APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1979, p. 431-435
0099-2240/79/09-0431/05$02.00/0
Vol. 38, No. 3

Improved Detection of Coliforms and *Escherichia coli* in Foods by a Membrane Filter Method

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Received for publication 25 June 1979

Analytical procedures based on filtration of homogenates through membrane filters, and particularly hydrophobic grid-membrane filters (HGMF), offer definite improvements in the enumeration of *Escherichia coli* and coliforms in foods. Whereas the counted specimen in pour plates may not usually be greater than 0.1 g, up to 1.0 g of ground beef, green beans, potato, cod, strawberries, or grapes could be filtered and counted on HGMF. Greatly improved limit of detection, reduced interference by noncoliforms, and complete removal of growth inhibitors such as polyphenols were demonstrated for HGMF, using violet red bile and mFC agars. In addition, counting on HGMF eliminated a false-positive reaction caused by sucrose in ice cream.

The theoretical limit of detection of a microbiological plate count procedure is determined by the maximum quantity of food plated. For conventional 90-mm petri dishes this is generally 0.1 g (e.g., 1.0 ml of a 10% homogenate). In practice, at least four problems may occur when organisms are present in very low concentrations.

(i) Greater weights of food must be used. Larger quantities of media must then also be used to maintain adequate dispersal. In plate counts this is often impractical, with the result that analyses tend to degenerate to presence/absence tests.

(ii) High concentrations of other species may prevent observation of the species of interest. For example, deaminating organisms growing in close proximity to *Escherichia coli* may interfere with development of acid-dependent color cues.

(iii) Inhibitory substances in the food, such as artificial preservatives or polyphenols, may affect multiplication if they are not diluted out sufficiently.

(iv) Insufficient dilution of other interfering substances may cause false-positive growth reactions. For example, sucrose carried into petri dishes from ice cream may cause sucrose-fermenting organisms to be mistaken for coliforms.

Membrane filter (MF) techniques are potentially attractive in this difficult area of analysis. Sharpe et al. (14), for example, recently demonstrated that suspensions containing at least 0.1 g of most foods could be easily filtered through conventional 47-mm-diameter, 0.45-μm-pore size MF and that filtration of much larger quantities of many foods was feasible. The MF may, therefore, directly improve theoretical limits of detection by permitting examination of larger quantities of food. It may also eliminate problems (iii) and (iv) by allowing soluble interfering materials to be completely removed.

A relatively new development, the hydrophobic grid-membrane filter (HGMF), offers two further advantages. It has a greatly improved numerical operating range (9, 11-13) compared with normal MF, pour plates, or surface-inoculated plates. The upper counting limit for the HGMF described in this paper (Fig. 1), for example, is around 15,000 growth units. In normal counting procedures this can minimize both labor needed for diluting and the probability of losing data through misjudging platable dilution ranges. At the limit of detection, however, by isolating colonies from one another far more effectively than MF or plates (Fig. 2), the HGMF should reduce interference with development and recognition of the species of interest.

In principle, therefore, MF (and particularly HGMF) appeared capable of solving the most vexing problems in enumerating low levels of particular species in foods. Their practical performance in detecting coliforms or *E. coli* in foods is reported here.

MATERIALS AND METHODS

HGMF. A cross-country ski wax, Swix Special Green (Astra Wallco, Oslo, Norway; GDL Ltd., Montreal, Que., Canada; Norfell Inc., Chelmsford, Mass., or most North American sports stores) had been found

superior to a dental sticky wax previously used for printing HGMF (data not shown). A 1984 grid-cell HGMF, printed on standard membrane filters (0.45-μm pore size, HAWP 0047; Millipore Corp.) with this wax, was used throughout the study. Repeated quality checks demonstrated the absence of contamination, and HGMF were not sterilized after printing.

Foods. Regular ground beef, green seedless grapes, an economy ice cream, and frozen cod, green beans, potatoes, and strawberries were purchased locally. In early experiments, foods were inoculated with a very dilute suspension of *E. coli* just before blending. In other experiments only natural contamination was analyzed.

