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New Screening Test To Determine the Acceptability of 0.45-μm Membrane Filters for Analysis of Water

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During routine membrane filter (MF) quality control testing, irregularities such as partial or complete inhibition of microbial growth at grid lines, abnormal spreading of colonies, growth in or along the grid lines, nonwetting areas, poor colony sheen and metallic sheen on the MF surface with mEndo agar, brittleness, decreased recovery, and severe wrinkling were observed with several lots of filters. To study these effects and to develop a more sensitive screening test for MF quality, we compared five different MFs with various types and degrees of defects by using five stock coliform cultures and five different media. Results showed that the *Enterobacter aerogenes*-tryptic soy agar test system detected more MF defects than any other combination did and was superior to the *Escherichia coli*-mFC agar American Society for Testing and Materials method for grid line inhibition. Filtered natural samples grown on the same media showed the same effects as were observed with the pure cultures. Poor colony sheen and sheen on the MF surface were best detected with *Enterobacter aerogenes* on mEndo agar. The use of tryptic soy agar and mEndo agar with this organism permitted the maximum detection of MF irregularities. Of the 142 MF lots tested by this method, 30% were acceptable, 10% were marginally acceptable, and 61% were unacceptable. This method provides a valuable screening test for determining the acceptability of 0.45-μm-pore-size MFs used for coliform and heterotroph analysis and may also be useful in conjunction with other methods.

The accurate recovery of bacteria from water samples by using the membrane filter (MF) method depends upon the quality of the filters used. Filters should provide easily countable colonies and be free of defects such as (i) toxic, inhibitory, or "bleeding" ink; (ii) chemicals, excess surface-wetting agents, or other extractables; (iii) stamping or pressure effects; (iv) irregular pore size and distribution; (v) hydrophobic or nonwetting areas; (vi) brittleness; (vii) warping; (viii) uneven flow rates; and (ix) poor retention of the desired microorganisms (15). Variations in filters may occur because of differences in manufacturing procedures, materials, storage conditions, and degree of quality control (3, 14, 15). A decline in the quality of the filters may have an adverse effect on counting, recovery, and bacterial colony morphology.

Routine comparisons of MF lots for bacterial recovery revealed irregularities in colony morphology of stock cultures with some lots of filters, but not with the reference lot. Types of defects seen were partial or complete inhibition of colony development at the grid lines, abnormal spreading of colonies, nonwetting areas, growth of the bacteria along the grid lines, poor or absent colony sheen and the formation of a metallic sheen on the surface of the filter on mEndo agar, brittleness, decreased recovery, and severe wrinkling, as well as various combinations of the above. This study was initiated to investigate these defects, to develop a sensitive screening test for determining the acceptability of MFs for our laboratory, and to determine the incidence of defects in filters from different manufacturers.

(A preliminary report of this research has been presented [K. P. Brenner, J. R. Menkedick, C. C. Rankin, and R. H. Bordner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q-89, p. 296].)

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MATERIALS AND METHODS

Bacterial cultures. Five bacterial cultures were used in the test development study. The four U.S. Environmental Protection Agency bacterial strains, *Enterobacter aerogenes* EPA 202, *Klebsiella pneumoniae* EPA 207, *Citrobacter freundii*, and *Enterobacter cloacae*, were isolated from natural water samples and maintained as lyophilized cultures at −20°C. *Escherichia coli* ATCC 11229 was obtained from the American Type Culture Collection, Rockville, Md. The five organisms were characterized and identified by using API-20E strips (Analytab Products, Plainview, N.Y.), and working cultures were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) (TSA) slants and stabs. Other cultures used to study MF defects associated with other MF methods (1, 3, 4, 9, 19–21, 32) were two U.S. Environmental Protection Agency bacterial strains, *Streptococcus faecalis* EPA 205 and *E. coli* EPA 206, and four American Type Culture Collection cultures, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Proteus vulgaris* ATCC 13315, grown from Difco Bactrol disks.

