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Utilization of Hydrated Petrifilm Coupled with Filtration in the Detection and Enumeration of *Escherichia coli* in Water Samples

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Hydrated 3M™ Petrifilm™ *E. coli* / coliform (EC) plates coupled with filtration (HPECF) was found to be suitable in the analysis of 100 mL water samples, which is required in standard protocols in food and pharmaceutical industries. This was demonstrated with the use of suspensions of pure *Escherichia coli* and *E. coli* mixed with *Enterobacter aerogenes* and *Pseudomonas aeruginosa* – with 93–100% cell recovery. The results indicated that the HPECF method could be valuable in standard water quality analysis in pharmaceutical companies as it provides an acceptable and faster way to comply with the 2017 Philippine National Standards for Drinking Water (PNSDW). Likewise, HPECF results can be obtained after 48 h instead of 120 h as being currently used in the Most Probable Number (MPN) method, making HPECF three times faster than MPN. Furthermore, a simple cost-benefit analysis revealed that the use of HPECF can reduce the cost of water testing by 50% as compared to the MPN method. Consequently, the HPECF method is a more economical and faster alternative to the standard MPN method for water quality analysis for manufacturing companies.

Keywords: coliform, *E. coli*, filtration, Petrifilm, water analysis

INTRODUCTION

Water quality is of primary importance in a manufacturing company to ensure safe and good quality products. Likewise, from raw materials to end-product testing, the company should guarantee that appropriate tests and analyses are carefully and correctly done in order to ensure that their products meet the standards required by regulatory agencies. During water analysis, coliforms – primarily *E. coli* – are commonly detected and enumerated. Coliforms are gram-negative, aerobic to facultative anaerobes that are able to ferment lactose and produce gas at 35 °C within 48 h of incubation (Yousef and Carlstrom 2003). Fecal coliforms serve as a biological indicator of fecal contamination in water samples. Among the fecal coliforms, *E. coli* is the best indicator used in drinking water analysis because it is normally found in mammal's gastrointestinal tract, and is known to survive the normal conditions of drinking water (Edberg *et al.* 2000). Moreover, the methods for its detection are relatively inexpensive compared to other coliforms.

As science and technology develop, new discoveries and inventions arise – leading to more efficient and cost-effective methods of analysis applicable in the field of microbiology. For water analysis required in manufacturing industries, the standard is the MPN method, which is tedious

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and time-consuming. Euro-med Laboratories Phil., Inc. uses MPN for their routine water analysis (Catsao, *pers. comm.*). Ready-to-use products have been developed to help microbiologists save time, energy, and money. One of these products is the 3M™ Petrifilm™ count plate, which makes it possible for microbiologists to detect and enumerate a number of microorganisms much easier and faster compared to traditional testing procedures (3M Food Safety 2014). To date, different 3M™ Petrifilm™ count plates are available and can be used to enumerate common microorganisms such as coliforms, *E. coli*, *Listeria sp.*, *Staphylococcus aureus*, Enterobacteriaceae species, yeasts, and molds. Moreover, 3M™ Petrifilm™ count plates can be used in the enumeration of total aerobic count (3M Food Safety 2010).

Since *E. coli* is the most common microorganism tested in water, the 3M™ Petrifilm™ count plates can be used to enumerate *E. coli* in water, but only if 100 mL water samples can be tested as required by regulatory agencies. However, the 3M™ Petrifilm™ count plates normally require only 1 mL water samples – a volume not acceptable in the standard microbial analysis of water samples. In order to solve this problem, Raymundo (personal communication) proposed that by hydrating the 3M™ Petrifilm™ count plates and filtering 100 mL sample – also called the Petrifilm™ Aqua Coliform Count (AQCC) Filtration System – the 3M™ Petrifilm™ count plates may give accurate results and can serve as an alternative method to MPN and standard plate count methods.

Therefore, this study was conducted to determine the suitability of using HPECF as an alternative to MPN. The cell recovery of *E. coli* in pure or in mixed populations of water suspensions using HPECF was compared with the direct inoculation on Petrifilm™ EC plates (DIPEC). The applicability of HPECF method was also verified using water samples from natural sources.

MATERIALS AND METHODS

Growing of Bacterial Cultures and Determination of Cell Concentration

The cell concentrations of 24 h cultures of *E. coli* ATCC 8739, *E. aerogenes* ATCC 13048, and *P. aruginosa* ATCC 9027 grown in tryptic soy broth were separately determined by spread plating 100 µL of 10^{-6}–10^{-8} dilutions of each culture on tryptic soy agar plates. Colonies were counted and CFU/mL was computed after 24 h incubation at 32°C. Based on the calculated cell concentration, 100 mL of pure *E. coli* suspension containing approximately 100 CFU was prepared using sterile distilled water. A 100 mL mixed cell suspension of *E. coli*, *E. aerogenes*, and *P. aeruginosa* was also prepared to contain approximately 100 CFU. Spread plating (as described above) was done to verify the viable bacterial count of the prepared bacterial suspensions. The prepared bacterial suspensions were used in the detection and enumeration of *E. coli* and other coliforms using three different methods: MPN, DIPEC, and use of HPECF. *E. coli* ATCC 8739 was used because it is a standard strain specified in the USP 39 NF 34 US Pharmacopeia National Formulary (2016) for method validations, and to offset antimicrobial challenges.