Microbial counts. Foods (10.0 g) were stomached (9) for 30 s in 90 ml of sterile 0.1% peptone solution, using a Colworth Stomacher 400 (Canadian Laboratory Supplies Ltd.). Samples for counting were pipetted by using 1- or 10-ml bacteriological pipettes with disposable tips specially made in our laboratory. These tips were capped with Spectramesh polyethylene mesh, 105-μm pore size (Cole-Parmer Instrument Co., Chicago, Ill.), which removed coarse debris. A preliminary study showed that bacterial recovery was not affected (manuscript in preparation). For HGMF, aliquots (1.0 and 10.0 ml) were added to 50 ml of sterile peptone solution in the filtration funnel and mixed thoroughly by using the pipette, before filtration. HGMF were laid on the surface of petri dishes containing approximately 20 ml of violet red bile (VRB) or mFC agar (Difco). For pour plates, 1.0-ml aliquots were mixed with 15 ml of the melted, tempered (45°C) agars. VRB plates were overlaid with 5 ml of agar. No further dilutions were made. Plates were incubated for 20 h at 35°C for VRB and at 44.5°C for mFC agar before counting. HGMF counts were converted to most probable numbers of growth units as described earlier (13).

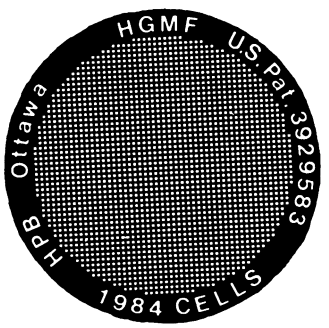

FIG. 1. *Ink facsimile of the 47-mm-diameter 1984 grid-cell HGMF used in this study. Central blocks contain 10 × 50 grid-cells. Wax occludes 48% of the area; filtration rate is approximately 52% of the corresponding MF.*

Filtration. The filtration apparatus was equipped with polished, cylindrical, graduated Plexiglas funnels, which allowed 50 ml of peptone dispersant to be easily dispensed. The apparatus was already available and was merely convenient; any standard commercial unit would have served. Filtration was carried out by using a water pump and a pressure differential of about 95 kPa, but without rinsing the cylinder down onto the HGMF. Between filtrations cylinders were scrubbed, rinsed in deionized water, and flooded with intense ultraviolet light for 2 min. In control experiments using a ground beef suspension containing 10^9 cells of *E. coli* per ml, the cylinders yielded no colonies after irradiation.

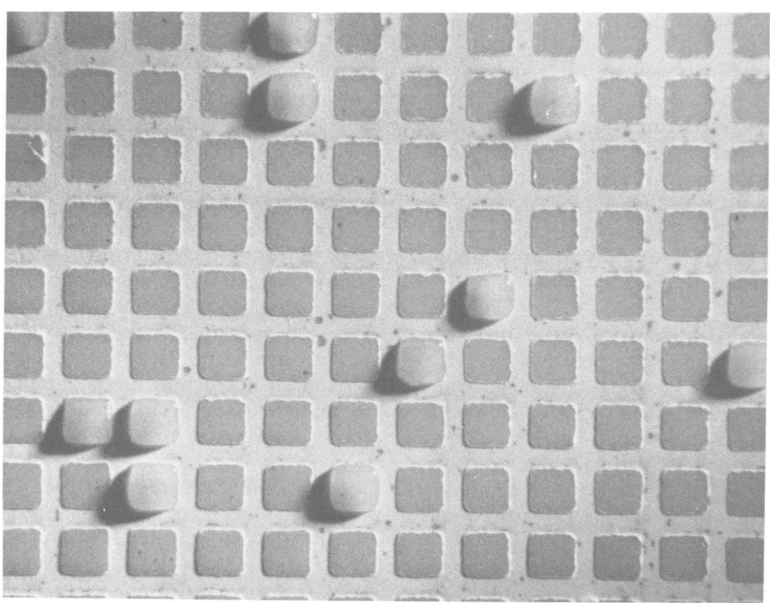

FIG. 2. *E. coli colonies on a 1984 grid-cell HGMF, photographed in oblique light.*

RESULTS

"Normal" recoveries by HGMF and pour plates for 0.1 g of food. Table 1 compares recoveries by HGMF and pour plates where the minimum count was 70 colonies, i.e., at analytical levels well above the limit of detection. To reach this level it was necessary to artificially inoculate most of the samples. Duplicate counts from individual suspensions were generally very close; however, large variations in apparent recovery efficiency were noted. For four uninoculated ground beef samples, counts of red colonies on HGMF were considerably higher than on pour plates. However, three other samples which had been inoculated with *E. coli* also yielded many very small colonies which turned red in VRB pour plates but remained colorless on HGMF. The ratio of HGMF and pour plate counts for these samples is low because of this. The larger colonies reacted as *E. coli* by the IMViC (indole, methyl red, Voges-Proskauer, citrate) test, whereas these small ones did not. Their failure to colorize on HGMF probably reflects insufficient stain in the VRB medium; appreciable quantities are adsorbed by the HGMF itself.

