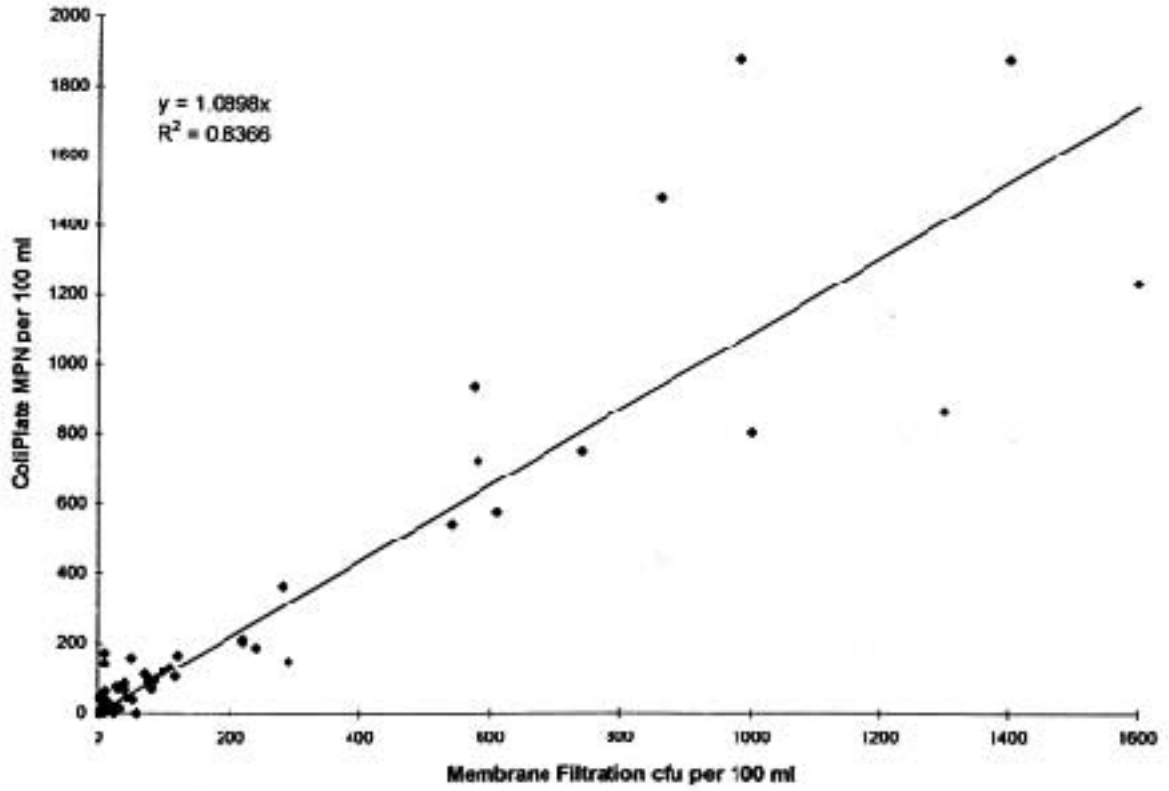

Fig. 1. Comparison between membrane filter and ColiPlate methods for enumerating total coliforms in natural water samples.