Media. All prepared media were obtained from Difco, except as noted. Plates (9 by 50 mm) containing 5 ml of each of the following media were used in the coliform comparison study: mEndo agar (1) (made from mEndo broth with 15 g of Bacto-Agar [Difco] added per liter), TSA, mFC agar (1), mHPC agar (1), and R2A agar (1). The mHPC and R2A agars were prepared by the cited methods early in the test development studies but were later obtained as prepared media.

Other media used, along with those described above, for the comparison study of MF defects exhibited by other microorganisms, were mTEC agar (9, 32), mT7 agar (20, 21), mHAR and mHARC agar (J. R. Haines and C. C. Rankin, personal communication), KF streptococcal agar (Difco and BBL Microbiology Systems, Cockeysville, Md.) (1), mE agar (32), XLD agar, Vogel-Johnson (VJ) agar (19), and mPA-C agar (4). All of these media, except the XLD agar,

TABLE 1. Number of 0.45-μm MF lots examined by the pure-culture screening test

Manufacturer	Total no. of MF lots tested
AMF Cuno, Meriden, Conn.	1
Amicon, Lexington, Mass.	4
Domnick Hunter, Durham, U.K.	4
Gelman Sciences, Ann Arbor, Mich.	37
Micro Filtration Systems (MFS), Dublin, Calif.	5
Millipore Corp. Bedford, Mass.	59
Micron Separations, Inc. (MSI), Honeoye Falls, N.Y.	6
Nalge Company, Rochester, N.Y.	2
Nuclepore Corp. Pleasanton, Calif.	8
Oxoid Ltd., London, U.K.	2
Schleicher & Schuell, Inc., Keene, N.H.	5
Sartorius, Hayward, Calif.	3
Whatman, Inc., Clifton, N.J.	6

which was used with *Salmonella typhimurium*, were used with other established MF methods (1, 3, 4, 9, 19–21, 32) for specific microorganisms or groups of organisms, i.e., *E. coli* (9, 20, 21, 32), fecal streptococci (1, 3), enterococci (32), staphylococci (19), and *P. aeruginosa* (4).

Membrane filters. We used 142 lots of membrane filters (diameter, 47 mm; pore size, 0.45 μm) from 13 different manufacturers (Table 1). Most of the filters were gridded and composed of mixed esters of cellulose, although some were cellulose nitrate (one lot from Nalge Co., Rochester, N.Y.; all lots from Schleicher & Schuell, Inc., Keene, N.H.; Sartorius Corp., Hayward, Calif.; and Whatman, Inc., Clifton, N.J.). A few were cellulose acetate (one lot from Nalge; two lots from Oxoid, London, England), polycarbonate (six lots from Nuclepore Corp., Pleasanton, Calif.), or polysulfone (three lots from Gelman Sciences, Inc., Ann Arbor, Mich.). One lot of mixed esters of cellulose (pore size, 0.7 μm; type HCWG; Millipore Corp., Bedford, Mass.) was also used in these studies because it exhibited a variety of different defects when tested with the *Enterobacter aerogenes* culture.

Four different reference lots (Millipore) were used in the course of these studies. No filters showed serious colony or other MF defects, and each lot averaged ≥88.5% of the heterotrophic plate count (1) recovery when tested with the pure culture of *Enterobacter aerogenes*.

Bacterial assay procedure used for the coliform comparison study. The five cultures used for the test development procedures were each inoculated into 10-ml tryptic soy broth (TSB) tubes, which were subsequently incubated for 24 to 48 h at 35°C. The cultures were transferred into fresh TSB the day before the experiment and incubated for 20 to 24 h at 35°C. Tenfold dilutions were made in phosphate-buffered dilution water (1, 3), and three 1-ml volumes of each of four dilutions (10^{-5} to 10^{-8} for *E. coli*; 10^{-6} to 10^{-9} for the other organisms) were filtered through membranes of each of the five lots. The MF funnels and bases used for the filtrations were autoclaved daily and placed in UV germicidal light cabinets between filtrations. The filters were placed on the 5-ml agar base plates. The procedure was repeated with each different type of agar and each different organism. All plates were incubated for 24 h at 35°C, except for the mFC plates, which were heat sealed in plastic bags and incubated for 24 h in a 44.5°C water bath. The colonies were counted, and the filters were observed for ink concentration and distribution, colony irregularities, and other MF defects.