Recoveries by HGMF and pour plates at the limit of detection. A summary of coliform and *E. coli* counts for foods naturally contaminated at very low levels is shown in Table 2. Out of 15 samples, at the 0.1-g level, for example, a total of only eight colonies were obtained on mFC pour plates, compared with 34 on HGMF. On the same medium at the 1.0-g level, however, HGMF yielded a total of 169 colonies—one filter containing 17 colonies and three containing more than 20. For some of the samples, considerable numbers of noncoliforms were also observed on both pour plates and HGMF.

TABLE 1. *Comparison of recovery by HGMF and pour plates at levels well above the limit of detection*

Food	Medium	No. of samples[a]	Recovery by HGMF/PP[b]	
			$\bar{x}$	σ
Ground beef	VRB	3	0.74	0.35
	mFC	3	0.88	0.50
	VRB[c]	4	2.04	1.16
Cod	VRB	4	1.06	0.44
	mFC	4	1.51	1.11
Green beans	VRB	6	1.01	0.31
	mFC	6	1.18	0.49
Potatoes	VRB	5	0.74	0.33
	mFC	5	0.63	0.37
Ice cream	VRB	2	0.98	0.20
	mFC	2	1.14	0.22

[a] Duplicate platings from each suspension.

[b] Values of HGMF/pour plates (PP) were calculated individually from means of duplicate platings for each sample. Means and standard deviations of these ratios were then calculated.

[c] All samples except these were artificially inoculated.

TABLE 2. *Comparison of recoveries by HGMF and pour plate for foods containing low levels of organisms (ground beef, green beans, cod, potato, and ice cream; total of 15 samples)*

Recovery on:	No. of samples showing growth	Total colonies from all samples
VRB[a]		
PP, 0.1 g[b]	1	5
HGMF		
0.1 g[b]	2	5
1.0 g[c]	2	20
mFC		
PP, 0.1 g[b]	3	8
HGMF		
0.1 g[b]	4	34
1.0 g[c]	4	169

[a] Not included are four ground beef specimens so highly contaminated (>150,000/g) with coliforms that plates and HGMF were saturated.

[b] PP, Pour plate. Duplicate platings from each specimen.

[c] Single platings.

Recoveries by HGMF and pour plates for foods containing growth inhibitors. *E. coli* inoculated onto strawberries and grapes failed to grow in pour plates prepared from 0.1 g of food three times, and only very poorly three other times, out of eight experiments. Good growth was observed each time on HGMF; moreover, growth on HGMF was not greatly affected by taking up to 1.0 g of the fruits. The data are shown completely in Table 3 for greater clarity.

False-positive coliform reactions from ice cream. A sucrose-fermenting *Streptococcus* sp. and a *Proteus* sp., both reliably yielding red colonies in pour plates prepared from 0.1 g of ice cream, were isolated. Ice cream inoculated with a mixture of these organisms and *E. coli* yielded both white and red colonies on HGMF but all red colonies on pour plates.

DISCUSSION

Higher overall counts on HGMF compared to pour plates from equivalent quantities of food when coliforms or *E. coli* were few and, in particular, when other species were numerous support the hypothesis that better isolation between centers of growth in HGMF may reduce interference with the development of acid-dependent color cues for these organisms. That the im-

TABLE 3. *Counts of E. coli on HGMF and pour plates from inoculated strawberries and grapes*

Food	No. of colonies[a]					
	VRB			mFC		
	PP, 0.1 g	HGMF		PP, 0.1 g	HGMF	
		0.1 g	1.0 g		0.1 g	1.0 g
Strawberries						
1	0	104	—[b]	0	92	—
2	0	34	—	0	8	—
3	4	255	—	1	142	—
4	2	41	154[c]	8	43	148[c]
5	97	329	—	223	237	—
6	168	314	—	147	277	—
Grapes						
1	0	18	—	0	25	—
2	2	22	205[c]	2	34	230[c]

[a] Mean of duplicates except where indicated. PP, Pour plate.
[b] —, Not done.
[c] Single platings.

provement was most noticeable with the mFC agar is reasonable, in view of its development for MF work.

The ability of HGMF to handle 1.0-g quantities of these foods, rather than 0.1 g, even when considerable numbers of other organisms were present, is reflected excellently by the relative counts. It would appear that use of HGMF or MF to filter 1.0 g of food will often permit these organisms to be enumerated, rather than simply listed as present or absent. The overall ratio of counts at the two levels was not exactly 10. Whereas this may reflect some interference by the other flora, it may also be due simply to the low statistical significance of counts at the lower level.