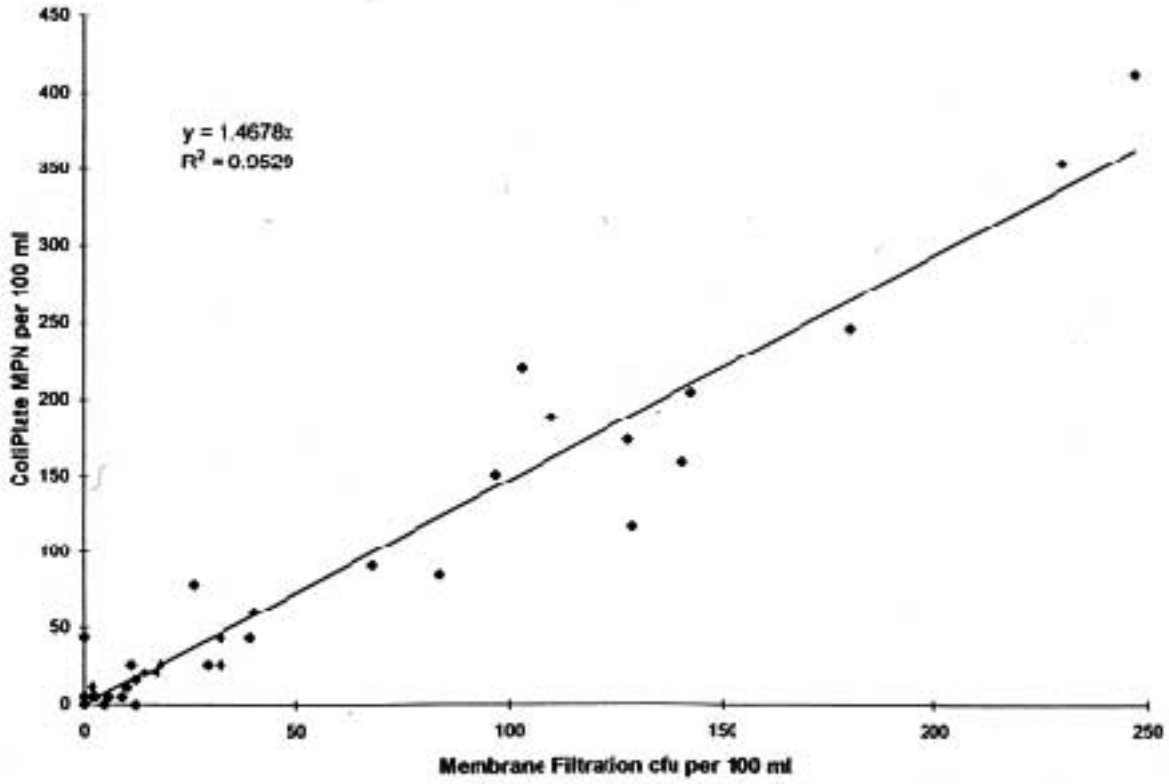

Fig. 2. Comparison between membrane filter and ColiPlate methods for enumerating *E. coli* in natural water samples.

