ELSEVIER

Enumeration of heterotrophs, fecal coliforms and *Escherichia coli* in water: comparison of 3M™ Petrifilm™ plates with standard plating procedures

H. Schraft*, L.A. Watterworth

Department of Biology, Lakehead University, Thunder Bay, ON, Canada P7B 5E1

Abstract

A total of 177 naturally contaminated water samples were analyzed by membrane filtration according to the Standard Methods for the Examination of Water and Wastewater published by the American Public Health Association. Filters were incubated in parallel on mHPC-agar and 3M™ Petrifilm™ Aerobic Count Plates (Petrifilm™ AC plates) for heterotrophic counts. Fecal coliforms and *Escherichia coli* were enumerated on mFC-agar and 3M™ Petrifilm™ *E. coli*/Coliform Count Plates (Petrifilm™ EC plates). Typical colonies on each media type were confirmed following standard procedures. Heterotrophic counts were between 10^3 and 10^4 CFU/mL and the average $\log_{10}$ counts obtained on Petrifilm™ AC plates were about two-fold lower than on mHPC-agar. Counts for fecal coliforms and *E. coli* were between 10^2 and 10^3 CFU/mL. Average $\log_{10}$ counts for confirmed fecal coliforms obtained on Petrifilm™ EC plates were slightly lower than on mFC agar with a correlation coefficient of 0.949. The average $\log_{10}$ counts for confirmed *E. coli* on Petrifilm™ EC plates and on mFC agar were statistically not different ($P=0.126$) with a correlation coefficient of 0.879. Specificity of Petrifilm™ EC plates and mFC agar was evaluated by comparing typical colony counts with confirmed counts. On mFC agar, counts for typical colonies were by 2 $\log_{10}$ CFU higher than the actual confirmed counts. In contrast, on Petrifilm™ EC plates typical colony counts were almost identical to confirmed colony counts for both fecal coliforms and *E. coli*. This comparison illustrates the high specificity of Petrifilm™ EC plates for enumeration of both fecal coliforms and *E. coli* in water.
© 2004 Elsevier B.V. All rights reserved.

Keywords: 3M™ Petrifilm™ plates; Water microbiology; Heterotrophic counts; Fecal coliforms; *E. coli*

1. Introduction

The waterborne outbreak linked to enterohemorrhagic *Escherichia coli* in 2000 in Walkerton, Ontario (Anonymous, 2000) has resulted in heightened surveillance of the microbiological quality of surface

---

* Corresponding author. Tel.: +1 807 343 8351; fax: +1 807 346 7796.
E-mail address: heidi.schraft@lakeheadu.ca (H. Schraft).

and drinking water. New regulations are implemented in Canada and in many other countries and in Ontario, they have resulted in a significant increase of microbiological water analyses (Ontario Safe Drinking Water Act, 2002).

Enumeration of total heterotrophic counts is commonly used as indicator of overall microbiological quality (APHA, 1995; WHO, 1993). To ensure absence of enteric pathogens, enumeration of appropriate indicator organisms is required. Some controversy still exists as to which group of organisms would be most suitable, but total coliforms, fecal coliforms and E. coli have been suggested. Total coliforms are defined as aerobic or facultatively anaerobic, Gram-negative rod-shaped, non-spore-forming, oxidase negative bacteria that ferment lactose at 35 °C with the production of acid and gas (APHA, 1995; WHO, 1993). Since coliforms are defined by biochemical properties, the group does not have taxonomic value and organisms of many genera, such as Escherichia, Citrobacter, Klebsiella or Serratia, are included. Total coliform counts are used to monitor treated water supplies with the objective to determine adequacy of the water treatment process and the integrity of the distribution system (APHA, 1995; WHO, 1993). To avoid the limitations of total coliforms, monitoring of fecal (or thermotolerant) coliforms has been suggested. This group includes those coliforms that ferment lactose at 44 °C. Although many coliforms are not active at the elevated temperature, non-fecal organisms, such as some species of Klebsiella, Enterobacter or Citrobacter, are detected by fecal coliform tests. Since levels of fecal coliforms are mostly directly correlated to those of E. coli, fecal coliform counts have been widely accepted for routine monitoring of water quality. However, confirmation of isolates as E. coli by additional biochemical tests is recommended (WHO, 1993). Today, E. coli is generally recognized as a good indicator for fecal contamination and it has been recommended as the indicator of choice for monitoring of drinking water (WHO, 1993).

Standard methods for the enumeration of heterotrophs, fecal coliforms and E. coli in water rely on membrane filtration with subsequent incubation of the filters on solid agar media. Preparation of such media is time consuming and expensive. Thus, ready-made media that would reduce time and labor required for

analyses are needed, particularly in light of the drastic increase of requirements for microbiological water testing. 3M™ Petrifilm™ plates provide such ready-to-use plating media which are widely accepted and approved for microbiological analysis in the food and beverage industry. In particular, 3M™ Petrifilm™ Count Plates could be useful as alternative for heterotrophic counts and 3M™ Petrifilm™ E. coli/ Coliform Count Plates for fecal coliforms and E. coli. However, only limited information has been published to date that allows an evaluation of Petrifilm™ plates for microbiological analysis of water by membrane filtration. The objective of this study was therefore to evaluate the application of ready-to-use 3M™ Petrifilm™ plates for the enumeration of heterotrophic counts, fecal coliforms and E. coli in naturally contaminated wastewater.

2. Materials and methods

2.1. Water samples

A total of 177 water samples were received from five different wastewater treatment facilities in Southern Ontario. The water samples were collected by plant personnel into sterilized 500 mL plastic bottles, refrigerated and shipped to the laboratory in cooled insulated containers. To ensure countable levels of microorganisms, all samples were taken before any disinfection step.

2.2. Membrane filtration

Within 24 h of collection, samples were analyzed by membrane filtration method for heterotrophic counts and fecal coliforms following the Standard Procedures for Water Analysis published by the American Public Health Association (APHA) (1995).

The samples were serially diluted in 0.1% peptone water and the dilutions filtered onto membrane filters (47 mm diameter and 0.45 μm pore size). Cellulose acetate filters (AMD Manufacturing, Mississauga, Ontario) were used for heterotrophic counts and fecal coliform counts were determined on nitrocellulose filters (Millipore). After filtration, each filter was aseptically placed on the appropriate growth medium.