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A COMPARISON OF SEITZ AND MILLIPORE MEMBRANE FILTERS FOR THE ENUMERATION OF FECAL COLIFORMS

By

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SØGAARD, HENRY: *A comparison of Seitz and Millipore membrane filters for the enumeration of fecal coliforms.* Acta vet. scand. 1976, 17, 25—31. — In a comparison of two commonly used membrane filters for enumerating fecal coliform bacteria it was demonstrated that Seitz type M filters recovered statistically more colonies of bacteria than did Millipore HAWG 047S1 filters from pure cultures of *Escherichia coli* incubated at 44°C. The membranes were grown on 0.4 % Teepol agar. On incubation at 37°C no significant discrepancy was found. As a reference method was used pour plating in plate count agar (Difco). It was demonstrated that incubation at 44°C did not per se inhibit propagation of fecal coliforms. Both types of filters examined were sterilized by the manufacturers with ethylene oxide. The discrepancy found can therefore not be due to sterilization procedures.

membrane filtration; fecal coliforms.

Detection of *Escherichia coli* is the method most commonly applied for the demonstration of fecal pollution of water samples. The bacteriological procedures usually include a quantitative determination of such organisms. According to the official Danish standard methods for examination of drinking water, which were published in 1974 (*Vandundersøgelse*), fecal coliform bacteria may be enumerated by two different methods: A most probable number procedure using MacConkey broth or Gray's glutamic acid medium, or by membrane filtration. After filtration, the membranes are transferred to Teepol agar or to filter paper pads soaked with Teepol broth.

The standard methods do not specify which commercial brand or type of membrane filters should be used. *Presswood &*

Brown (1973) demonstrated that one commercial brand of filters recovered statistically more colonies of bacteria from pure cultures of *E. coli* than did another one. This discrepancy could be demonstrated at either 35°C or 44.5°C.

In our laboratory we therefore decided to make a comparative study of the two commercial brands of membrane filters which we use. The study was designed to produce a number of data sufficient for a statistical analysis. Pure cultures of *E. coli* strains were studied and the filters were incubated at 37°C and 44°C parallelly. As a method of reference was used pour plating.

It should be emphasized that other commercial brands of membrane filters are available in the market. The results of the present study, therefore, do not indicate which brand is the one of choice.

MATERIALS AND METHODS

Bacterial strains

A total of 20 strains of *Escherichia coli* were included in the study. They were all isolated from drinking water samples. The identity of the strains were established according to typical reactions in the following tests: growth in semi-solid agar, Voges-Proskauer reaction, indole production, fermentation of glucose and malonate, gelatin liquefaction, H₂S-production. Media used for these tests were as described by *Lautrop* (1956). Furthermore, determination of lysin and ornithine decarboxylase was done according to *Møller* (1955).

Membrane filters

The following two commercial brands of 0.45 µm porosity membrane filters were compared: Millipore, HAWG 047S1 (Millipore Corporation, Bedford, Massachusetts 01730, USA) and Seitz type M, catalogue no. 0045 1 047 4211 (Seitz-Asbest-Werke, Theo u. Geo Seitz, Bad Kreuznach, Germany). Both brands of filters are made from mixed esters of cellulose and they are sterilized by the manufacturers with ethylene oxide. The same lot of the two filters were used throughout the study.

Preparation of bacterial suspensions

The strains were grown overnight in nutrient broth (Difco) at 37°C. In the following morning fresh broth was inoculated

from these cultures. The cell density was followed in a spectrophotometer (Spectronic 20) at 450 nm. Incubation was interrupted at O.D. 1.0 which corresponded to an approximate cell concentration of 10^8 per ml. From the cultures, ten-fold dilutions were made in quarter strength Ringer solution. Two l of 10^{-8} dilution were made by adding 20 ml of 10^{-6} dilution to 1980 ml of Ringer solution. The final suspension thus contained a concentration of cells within a workable range for membrane filtration, i. e. approx. 10^2 per 100 ml.

Procedures

From each of the cell suspensions 20 replicate cultures were prepared on Millipore filters, and 20 on Seitz filters. Fifty ml were filtered through each filter by use of a Millipore filtering apparatus. The filters were transferred to Teepol agar plates. Of the replicate samples half were incubated at 37°C and half at 44°C for 24 hrs. After incubation the number of colonies were counted under a stereo-microscope at a $7\times$ magnification.

For pour plates, six replicate samples were made in plate count agar (Difco). Of these, three were incubated overnight at 37°C and three at 44°C . Number of colonies were counted in a Quebec colony counter.

RESULTS

A mean value was calculated from all lots of replicate samples. These data are presented in Table 1. Results from pour plating represent the number of colony forming units per ml of the 10^{-6} dilutions whereas figures from membrane filters are derived from 50 ml of 10^{-8} dilutions.

In paired experiments, the differences of two partners receiving two kinds of treatment can be assumed to be normally distributed. The differences can therefore be applied to a t-test by using the following equation:

$$t = \frac{\bar{d}}{s_d/\sqrt{n}},$$

where \bar{d} is the mean of the differences between paired lots of replicate samples, s_d is the standard deviation, and n is the number of paired experiments.

Table 1. Mean counts of 10 replicates on membrane filters and three replicates by plate counts of 20 strains of *Escherichia coli*.