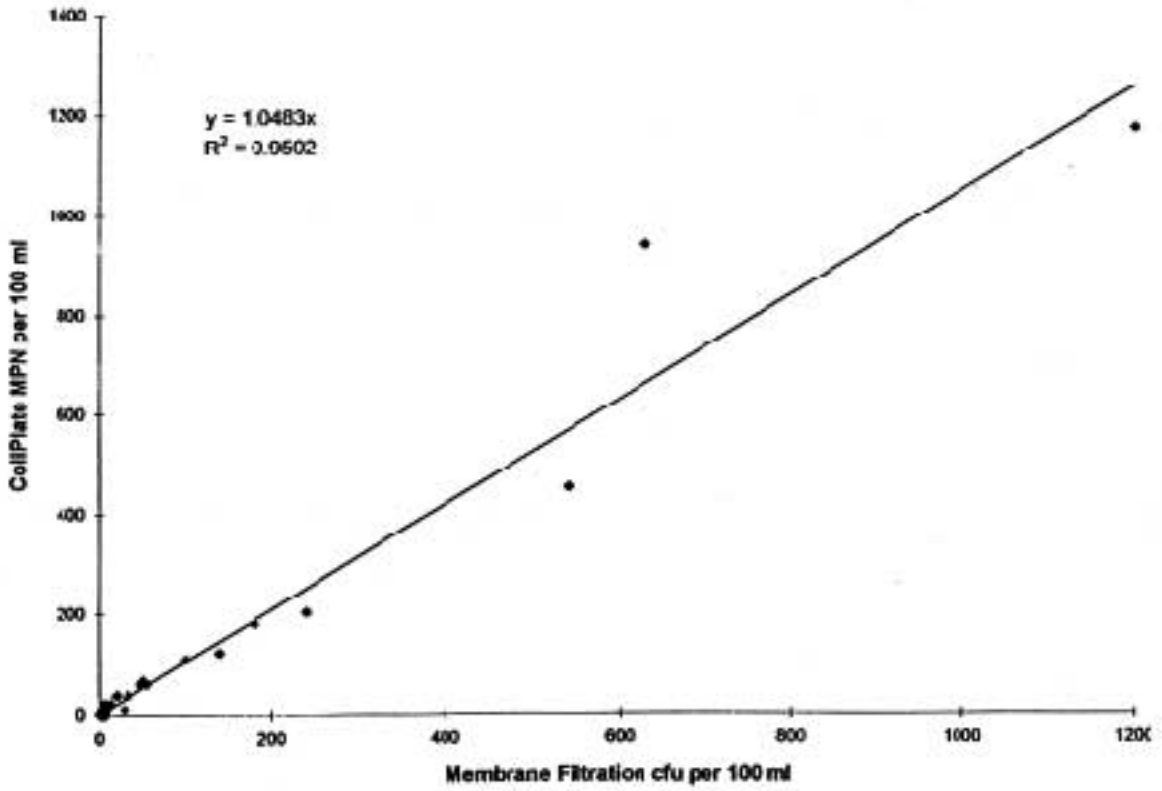

Fig. 3. Comparison between membrane filter and ColiPlate methods for enumerating total coliforms in sterile water spiked with culture-grown *E. coli* cells.