Collection of Water Samples

About 500 mL of different water samples were aseptically collected. The samples included the following: 1) commercially available bottled drinking water purchased from a store near Euro-med Laboratories, Phil., Inc., Dasmariñas City; 2) deep well water collected at Euro-med Laboratories, Phil., Inc., Dasmariñas City, Cavite; 3) rainwater collected while raining at Euro-med Laboratories Phil., Inc., Dasmariñas City, Cavite; 4) pond water collected from a mall in Biñan, Laguna; and 5) tap water from a household in Macabling, Santa Rosa, Laguna (this sample was neutralized with sodium thiosulfate prior to analysis). The samples were kept at 4 °C until they were processed. The undiluted water samples were directly used in the detection and enumeration of *E. coli* and other coliforms using the MPN method and HPECF. The setup for each water sample tested performed in triplicates.

Detection and Enumeration of *E. coli* and other Coliforms

For the standard MPN protocol, the method of Fernandez *et al.* (2008) was followed. The second method involved the DIPEC method using 1 mL of each of the samples. The inoculated EC plates were incubated at 32 °C for 24–48 h. In the third method using HPECF, the medium of 3M™ Petrifilm™ EC plate was first hydrated with 1 mL sterile distilled water and allowed to stand for at least 1 h for the gel to solidify and stick to the cover of the EC plate. The 100 mL prepared bacterial suspensions and 100 mL of the collected water samples were passed through the Petrifilm™ AQCC Filtration System – after which the membrane filters (Advantec, Tokyo, Japan) were transferred to the hydrated EC plates, ensuring that the side of each of the membrane filters with the filtered microorganisms got in contact with the gel. EC plates were incubated 32 °C for 24–48 h. All the inoculations were performed in triplicates.

Computation of Percent Recovery

The percent recovery was computed for the DIPEC and HPECF. The average count of three plates for each setup was divided by the original viable count obtained and then multiplied by 100 to get the % recovery.

RESULTS AND DISCUSSION

The three enumeration methods used yielded different results when 117 CFU / 100 mL pure *E. coli* suspension was used. DIPEC resulted in 67.5 CFU/100 mL, while the use of HPECF yielded 109 CFU / 100 mL. The standard MPN method, on the other hand, yielded 35 MPN / 100mL (Table 1). The results indicated a 92.8% recovery of *E. coli* cells using HPECF and only 57.8% recovery for DIPEC.

Table 1. Colony-forming unit (CFU), MPN, and % cell recovery of *E. coli* obtained from MPN, DIPEC, and use of HPECF. Original viable count of the *E. coli* suspension is 117 CFU / 100 mL.

Method	Colony count [a]	% Cell recovery[d]
MPN	35[b]	Not determined
DIPEC	67.5[c]	57.5
Use of HPECF	109[c]	92.8

[a]Average of three plates
[b]MPN / 100 ml
[c]CFU

When *E. coli* was mixed with another coliform (*E. aerogenes*) and non-coliform (*P. aeruginosa*), HPECF recovered 100% of the *E. coli* cells and 21.4% of the *E. aerogenes* cells (Table 2). The *E. coli* cell recovery in using HPECF was 4.7 times higher than the direct inoculation on 3M™ Petrifilm™ EC plates. This can be explained by the higher volume of sample used in HPECF, wherein 100 mL sample was filtered compared to the 1 ml used in DIPEC. The filtration step in the HPECF method aided to trap the *E. coli* cells on the surface of the filter used.

Table 2. Cell recoveries obtained from the mixed culture using DIPEC and use of HPECF.

Bacterial culture	DIPEC		Use of HPECF	
	CFU[a]	% Recovery[b]	CFU[a]	% Recovery[b]
Escherichia coli	14	24.6	57	100
Enterobacter aerogenes	4	14.3	6	21.4
Total coliforms	18	21.2	63	74.1

[a]Average of three plates
[b]Original CFU of the mixed suspension = 111 CFU (57 CFU of *E. coli*; 28 CFU of *E. aerogenes*; 26 CFU of *P. aeruginosa*)

To validate the results, HPECF and the MPN methods were used in detecting and enumerating *E. coli* and other coliforms using different water samples. As expected, there was neither *E. coli* nor other coliforms detected in the bottled drinking water samples using HPECF or MPN (Table 3). With samples from deep well, pond, rain, and domestic tap water, HPECF was able to detect *E. coli* but not with MPN technique; higher numbers of coliforms were also observed in the former method. Since there is a low number of *E. coli* in the aforementioned samples, HPECF was able to detect the presence of the bacterium but not with MPN – indicating the higher sensitivity detection level of this proposed methodology. It can be concluded that HPECF is a better method in detecting and enumerating *E. coli* and non-*E. coli* coliforms in various sources of water samples.

