

EVALUATION OF PETRIFILM™ EC AND HS FOR TOTAL COLIFORMS AND *ESCHERICHIA COLI* ENUMERATION IN WATER

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Submitted: July 17, 2002; Returned to Authors for corrections: October 10, 2003; Approved: November 06, 2003

SHORT COMMUNICATION

ABSTRACT

Petrifilm™ EC and HS were compared to the MPN method to determine their efficacy to enumerate total coliforms and *E. coli* in 145 samples of water (76 water *in natura*, 69 drinking water). For water *in natura*, Petrifilm™ HS and EC showed good correlation with MPN method. In chlorinated water (≤ 20 col/100 mL and negatives) the agreement was low. For *E. coli* enumeration, Petrifilm™ EC showed a good correlation with MPN method. The results indicated that Petrifilm™ EC and HS are accurate to enumerate coliforms and *E. coli* in water when the expected counts are higher than 20/100 mL.

Key words: water, coliforms, *Escherichia coli*, Petrifilm™.

The determination of total coliforms and *Escherichia coli* in water can be used to assess the sanitary quality. These microorganisms are good indicators, mainly because they are simple to identify and enumerate (10,12). The traditional multiple tubes technique (MTT) for microbiological water analysis (1) is troublesome and slow. Recently, new methods and instruments have been developed as alternatives to the conventional methods (5), based in defined substrate technology with chromogenic and fluorogenic indicators.

Petrifilm™ plates (3M Company, St. Paul, MN USA), developed in the 80's, are an alternative method to enumerate food hygiene indicators microorganisms. Petrifilm™ EC plates are recognized by several international organizations (AOAC and APHA) as official methods for coliforms and *Escherichia coli* enumeration in all types of food (2). In water, the use of Petrifilm™ plates is limited due to the small sample volume sowed on the plates.

This study was carried out to assess the efficiency of Petrifilm™ *E. coli* (EC) and Petrifilm™ High Sensitivity (HS) plates for total coliforms and *E. coli* enumeration in various types of water in Londrina, PR, Brazil using to the standard most probable number (MPN) method by the MTT as reference method.

A total of 145 water samples (31 from water supply network, 08 from swimming pools, 30 from wells, 07 from springs, 44 from rivers, 03 from streams, 19 from lakes and 03 from ponds) were obtained in Londrina region, North Paraná, Brazil. Water samples from the supply network and swimming pools were chlorinated and the remaining samples were *in natura*. The samples were collected in sterilized amber glass flasks and kept at 4°C until processing. Chlorinated water samples were collected in flasks containing 18% sodium tiosulfate solution (0.1mL). Serial decimal dilutions until 1:10,000 were made in phosphate buffer with 1M magnesium chloride and pH 7.0 (11). River water samples which received sewage outlet were submitted to greater dilutions.

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The MTT was used to analyze 54 *in natura* water samples with five or more series of five tubes containing Lactose Broth (LB) (Merck, Darmstadt, Germany). Lauryl Sulphate Tryptose (LST) broth was used (Difco, Sparks, USA) (10) to analyze another 22 samples of *in natura* water. Series of five tubes containing double concentrated LB were sown with 10 mL of the sample without dilution. The others tubes received 1 mL of the sample without dilution or 1:10, 1:100 and 1:1000 dilutions. The ten tubes technique was used to analyze 40 chlorinated water samples from the water supply network and 30 water samples for human consumption without disinfection. The tubes of these series contained 10 mL of double concentration LB and 10 mL of the sample without dilution were sowed in each tube. The sown tubes were incubated at $35 \pm 1^\circ\text{C}$ for 24-48 h. Positive tubes were those that presented clouding in the culture medium and gas (Durhan tubes) (1).

An aliquot of 0.03 mL from each positive tube in the presumptive test was transferred to tubes containing 7 mL Brilliant Green Lactose Bile broth (BGCL) (Oxoid, Basingstoke, Hampshire, England) and incubated at $35 \pm 1^\circ\text{C}$ for 24-48 h. Positive tubes were those that presented clouding in the culture medium and gas production (Durhan tubes). The MPN of total coliforms in 100 mL was determined by the number of positive tubes, correlating them in a MPN table (1).