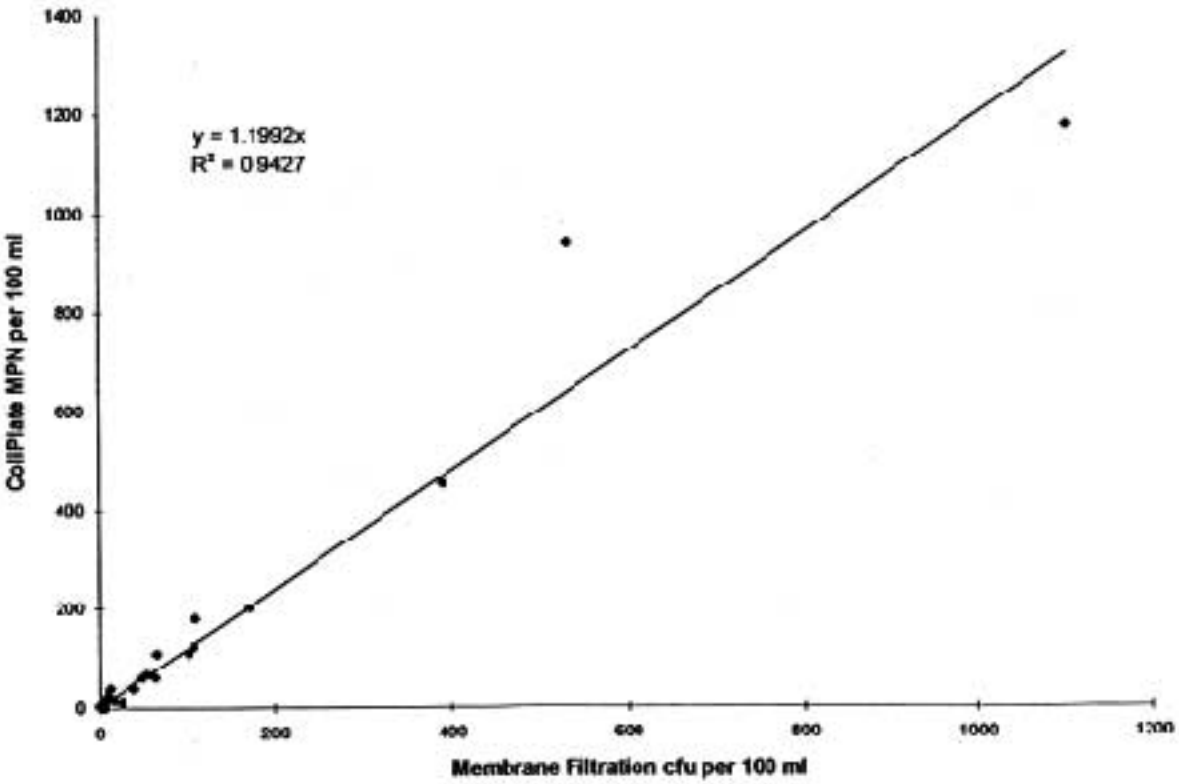

Fig. 4. Comparison between membrane filter and ColiPlate methods for enumerating *E. coli* in water spiked with culture-grown *E. coli* cells.

two methods were strong for both total coliform (R^2 = 0.9501, p <0.01) and for *E. coli* (R^2 = 0.9427, p <0.01). Counts of total coliform density did not differ significantly between the methods (slope = 1.0483). However, counts of *E. coli* density obtained by the CP method were 19.9% higher than counts obtained by MF (Fig .4). With the CP method, counts of total coliform in these samples were always identical to *E. coli*. However, with the MF method, while counts of *E. coli* correlated very strongly to total coliform counts (R^2 = 0.9888, Fig. 5), 13.3% of the *E. coli* cells which were detected as coliform were not identified as *E. coli.*

Comparison of the CP and MF Methods for Enumerating Total Coliform and *E. coli* in Water Spiked with Rehydrated Lyophilized (Freeze-Dried) *E. coli* Cells

Counts of total coliform and *E. coli* in 17 spiked samples ranged from non-detectable to 3400 cells per 100 mL (Figs. 7 and 6). Correlation coefficients between the two methods were strong for both total coliform (R^2 = 0.9344, p ⩽0.01) and for *E. coli* (R^2 = 0.9349, p <0.01). Counts of total coliform and *E. coli* differed significantly between the methods. Total coliform and *E. coli* densities estimated by CP were 37.91 and 168.17% higher, respectively, than values estimated by MF. In addition, with the CP method, counts of total coliforms in these samples were always identical to *E. coli*. In comparisons between counts of *E. coli* vs. total coliform obtained by MF, there was a very strong correlation between coliform and *E. coli* counts (R^2 = 0.9879, p ⩽0.01, Fig. 8). However, over 48% of the *E. coli* cells, which were detected by MF as coliforms, were not detected by the MF method as *E. coli*.

DISCUSSION

This study compared the new ColiPlate™ (CP) kit with the standard membrane filter (MF) method for quantifying total coliforms and *E. coli* in water. The comparative analysis of *E. coli* density in natural water samples revealed that while there was a strong correlation between the two methods, considerably higher counts were determined by CP. There are several possible