Table 3. Enumeration of *E. coli* and other coliforms obtained from various water samples using HPECF and MPN method.

Water sample	MPN method (MPN / 100 ml)		Use of HPECF[a] (CFU / 100 ml)	
	E. coli	Other coliforms	*E. coli*	Other coliforms
Bottled drinking water	<1	<1	0	0
Deep well water	<1	5	4	TNTC
Pond water	<1	16	9	TNTC
Rain water	<1	18+	1	TNTC
Domestic tap water	<1	9	2	TNTC

[a]Average of three plates

Both MPN and HPECF methods allow the detection of *E. coli* and other coliforms in 100 mL water samples. However, MPN requires additional materials such as Durham tubes for the presumptive test, Levine EMB agar plates for the confirmatory test, and nutrient agar slant with a Durham tube for the completed test. Gram staining and microscopy are also needed. MPN, therefore, could be costly and entails five days since it involves three different tests to identify and count. On the other hand, HPECF results are obtained within 48 h, making HPECF three times faster than MPN. In the industry, the quicker release of results is very essential. In Euro-med Laboratories Phil., Inc., MPN is used for routine water analysis where results are obtained after five days (Catsao, *pers. comm.*). According to her, results are needed immediately because results are required prior to the release of the product (for bottled drinking water), and prior to the use of the water for sanitation of the rooms. Also, more time is being used by the microbiologist in the preparation of materials and media than in doing the actual test. The use of HPECF, therefore, reduces the time consumed during media preparation and expedites the release of results.

In this study, HPECF was found to be applicable in analyzing water samples with low bioburden. This would be practical for pharmaceutical companies where water supply is routinely tested for coliforms. HPECF can also be used for water samples from natural sources with relatively high bioburden if the objective is just to detect the presence of *E. coli.* Furthermore, the use of HPECF in water analysis provides an easier way to comply

with the PNSDW (DOH 2017), as HPECF satisfies the required minimum volume of 100 mL of water samples for analysis. Also, the reliability, relatively low cost, easy storage, and uncomplicated utilization make Petrifilm™ plates suitable for volunteer-based and educational water quality monitoring applications – particularly when used as a preliminary screening method to identify problem sites (Vail *et al.* 2003).

Another attractive feature of HPECF is the economic savings when it is applied in the industry. The cost amounts to Php 332.80/test for MPN *vs.* Php 172.18/test for HPECF. The calculated savings that can accrue due to the replacement of MPN with HPECF is Php 160.62/test (Table 4). These savings exclude the cost of glassware, space occupied inside the incubator, and electricity. Greater savings can be earned if 3M™ Petrifilm™ EC count plates are purchased in bulk. Considering the amount of water analysis being conducted on a monthly basis in a pharmaceutical company, annual savings can be significant.

Overall, the use of HPECF offers several advantages over the MPN method: a) saves a lot of time since the MPN method is divided into three steps/phases such as the presumptive, confirmatory, and completed tests that must be performed first before confirmed results are obtained; b) minimizes cost since fewer media and glassware are needed; c) saves energy of laboratory technician/microbiologists, enabling them to perform more tasks in a given period of time; d) saves incubator space since Petrifilm™ with filter used is thin and small; and e) lessens waste materials. Similarly, the advantage of HPECF over the DIPEC method is that HPECF makes it possible to test the required 100 mL for standard water sampling. It should be noted that the standard volume tested using the DIPEC is only 1 ml, and this volume is not acceptable for water sampling. Although the results of this study indicate the use of HPECF as a practical and economical alternative to the standard MPN, validation of the proposed protocol can be done using a wider array of water samples from varied sources.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table 4. Cost-benefit analysis comparing the use of the MPN method and HPECF.

Items	Cost comparison	
	MPN method	HPECF
1. Culture media		
a. Lactose broth	Php 12.00 / 100 mL	N/A
b. Brilliant green lactose Broth	Php 19.20 / 60 mL	N/A
c. Eosin methylene blue Agar	Php 22.80 / 120 mL	N/A
2. Materials		
a. 3M™ Petrifilm™	N/A	Php 120.00 / piece
b. 0.45 µm membrane filter	N/A	Php 11.18 / piece
3. Labor for a regular microbiologist		
a. Washing of glassware	45 min	N/A
b. Media preparation	50 min	N/A
c. Sterilization of culture media/materials	30 min	10 min
d. Dispensing of media	15 min	N/A
e. Preparation forDecontamination	15 min	5 min
f. Test proper	45 min	15 min
Total labor cost[a]	Php 278.80	Php 41.00
Total cost	Php 332.80	Php 172.18
Total savings	**Php 160.62**	

[a]The rate for a microbiologist is Php 82/h assuming that the monthly salary is Php 17,000.

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