Natural sample comparison study. Tenfold dilutions of two natural water samples were filtered in triplicate through a reference lot, Oxoid Nuflow 0.45-μm filters, and Millipore HCWG 0.7-μm filters. The water samples were obtained from Tylersville artesian well, located north of Cincinnati, Ohio, and Burnet Woods Lake in Cincinnati, Ohio. The filters were placed on base plates of the same five media used for the coliform comparison study to see whether defects visible with the pure cultures also occurred with environmental samples. All plates were incubated, and the filters were observed for irregularities as previously described.

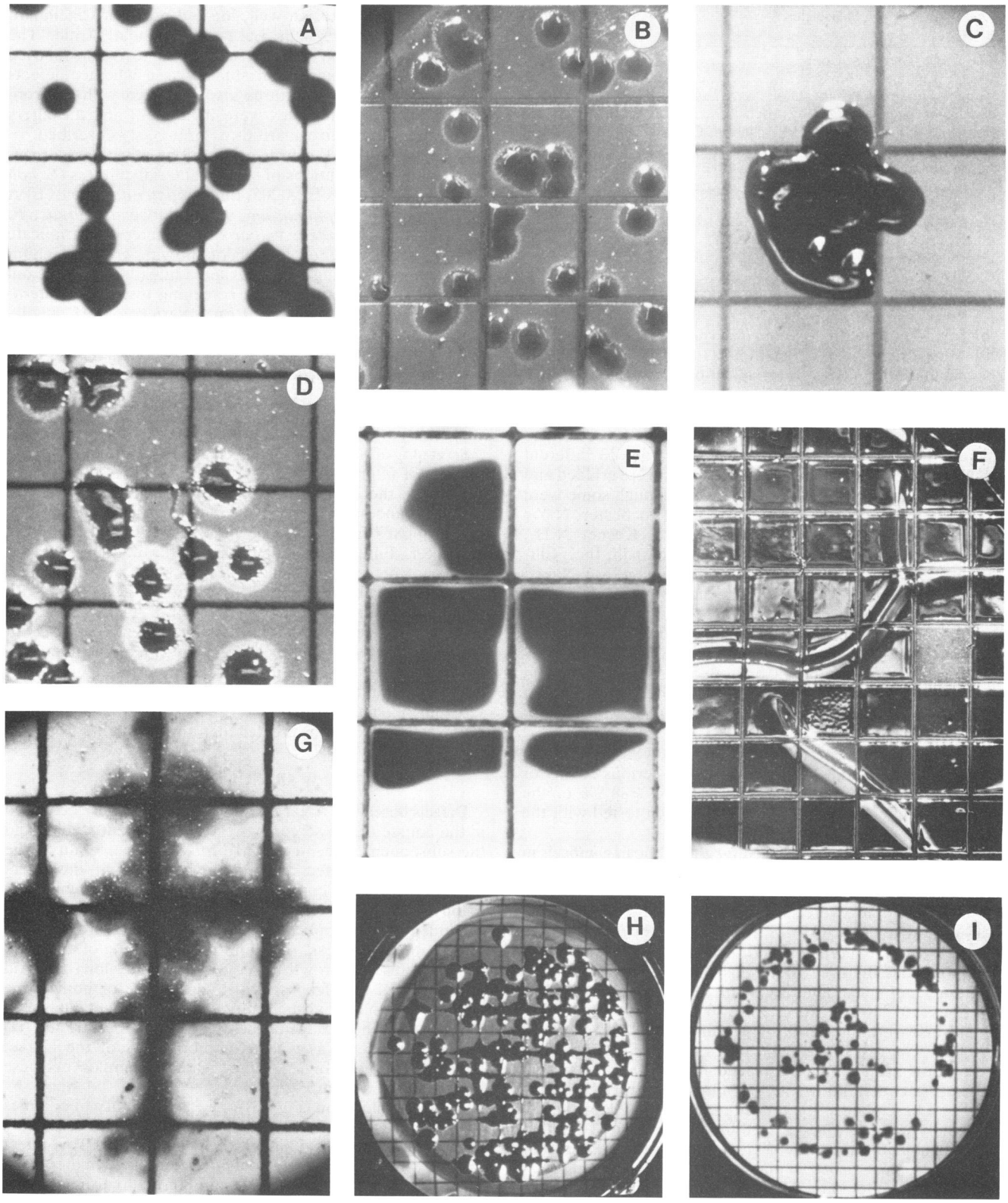
Comparison of MF defects produced by other microorganisms and media. Dilutions of seven bacterial cultures (*E. coli* EPA 206, *S. faecalis* EPA 205, *Enterobacter aerogenes* EPA 202, *Salmonella typhimurium*, *Staphylococcus aureus*, *P. aeruginosa*, and *Proteus vulgaris*) were filtered in triplicate through a reference lot, a filter lot showing grid line inhibition, and one showing abnormal spreading (determined from the results of the pure culture screening test using *Enterobacter aerogenes*) and placed on a variety of MF media. Three nonselective media, TSA, mHPC, and R2A, were used with all cultures, whereas the selective media were used only with specific cultures. All organisms grown on the selective media were incubated at their recommended temperatures (3, 4, 9, 19–21, 32), except for *Enterobacter aerogenes*, which was always incubated at 35°C.