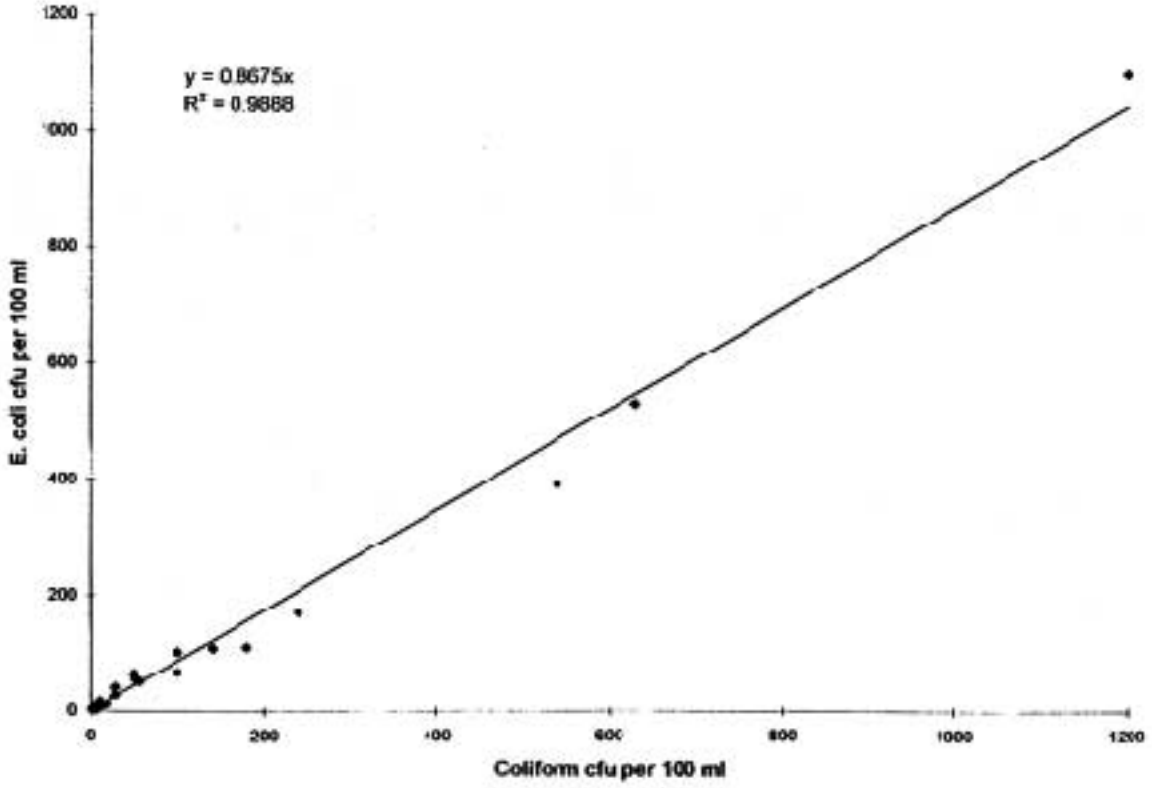

Fig. 5. Comparison between membrane filter counts of total coliforms and *E. coli* in sterile water spiked with culture-grown *E. coli* cells.