For fecal coliforms enumeration, 0.03 mL from each positive tube in the presumptive test was transferred to tubes containing *Escherichia coli* (EC) broth (Micromed, Jacaré, Rio de Janeiro, Brazil) and incubated at $44.5 \pm 1^\circ\text{C}$ for 24 h in water bath. Positive tubes were those that showed clouding in the culture medium and gas production (Durhan tubes). The MPN of total fecal coliforms in 100 mL was determined by the number of positive tubes in each dilution, and correlating them in a MPN table (1).

Aliquots of 1 mL of each sample (or chosen dilution) were sowed in Petrifilm™ EC plates and incubated at $35 \pm 1^\circ\text{C}$ for 24-48 h. Total coliforms and *E. coli* colonies were enumerated according to the manufacturer instructions. The results obtained were multiplied by 100 to obtain the results for 100 mL of sample.

The Petrifilm™ HS plates for total coliforms enumeration were sown with 5 mL of the chosen dilution and incubated at $35 \pm 1^\circ\text{C}$. According to the manufacturer recommendations, readings were made after 24 h incubation, and an additional reading was made after 48 h. The total coliforms colonies were enumerated according to the manufacturer instructions. The results were multiplied by 20 to obtain the results for 100 mL of sample.

The results were converted into \log_{10} and submitted to correlation, data dispersion and linear regression analyses using EXCELL (Microsoft Office 2000) and Statistica (1993). The results were analyzed according to the sample treatment and to the medium used in the presumptive stage. The results of the analyses of water for human consumption along with the water samples for recreation, which showed low counts and negative

results, were submitted to the Kappa test to verify the agreement among the methods (6).

Results using standard method and Petrifilm™ EC (0.93) presented good correlations regardless the culture medium used in the presumptive phase, with 0.09 of mean variance (*mv*) (Fig. 1). Good correlations were also obtained using the traditional method and Petrifilm™ HS (0.90) with *mv* 0.14 (Fig. 2). The correlation between the methods for enumeration of total coliforms in 54 samples of *in natura* water using LB in the presumptive phase of the traditional method and Petrifilm™ EC was 0.93 (*mv*: 0.10). Under the same conditions, the correlation with the Petrifilm™ HS was 0.94 (*mv*: 0.28).

When LST was used in the presumptive stage of the standard method for analysis of 22 *in natura* water samples, the correlation with Petrifilm™ EC was 0.90 (*mv*: 0.06); the correlation with the Petrifilm™ HS was 0.73 (*mv*: 0.28). The decrease in the correlation and increase in the variance may be attributed to higher counts in Petrifilm™ HS compared to the standard method in 68% of the samples indicating a high sensitivity in Petrifilm™ HS. Our results are similar to the ones reported by Curiale *et al.* (4), who compared MPN and plate count techniques.

The analysis of 39 chlorinated water samples for human consumption by standard method and Petrifilm™ EC and HS resulted in absence of coliforms. Analysis of 31 samples without disinfection for human consumption, the agreement between traditional method and Petrifilm™ HS (48 h reading) was considered substantial (Kappa:0.78) and the agreement with the Petrifilm™ EC was considered moderate (Kappa:0.53) (3,5,9).

In Petrifilm™ HS, samples with less than 20 coliforms/100 mL may not show any count. The same occurs in Petrifilm™ EC

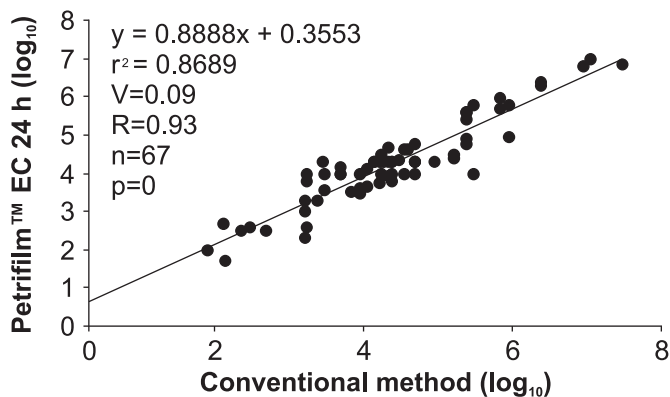


Figure 1. Dispersion of the enumeration results for total coliforms in *in natura* water samples using the EC Petrifilm™ read at 24 h and the multiple tube technique with Lactose Broth, and Lauryl Sulphate Tryptose Broth in the presumptive step and Brilliant Green Bile Lactose Broth in the confirming step.