Screening of MF lots from several manufacturers. A total of 142 lots of filters from 13 manufacturers were screened for defects by the bacterial assay procedure described above with TSA and mEndo agar base plates. Whenever possible, several boxes from the same lot were tested, using every fifth filter. Filters were rated acceptable if there were no significant defects, marginally acceptable if a slight defect was present, or unacceptable if obvious (i.e., those with numerous colonies affected), severe, or multiple defects occurred. A reference lot was tested along with each MF lot or group of MF lots screened. Filter lots were considered to have decreased recovery if they recovered less than 85% of the reference filter lot concentration on either or both of the screening media; recovery was acceptable if the mean count ratios were equal to or greater than this value.

RESULTS

Defects observed. A variety of irregularities were observed in the course of the screening test development studies and the subsequent screening of MF lots for acceptability. The major types of defects in colony growth and the filters themselves were (i) complete or partial grid line inhibition of colony growth, (ii) compressed areas of the filter that were produced by the application of excess pressure during the grid-stamping process, (iii) abnormal spreading of colonies, (iv) wrinkles, (v) growth of colonies in and along the grid lines, (vi) MF brittleness, (vii) hydrophobic or nonwetting areas, (viii) poor or absent colony sheen and metallic sheen formation on the surface of the MF on mEndo agar, and (ix) reduced recovery, as well as combinations of the above. Normal colonies (Fig. 1A) and some examples of these defects are shown in Fig. 1.

Grid line inhibition (Fig. 1B), i.e., the inability of colonies to grow across the grid, was demonstrated by the formation of flat-sided colonies adjacent to the grid lines. This effect may be caused by bacteriocidal or bacteriostatic materials in the ink or by lack of nutrients resulting from hydrophobic ink or from pores distorted and crushed during the grid-stamping process. The thickness of the grid and the darkness of the ink were not correlated with the presence or severity of the grid


A
B
C
D
E
F
G
H
I

line inhibition. Partial grid line inhibition, shown by the production of partially flat-sided colonies (Fig. 1C), was occasionally observed. This effect was probably caused by irregularities in ink application or uneven pressure during the grid-stamping process.

The stamping effect (Fig. 1B), formed by excessive pressure during the printing of the grid lines, was demonstrated by visible grooves or channels at the grid line or in the bacterial growth crossing the grid lines. Flat-sided colonies were frequently found next to the grooves, suggesting that this effect may mechanically block or retard microbial growth or prevent nutrient diffusion through distorted or crushed pores.

The abnormal spreading of colonies ranged from convex colony centers surrounded by a thin, narrow halo of growth (Fig. 1D) to a severe condition with flat or only very slightly raised centers with larger halos merging to form a confluent background (Fig. 1E). The latter condition made counting very difficult or impossible. Furthermore, counts obtained when this severe condition was present were often reduced and of questionable accuracy. This spreading may be caused by abnormal pore structure and/or by surfactants, wetting agents, and conditioners added to the MF during the manufacturing process. Studies in this laboratory (data not shown) showed that spreading can be induced by treating acceptable filters with surfactants and certain medium ingredients prior to filtration of the bacteria.