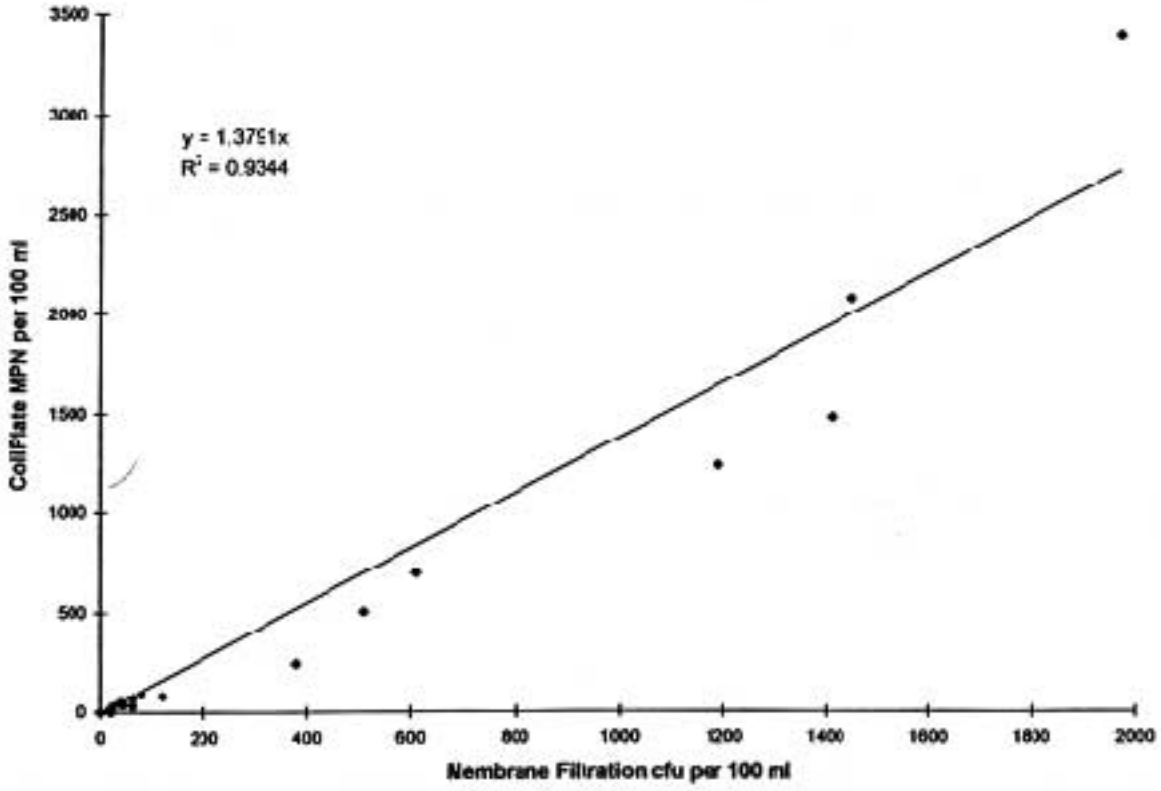

Fig. 6. Comparison between membrane filter and ColiPlate methods for enumerating total coliforms in sterile water spiked with rehydrated freeze-dried *E. coli* cells.