E. coli strain no.	37°C			44°C		
	Plate count	Milli-pore	Seitz	Plate count	Milli-pore	Seitz
1	176	60	70	132	67	82
2	393	134	110	352	87	131
3	372	139	154	358	148	169
4	293	93	101	222	100	108
5	325	179	179	282	168	190
6	280	137	132	264	119	148
7	295	139	136	284	146	154
8	181	47	45	182	53	47
9	133	38	40	130	41	40
10	255	92	115	271	100	102
11	244	86	97	251	91	107
12	198	94	111	205	93	116
13	216	68	91	222	70	87
14	165	68	76	169	64	77
15	342	118	137	345	114	135
16	324	151	164	317	148	163
17	240	74	71	235	77	76
18	277	118	110	261	115	112
19	292	135	133	277	135	134
20	249	100	95	263	86	94
Average	263	104	108	251	101	114

In Table 2 a summary is given of comparisons between the various methods which were tested. Since figures from membrane filtrations and pour plates are based on different dilutions, no direct comparison has been made between these procedures. The results given in Table 1, however, indicate that the number of colony forming units seems to be somewhat less when determined by the membrane filtration procedures. Pour plates were included in the study in order to assess whether an incubation temperature of 44°C per se had a detrimental effect on the growth capacity of *E. coli*. According to Table 2, this apparently is not the case. On a selective medium like Teepol agar, 44°C does not inhibit *E. coli* cells in the logarithmic growth phase to a significant degree. This can be seen by comparing results for identical filters at two temperatures. Likewise, no discrepancy

Table 2. Summary of statistical analyses of the differences between methods for enumeration of fecal coliforms.

	t-value
Plate count 37°C vs Plate count 44°C	2.20
Millipore 37°C vs Millipore 44°C	0.91
Seitz 37°C vs Seitz 44°C	2.73
Millipore 37°C vs Seitz 37°C	2.01
Millipore 44°C vs Seitz 44°C	4.47

was demonstrable when comparing the efficiency of Millipore and Seitz filters at 37°C. A statistically highly significant discrepancy, however, was in evidence when the two brands of filters were compared at 44°C ($t = 4.47$, $P < 0.001$).

DISCUSSION

Membrane filtration as a method for enumeration of coliforms and fecal coliform in drinking water offers several advantages when compared to MPN-methods. Bacteria can be concentrated from larger samples. It is less labor consuming, which increases the capacity of a routine laboratory. Furthermore, results are available in a shorter time. The main objection which can be raised against membrane filtration is that gas production is not detectable. This may necessitate confirmatory steps.

Whereas MPN-methods are well established in Danish laboratories, little experience is available as regards membrane filtration. In our laboratory we have therefore initiated preliminary work in order to gain some experience. The present study is part of this work. The data presented, clearly indicate that two commercial brands may differ in their capacity to recover fecal coliforms. In a similar investigation, *Presswood & Brown* (1973) arrived at the same conclusion. They compared Gelman and Millipore membrane filters and found that Gelman GN-6 filters recovered statistically more colonies from pure cultures of *E. coli* than did Millipore HAWG 047SO filters. This difference was demonstrated at either 35°C or 44.5°C. Comparative examinations of river water for fecal coliforms gave results comparable to those obtained for pure cultures. *Schaeffer et al.* (1974) also examined natural samples and found no significant differences in the efficiency of the recovery of fecal coliforms on Gelman

or Millipore filters. However, higher counts of total coliforms were obtained with Gelman filters.

Presswood & Brown suggested that different methods of sterilization of the filters could be the reason for the difference in the extent of effects on the growth of bacteria.

Dutka et al. (1974) compared autoclave- and ethylene oxide-sterilized membrane filters manufactured by Gelman, Millipore, and Sartorius. Their studies indicated that the various brands differed as regards recovery of total coliforms, fecal coliforms, fecal streptococci, and heterotrophs. In general, ethylene oxide-sterilized filters were less efficient than those sterilized by autoclave. *Sladek et al.* (1975) demonstrated that the morphological structure of the filter material was a critical factor. The surface opening diameter seems to be a primary determinant of fecal coliform growth on membrane filters.

The present study supports the view that the method of sterilization is not the crucial factor determining differences between filters of different origins, since the two brands which have been compared were both sterilized by ethylene oxide. The study has also confirmed that selecting membrane filters from a variety of brands which are commercially available should be done with some criticism.

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SAMMENDRAG

En sammenligning af Seitz og Millipore membranfiltre til påvisning af fækale kolibakterier.

To almindeligt anvendte membranfiltre til bestemmelse af fækale kolibakterier i vand blev sammenlignet. Det er påvist, at Seitz type M filtre gav vækst af et statistisk sikkert højere antal bakteriekolonier fra renkulturer af *Escherichia coli* ved 44°C. Membranfiltrene blev dyrket på 0,4 % Teepol agar. Ved 37°C kunne ikke påvises statistisk signifikante forskelle. Som referencemetode er anvendt kimtælling i plate count agar (Difco). Det kunne vises, at en dyrkningstemperatur på 44°C ikke i sig selv hæmmede væksten af *E. coli*. Begge typer af filtre er steriliseret af fremstillerne med ethylenoxid. Den fundne forskel kan derfor ikke tilskrives steriliseringsprocedurer.

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