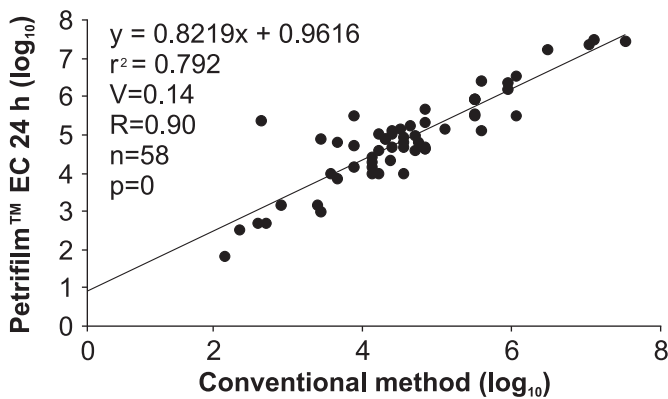


Figure 2. Dispersion of the total coliform enumeration results in *in natura* water samples using the HS Petrifilm™ read at 24 h and the multiple tube technique with Lactose Broth, and Lauryl Sulphate Tryptose Broth in the presumptive step and Brilliant Green Bile Lactose Broth in the confirming step.

with samples presenting less than 100 coliforms/100 mL. The poor agreement obtained in samples with low counts may be attributed partially to differences in the volume sowed in standard method (100 mL) and in Petrifilm™ HS (5 mL) and EC (1 mL) plates. For water for human consumption, in 5 (15%) of the 30 samples the counts were lower than 20 coliforms/100 mL in the traditional method, and Petrifilm™ HS was able to detect coliforms in four of them. In addition, 2 (6.66%) samples presented counting in the Petrifilm™ HS which were not detected by the traditional method. This confirms the great recovery capacity of coliform cells in Petrifilm™ HS, which is better than the medium used in the standard method.

For *E. coli* enumeration, results in Petrifilm™ EC presented correlation of 0.88 ($mv: 0.28$) with results obtained by traditional method using EC Broth. As expected, the countings in Petrifilm™ EC were lower than in EC broth, once in the standard method three other microorganisms are included in the fecal coliforms group besides *E. coli*.

The Petrifilm™ manufacturer recommends not to use tiosulphate in samples to be sown in Petrifilm™ HS and EC, but this practice did not show any interference in the countings in chlorinated water samples. A parallel experiment showed no significant differences between Petrifilm™ HS and EC counting of samples with or without tiosulphate (data not shown).

Despite allowing the analysis of a greater sample volume, traditional methods for MPN determination present little repeatability. In addition, isolation of microorganisms for identification is difficult. Using five tubes for each dilution, the MPN estimation fluctuates between 70% to 260% of the real counting. The colony counting procedure using Petrifilm™ offers greater precision and provides isolated colonies for identification, when necessary (8).

According our results, Petrifilm™ HS and EC can be used safely when more than 20 total coliforms/100 mL are expected, as occurs in *in natura* waters from rivers, lakes and ponds. For *E. coli* enumeration, the Petrifilm™ EC showed a good correlation with standard method, as reported by other authors comparing the same techniques (4,7). Analysis of the results indicates that the volume of water tested did not influence the result of the counting, as long as the sample was sufficiently homogenized and that the quantity of microorganisms was superior to the detection limit of the method used.

Petrifilm™ is four times quicker than the MPN method for total coliform enumeration and twice as fast for *E. coli* enumeration. Furthermore, Petrifilm™ HS and EC plates are more practical to use and read the results, avoiding replications and use of great quantity of glassware and culture medium, as used in the standard method.

RESUMO

Avaliação do Petrifilm™ EC e HS para enumeração de coliformes totais e *Escherichia coli* em água

Petrifilm™ EC e HS foram comparados ao método do Número Mais Provável (NMP) para determinar sua eficiência na enumeração de coliformes totais e *E. coli* em 145 amostras de água (76 de água *in natura* e 69 de água de abastecimento). Em água *in natura*, Petrifilm™ HS e EC mostraram boa correlação com o método de NMP. Em água clorada (≤ 20 col/100 mL e negativas) a concordância foi baixa. Para enumeração de *E. coli*, Petrifilm™ EC mostrou boa correlação com o método de NMP. Os resultados indicaram que Petrifilm™ EC e HS podem ser usados com segurança para enumeração de coliformes totais e *E. coli* em água, desde que as contagens esperadas sejam maiores que 20/100 mL.

Palavras-chave: água, coliformes, *Escherichia coli*, Petrifilm™.

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