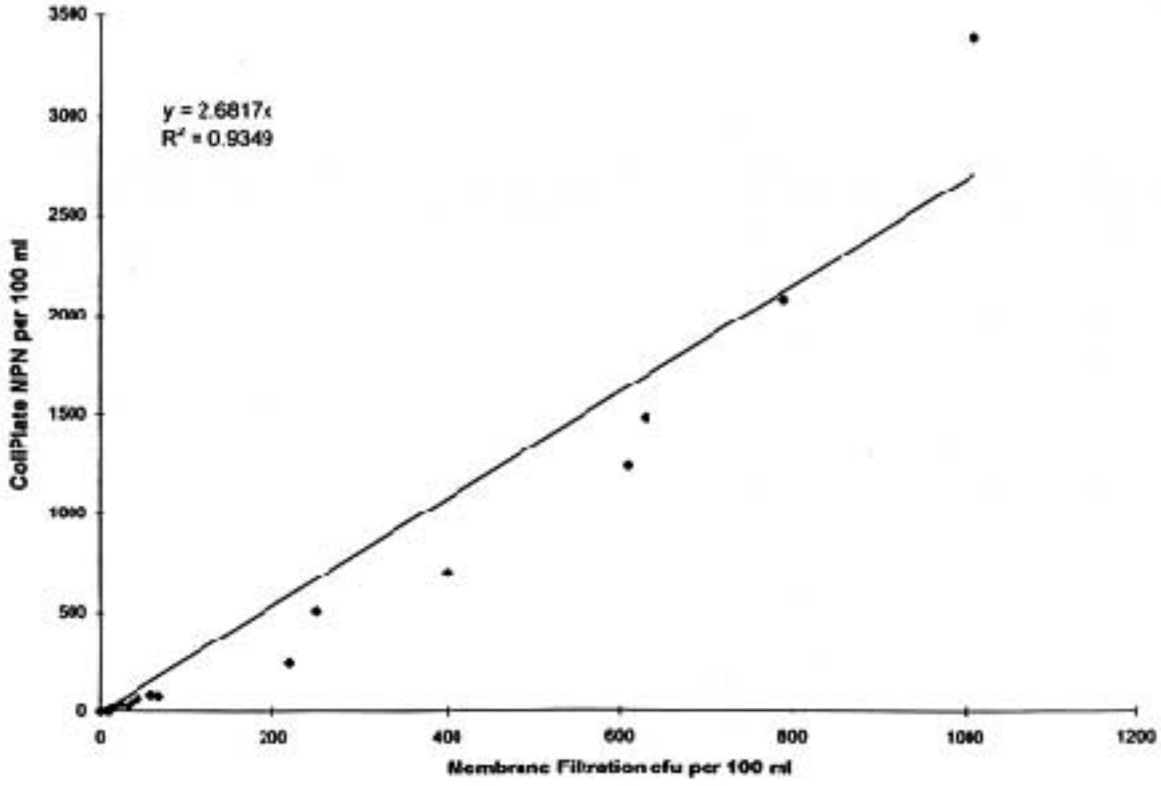

Fig. 7. Comparison between membrane filter and ColiPlate methods for enumerating *E. coli* in water spiked with culture-grown *E. coli* cells.

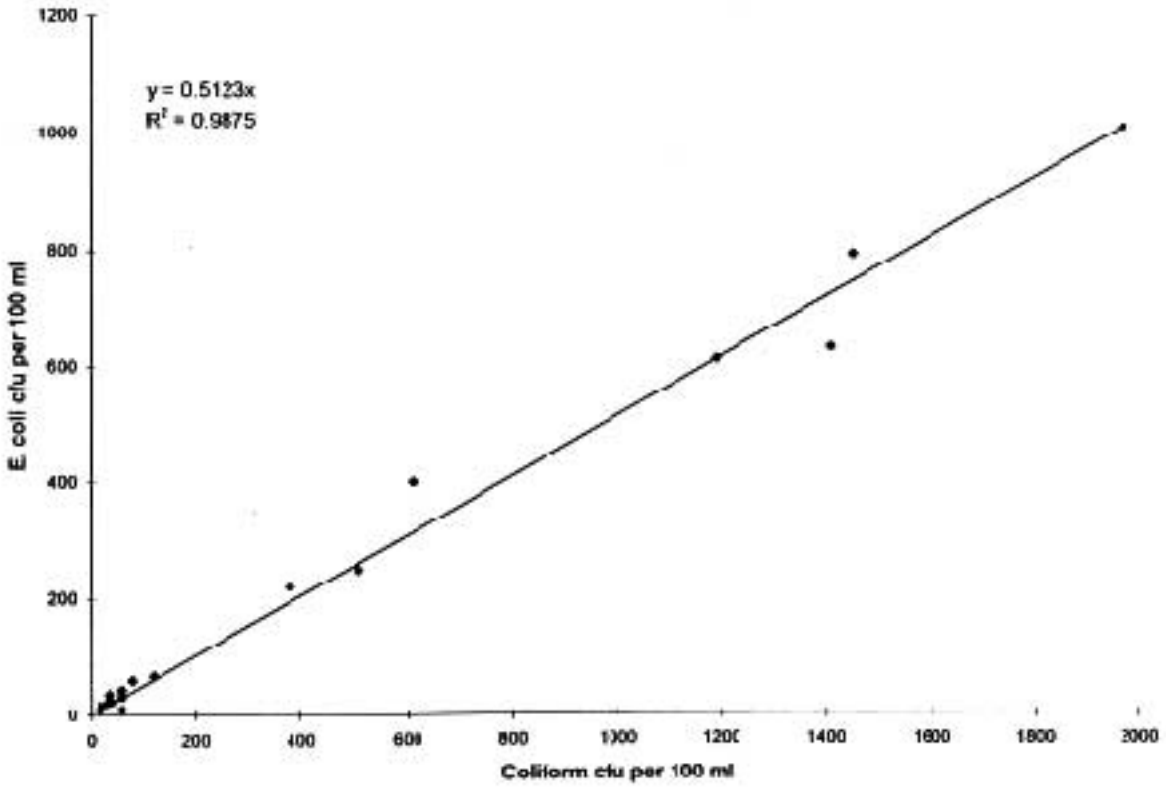

Fig. 8. Comparison between membrane filter counts of total coliforms and *E. coli* in sterile water spiked with rehydrated freeze-dried *E. coli* cells.

explanations for the discrepancy between the two techniques:

(i) A purely mathematical overestimation in calculating the MPN values, perhaps based on erroneous assumptions. However, estimating total coliforms in natural water samples, as well as in water artificially spiked with pure culture-grown cells resulted in similar counts between the two methods. Therefore, the observed discrepancies were not likely caused by a mathematical error in calculation, but were rather due to differential biological characteristics of cells and the methods of enumeration.