Small wrinkles were fairly common and seldom presented much of a problem. However, large and/or numerous wrinkles (Fig. 1F) did not always allow the proper seating of the filters on the agar, thereby preventing the diffusion of nutrients from the medium to the bacteria on the wrinkle surface. Extensive wrinkling problems may adversely affect recovery.

Occasionally, colonies grew in grooves formed by excessive stamping pressure or along the grid lines (Fig. 1G and H). When only a few isolated colonies were affected, accurate counts could be obtained, but extensive microbial growth along the grid lines (Fig. 1H) resulted in filters that could not be enumerated. This anomaly may be due to mechanical blockage of growth, pooling of media by grid line channels, or the presence of growth-stimulating materials in the ink.

Brittleness was observed with a few filters. Difficulties in handling and filtering were the primary problems encountered, but the possibility of decreased recovery resulting from minute cracks in the filter cannot be ruled out. Brittleness may be caused by deterioration of the filters due to age or lack of materials that maintain the suppleness of the filter. With most MFs tested, age did not correlate with brittleness.

Hydrophobic or nonwetting areas of the filter (Fig. 1I) caused colonies to be clustered in small areas of the filter; this made the colonies difficult or impossible to count. This defect may result from the absence of wetting agents, abnormal surface and/or pore structures in the affected areas, the presence of other hydrophobic substances such as oil, or defects in the MF supporting base screens. Work in this laboratory has shown that the shapes of nonwetting areas formed on some MFs were identical to scratches or dents on the filter support screens used. Filters placed on glass or plastic supports did not show the same problems.

Two defects were observed occasionally on mEndo agar: poor or nonexistent colony sheen and the formation of a golden-green metallic sheen on the surface of the filter. Both sheen conditions can interfere with the differentiation of coliforms from noncoliforms and hence with accurate enumeration of the target organisms. Figure 2 shows a comparison of a normal filter on mEndo agar with one showing the abnormal surface sheen. This defect was often seen on MFs that also demonstrated the abnormal spreading of coliform colonies. No spreading is evident in Fig. 2 because *K. pneumoniae*, the organism used in this case, did not exhibit spreading on mEndo agar.

Some filters demonstrated decreased recovery, i.e., had mean counts less than 85% of the mean count of their respective reference lot on one or both of the screening media. This defect may be caused by the composition of the filter material, inhibitory compounds in the filter, or the inability of nutrients to diffuse through the filter as a result of blocked pores, abnormal pore structure, or electrostatic interactions.

Various combinations of defects described above were also seen. In fact, more than one-third of the filter lots screened (about 60% of the unacceptable lots) had two or more defects. The formation of confluent squares was particularly troublesome to counting and recovery. This defect, a combination of grid line inhibition and abnormal spreading, often made counting impossible (Fig. 1E).

Coliform culture comparison study. The results of a comparison study of the colony and MF irregularities observed on a variety of filters with five coliform cultures and five different media are shown in Table 2. The cultures used were chosen for the following reasons: (i) *E. coli* ATCC 11229 is the recommended test organism in the American Society for Testing and Materials grid line inhibition procedure (2); (ii) *Enterobacter aerogenes*, the standard test organism for the water suitability test (1, 3), and *Enterobacter cloacae* are sensitive to toxic materials; (iii) *K. pneumoniae* and *C. freundii* are other representative coliform species. The five filter lots were chosen because they exhibited the presence or absence of a variety of MF defects when filters with *Enterobacter aerogenes* were grown on TSA. MF 1 was used as the reference filter because it demonstrated satisfactory recovery and performance with several media and did not produce any of the undesirable defects described above. MF 2 showed obvious grid line inhibition, whereas MF 4 showed only a marginal inhibition. MF 3 demonstrated abnormal spreading, and MF 5, with its larger pore size (0.7

