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Evaluation of Four Membrane Filter Materials for Use with 3M™ Petrifilm™ *E. coli* Coliform Count Plates to Enumerate *Escherichia coli* in Water Samples

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ABSTRACT

Testing was conducted to evaluate the performance of 3M™ Petrifilm™ *E. coli* / Coliform Count [EC] Plates in recovering varying concentrations of *Escherichia coli* from water samples. The membrane filtration method specified by the American Public Health Association [SM 9222B] was adapted to incorporate 3M Petrifilm EC Plates as the growth medium. Four commercially available membrane filter types were assessed. All filters showed statistically similar recoveries at a low level of inoculation. A statistically significant difference ($P<0.05$) was observed for inoculum level in the 100-150 CUF/100 mL range. This demonstrates that although there may be variability amongst filter manufacturers, the 3M Petrifilm EC Plate platform is an adequate culture media for the recovery and enumeration of *E. coli* from water samples.

INTRODUCTION

The American Public Health Association Standard Method 9222B for enumeration of *E. coli* in drinking-water requires filtration of 100 mL water through a membrane filter, followed by plating the filter onto a selective and differential growth medium. 3M Petrifilm EC Plates have global method approvals for use within the food industry, but no method validations for use in drinking or bottled water.

OBJECTIVES

The goals of this project were to:

- Investigate the feasibility of using 3M Petrifilm EC Plates coupled with membrane filters to detect and enumerate *E. coli* in drinking water samples.

- Compare performance of commercially available membrane filters in the above plating application

METHODS

Membrane filters from four manufacturers were evaluated as candidates for use with 3M Petrifilm EC Plates. The following filters (all 0.45 µm pore size and 47 mm in diameter) were utilized for this study:

- Pall GN-6 Metricel Grid
  - Hydrophilic mixed cellulose ester membrane
- Cuno NM04708 BNA045
  - Polyethersulfone membrane
- Whatman Nucleopore
  - Polycarbonate track etch membrane
- Millipore S-PAK Membrane
  - Mixed cellulose

A total of 264 corresponding samples of water were inoculated with *E. coli* ATCC 25922. Sterile phosphate buffered saline (pH 7.0) was utilized as the test water. Half of the samples were inoculated at a "low" concentration of 10 to 50 CFU/100 mL and the other half at a "high" concentration of 100 to 150 CFU/100 mL. Inoculum levels were confirmed via spread plating appropriate dilutions on Tryptic Soy Agar (with incubation under the same conditions as specified in SM 9222B)

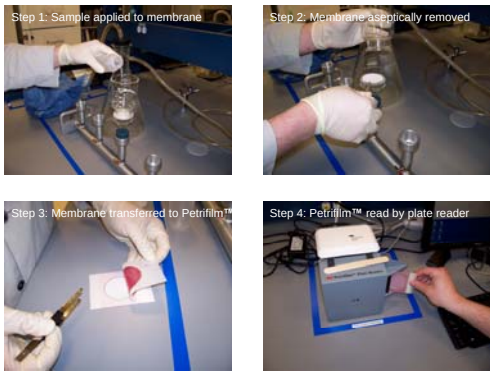

Figure 1. Illustration of the work flow process for coupling membrane filtration with 3M™ Petrifilm™ EC Plates. All plates were incubated at 35 ± 0.5 °C for 24 ± 2 hr.

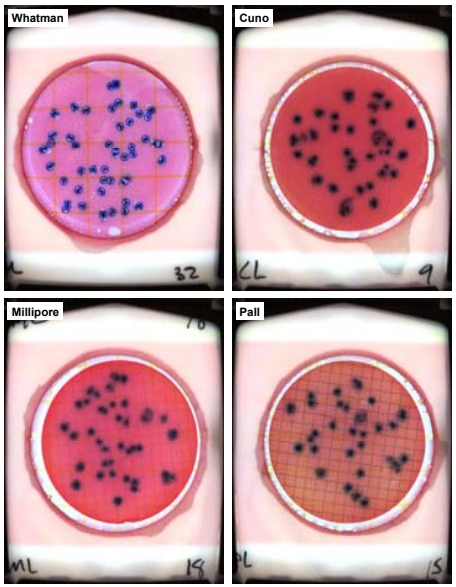

Figure 3. Examples of 3M Petrifilm EC Plates with membrane filters of each of the filter types evaluated. All images were acquired via the 3M™ Petrifilm™ Plate Reader. Difference in media coloration and colonial morphologies can be observed.