(ii) Failure of the MF technique to recover injured or weakened cells. This study has shown that considerably lower counts (ca. 38%) of total coliforms were obtained by MF, compared with the CP method, in samples spiked with highly stressed (previously freeze-dried) cells of *E. coli*. This is consistent with previous studies that reported lower sensitivity of MF in reviving stressed coliforms compared with the multiple fermentation tube method (Bissonette et al., 1975; Jacobs et al., 1986; Shipe and Cameron, 1954).

(iii) Further failure of the MF technique to detect stressed *E. coli* even when the cells are recoverable as coliforms. Evidently, in this study the MF failed to detect ca. 48% of the *E. coli* population, compared with counts obtained by MF for total coliform (Fig. 8). These observations suggest that conditions for growth and phenotypic expression of injured or weakened *E. coli* cells are considerably more conducive under the CP procedure than under the popular MF method. Our results are consistent with a recent study conducted by G.E. Horsnell (Ontario Ministry of Environment and Energy, unpublished data), which compared the MF method with the Colilert Quanti-trayTM method (which like CP is also based on the multiple fermentation tube principle) for enumerating *E. coli* in surface water. His study shows a strong linear correlation (R^2 = 0.90) between the two methods, but with 32% higher counts by the Quanti-tray method.

In conclusion, this study has demonstrated that the sensitivity of the CP test for enumerating coliforms and *E. coli* in water is comparable or superior to the

standard membrane filter technique. The CP method is particularly more reliable than MF in detecting injured or weakened *E. coli* cells. Since the CP test is also more convenient to perform (i.e., the kit is ready to use, easy to perform, and requires no sample dilution), it offers an attractive alternative to the traditional MF technique

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REFERENCES

American Public Health Association. 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Inc., Washington, D.C.

Bissonette, G.K., J.J. Jezeski, G.A. McFeters, and D.G. Stuart. 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Environ. Microbiol. **29**:186-194.

Ciebin, B.C., M.H. Brodsky, R. Eddington, G. Horsnell, A. Choney, G. Palmateer, A. Ley, R. Joshi, and G. Shears. 1995. Comparative evaluation of modified m-FC and m-TEC media for membrane filter enumeration of Escherichia coli in water. Appl. Environ. Microbiol. **61**:3940-3942.

Jacobs, N.J., W.L. Zeigler, F.C. Reid, T.A. Stukel, and E.W. Rice. 1986. Comparison of membrane filter, multiple-fermentation-tube, and presence-absence techniques for detecting total coliforms in small community water systems. Appl. Environ. Microbiol. **51**:1007-1012.

Ossmer, R. 1993. Simultaneous detection of total coliforms and E. coli-Flurocult® LMX broth. In Proceedings of the Fifteenth Symposium on Food Microbiology and Hygiene, Bingen, Germany, August 31-September 3, 1993.

Reinhartz, A., I. Lampert, M. Herzberg, and F. Fish. 1987. A new short term sensitive bacterial assay kit for detection of toxicants. Toxic. Assess. **2**:193-206.

Shipe, E.L., and G.M. Cameron. 1954. A comparison of the membrane filter with the most probable number method for coliform determinations from several waters. Appl. Microbiol. **2**:85-88.

Thomas, H.A., Jr. 1942. Bacteria densities from fermentation tube tests. J. Amer. Water Works Assoc. 572-576.