FIG. 1. MF irregularities. (A) Normal *Enterobacter aerogenes* colonies, grown on TSA and stained with 1% (wt/vol) 2,3,5-triphenyl-2H-tetrazolium chloride (TTC). (B) Combination of excess grid-stamping pressure and complete grid line inhibition and very slight spreading of *Enterobacter aerogenes* colonies on mEndo agar. (C) A natural coliform from the Burnet Woods Lake sample showing partial grid line inhibition on mEndo agar. (D) Slight to moderate spreading (halo cells) of *Enterobacter aerogenes* colonies on mEndo agar. (E) Combination of severe spreading and complete grid line inhibition of *Enterobacter aerogenes* colonies and a zone of inhibition or toxicity between the colonies and the grid. The organisms were grown on TSA and stained with 1% TTC. (F) Combination of confluent squares, resulting from grid line inhibition and abnormal spreading of *Enterobacter aerogenes* colonies, and severe wrinkling on mEndo agar. (G) One natural fecal coliform from the Burnet Woods Lake sample growing along the grid line on the two-layer mFC agar (28). (H) Lattice effect with growth of *Enterobacter aerogenes* colonies on the grid lines of filters that had been treated with the detergent polyoxyethylene ether W-1 (Sigma Chemical Co., St. Louis, Mo.) and placed on mEndo agar. (I) A filter grown on mFC agar showing hydrophobic or nonwetting areas and crowding of *E. coli* EPA 206 colonies.

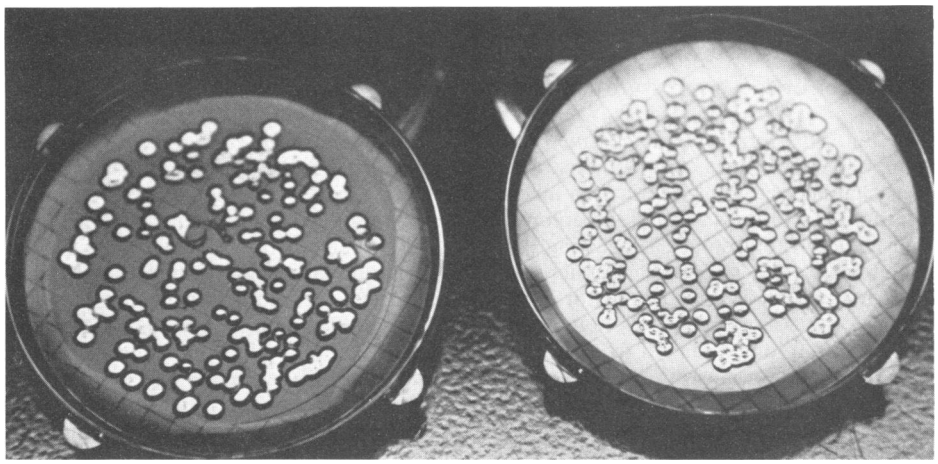


FIG. 2. Comparison of a filter showing normal colony sheen development on mEndo agar (left) with one exhibiting the abnormal surface sheen (right). The same dilution of *K. pneumoniae* was used for both filters, which were analyzed concurrently.

μm), showed a combination of grid line inhibition, stamping effect, and abnormal spreading.

Five types of irregularities were observed separately or in combination in this study: complete and partial inhibition of colony growth, abnormal spreading of colonies, poor or absent colony sheen and sheen on the surface of the filter with mEndo agar, and the stamping effect. Grid line inhibition was seen with all three of the filters affected (MF 2, MF 4, and MF 5) on all five types of media to a greater or lesser degree depending on the test organism used. This defect was most obvious with *Enterobacter aerogenes* on TSA. The other combinations of media and organisms often demonstrated less pronounced effects or were concentration dependent (i.e., the effect was obvious when a large number of bacteria were present on the filter but was less noticeable or absent at lower concentrations). Grid line inhibition was rarely observed with mEndo agar in this comparison study, but it was often seen during the screening studies of MF lots. The reason for this is not known.

Both *Enterobacter aerogenes* and *Enterobacter cloacae* showed abnormal spreading of colonies on TSA with MF 3 and MF 5. Although the effect was also seen on mEndo and mHPC agars with these organisms, it was less frequent and less obvious than with TSA. With all three media, the effect with *Enterobacter aerogenes* was more pronounced than that with *Enterobacter cloacae*. Spreading was seen only with *C. freundii* on mEndo agar and MF 3 and with *E. coli* on mHPC and mFC agars and MF 3 (one filter with each medium). No spreading was observed on R2A agar with any of the organisms, and no spreading was observed with *K. pneumoniae*.