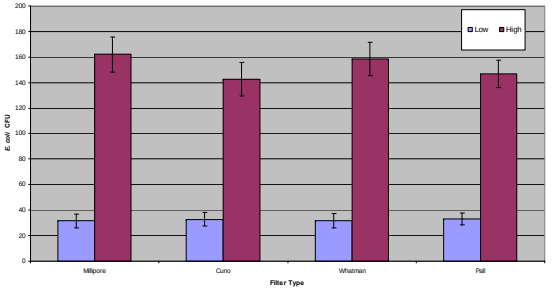

Figure 2. Comparison of *E. coli* recovery using membrane filtration coupled to 3M Petrifilm EC Plates. The target for the low inoculum spike was 10 to 50 CFU/100 mL. The target for the high inoculum spike was 100 to 150 CFU/100 mL. Four commercially available membrane filters were evaluated. All filters possessed a pore size of 0.45 µm.

LOW CONCENTRATION E. COLI COMPARISON

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Millipore	33	1041	31.54545	28.38068
Cuno	33	1083	32.81818	30.46591
Whatman	33	1050	31.81818	31.52841
Pall	33	1097	33.24242	20.93939

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	64.20455	3	21.40152	0.769048	0.513386	2.675385
Within Groups	3562.061	128	27.8286			
Total	3626.265	131				

HIGH CONCENTRATION E. COLI COMPARISON

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Millipore	33	5349	162.0909	190.2102
Cuno	33	4706	142.6061	169.1837
Whatman	33	5231	158.5152	167.7576
Pall	33	4843	146.7576	113.0644

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8548.083	3	2849.361	17.8025	1.02E-09	2.675385
Within Groups	20486.91	128	160.054			
Total	29034.99	131				

Table 1. ANOVA results for filter comparison study. Results from low and high *E. coli* concentration spikes are presented. α = 0.05. A significant difference was only observed at the high concentration, and may be attributed to these targets sitting at the edge of the upper limit of the method.

Filter Comparison	T Critical (one tailed)	T Stat Value	P Value (α = 0.05)
Millipore vs. Whatman	1.669	1.041	0.15
Pall vs. Cuno	1.669	-1.478	0.07
Cuno vs. Whatman	1.669	-5.038	2.05×10^{-6}
Millipore vs. Cuno	1.669	5.787	1.17×10^{-7}
Pall vs. Whatman	1.669	-4.031	7.51×10^{-5}
Millipore vs. Pall	1.669	4.886	3.61×10^{-6}

Table 2. Student T-test results for filter comparison study. Results from the high *E. coli* concentration spikes are presented. α = 0.05.

METHODS

Samples were filtered through each of the four membranes, plated onto hydrated 3M Petrifilm EC plates, incubated and enumerated both manually and using the 3M™ Petrifilm™ Plate Reader (Figure 1). Comparison of the filter recovery was performed using Student *t*-tests and ANOVA (α = 0.05).

RESULTS

The results are presented in Figure 2 and 3 as well as Tables 1 and 2. Analysis of data (both pre and post logarithmic transformation) showed counts between the four membranes were not statistically different for low count samples ($P > 0.05$). When comparing all 4 filter recoveries at the high range, a small, but significant difference was observed ($P = 1.40$ x 10-9). Student *T*-tests revealed that the four filters could be grouped into two statistically similar pairs. Review of images captured by the 3M Petrifilm Plate Reader indicate other factors may potentially affect results: membrane appearance (transparent versus opaque); incubation conditions such as height of plate stacks, location of plate within the stack, and plate moisture loss. The impact of these factors will be investigated in future studies.

SIGNIFICANCE

- The differences observed between filters at the high inoculum spike level may be due to the upper limitations of the method and not necessarily to the filters themselves.
  - Preferred counting range for 3M Petrifilm EC Plates is 15-150 CFU per plate[3]
- These preliminary studies indicate that the 3M Petrifilm EC Plate coupled with membrane filtration shows promise for consideration as an acceptable alternative quantitative *E. coli* method to SM 9222B.

REFERENCES

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