Both grapes and strawberries contain polyphenols or other growth inhibitors (5, 6, 15). In the pour plate method these substances remain in the growth medium, diluted 10 or 15 times at most from the homogenate. In the HGMF method described here, an initial 50-fold dilution occurred in the dispersant before filtration. Approximately 0.19 ml of liquid remained in filters after filtration. Solutes in this can be expected to have been diluted a further 50-fold by diffusion into the agar during incubation, giving an overall 2,500-fold dilution of any inhibitor. Further dilution could obviously be obtained by rinsing the filter before plating. Busta and Speck (4) have already described the use of an MF technique to eliminate inhibitors present in milk. It was felt that the results obtained here are an adequate demonstration of the ability of HGMF- or MF-based procedures to remove such inhibitors from other foods.

The possibility of false-positive coliform reactions in pour plates prepared from ice cream has been noted for many years (2, 3, 8). *Standard Methods for the Examination of Dairy Products* (7), for example, recommends that "because of the presence of sucrose in ice cream, coliform-like colonies on VRB agar should be confirmed." The need for confirmation may delay the analytical result by at least 24 h. In 1959, Nutting et al. (8) used MF to eliminate sucrose, diluting samples in warm Triton X-100 surfactant to expedite filtration. The result reported here confirms that the massive dilution of sucrose effected by membrane filtration is quite sufficient to eliminate false-positives. The HGMF procedure can thus reduce the time and labor needed for microbiological analysis of ice creams.

Overall, therefore, counting techniques using filtration of foods through HGMF or MF have considerable attractions in the difficult area close to the limit of detection of coliforms or *E. coli*. The limit of countability may be improved, and interference by other organisms or materials may be minimized. This investigation seems rather timely, in view of increasing interest in rapid identification of *E. coli* biotype 1, by an MF procedure (1) which has recently been subjected to collaborative study under the auspices of the International Committee for Microbiological Specifications of Foods (M. K. Rayman et al., manuscript in preparation). The method, as described, calls for no more than 1.0 ml of a 10 or 20% homogenate (i.e., no more than 0.2 g of food) to be spread over an 85-mm-diameter MF before plating. This is the maximum quantity of liquid the MF can be expected to absorb. The pertinence of the work described here and demonstration of the feasibility of actual filtration of food suspensions through MF (14) need little further comment.

Figure 3 shows *E. coli* biotype 1 colonies fil-

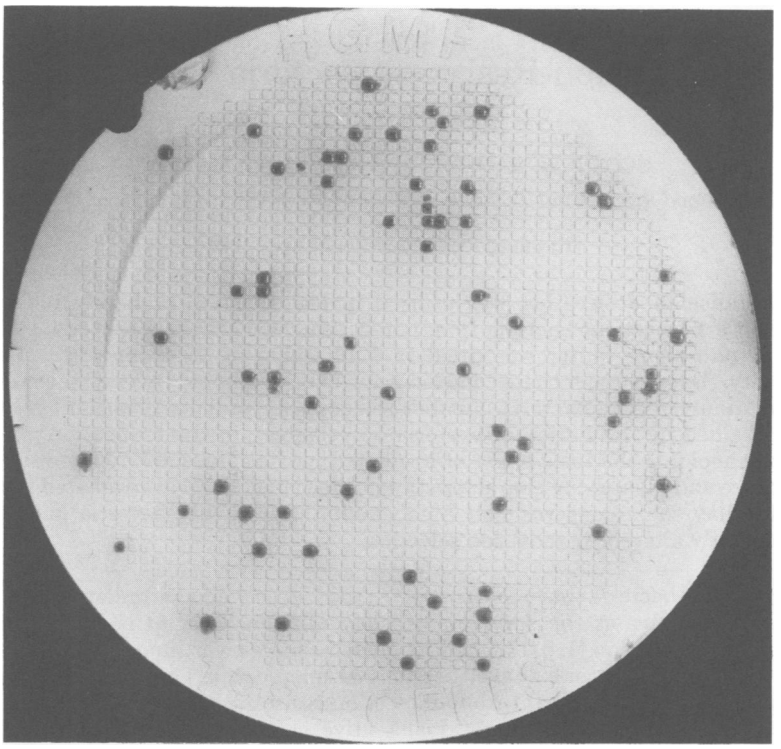

FIG. 3. *E. coli biotype 1 colonies on a 1984 grid-cell HGMF, stained for indole by the Anderson-Baird-Parker procedure (1).*

tered onto a 1984 grid-cell HGMF and stained for indole by using the Anderson-Baird-Parker procedure (1). Whereas some staining may occur in grid-cells adjacent to *E. coli* colonies, no particular problems are evident in either the use of HGMF or filtration for enumeration of this organism.

ACKNOWLEDGMENTS

We are very grateful to A. Hurst for bringing the matter of sucrose fermenters in ice cream to our attention, and to I. Dudas for construction of the filtration apparatus.

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