The other defects were not seen as often as grid line inhibition and abnormal spreading. Colony and MF surface sheen problems could be detected only on mEndo agar. The stamping pressure effect was detected most often on mEndo agar, although it could also be seen occasionally with the other media.

Results showed that the *Enterobacter aerogenes*-TSA test detected the greatest number and degree of defects of all the combinations tested. Defects were easy to see, were usually more severe than on the other media, and were rarely concentration dependent. Other advantages of this test system are the stability of the culture and the ease of preparation of the medium. In addition, this test was superior to the American Society for Testing and Materials method (2) (with *E. coli* and mFC agar) for the detection of grid line inhibition because it was not concentration dependent and had the advantage of detecting other MF defects as well.

The *Enterobacter aerogenes*-mEndo agar test, the second-best combination, was useful for detecting three additional defects. Two of these, poor or absent colony sheen development and the formation of a metallic sheen on the surface of the MF, were visible only on mEndo agar, whereas the stamping pressure effect was seen most easily on this medium. As a result of this comparison study, the *Enterobacter aerogenes*-TSA and *Enterobacter aerogenes*-mEndo agar test systems were used in combination for screening filter lots because they permitted the maximum detection of filter defects.

Natural sample comparison study. Because MF irregularities may similarly affect the morphology and recovery of microorganisms found in natural samples, we compared the defects shown for the pure culture with those obtained by using natural samples. A reference filter (MF 1) and two filters that demonstrated a wide variety of defects when tested with *Enterobacter aerogenes* on TSA and mEndo agar were tested with five different MF media. The results (Table 3) showed that the defective MFs produced the same types and intensities of irregularities observed with the pure culture and that acceptable filters, i.e., those free of defects with the pure culture, produced no observable colony defects with the natural samples. Grid line inhibition, abnormal spreading, and confluent squares were frequently evident on defective MFs, whereas wrinkles, the stamping pressure effect, MF surface sheen, nonwetting areas, and channel growth were seen only occasionally. Obvious defects (i.e., filters with numerous colonies affected) were found with all of the media. On mEndo and mFC agars, morphological irregularities could be seen with background (i.e., noncoliform) colonies as well as with target (coliform) colonies. With the natural samples in this study, the pure culture test accurately predicted the occurrence of morphological irregularities. In addition, 54 other natural water samples (data not shown) have demonstrated a variety of defects, including most of those previously described, on 21 different filter lots from three manufacturers.

Comparison study of MF defects observed with other microorganisms and media. A comparison of the MF irregular-

TABLE 2. Comparison of colony and MF irregularities observed on acceptable and unacceptable filters with five coliform cultures and five different media

Organism	Medium	MF[a]	Colony and MF irregularities observed[b]:						
			None	Grid line inhibition		Abnormal spreading	Poor sheen	MF sheen	Stamping
				Complete	Partial				
Enterobacter aerogenes EPA 202	TSA	1	+	−	−	−	NA[c]	NA	−
		2	−	++	±	±	NA	NA	−
		3	−	−	−	++	NA	NA	−
		4	−	+	+	−	NA	NA	−
		5	−	++	−	++	NA	NA	−
	mEndo	1	+	−	−	−	−	−	−
		2	−	+[d]	−	−	−	−	+
		3	−	−	−	++	+	−	−
		4	−	±	±	−	−	−	−
		5	−	+	−	++	−	+	+
	mHPC	1	+	−	−	−	NA	NA	−
		2[e]	−	++	±	−	NA	NA	−
		3	−	−	−	+[d]	NA	NA	−
		4	−	+	±	−	NA	NA	−
		5	−	++	−	++	NA	NA	±
	R2A	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	+	+	−	NA	NA	−
		5	−	+	−	−	NA	NA	−
E. coli ATCC 11229	TSA	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	++[d]	+	−	NA	NA	−
		5	−	++[d]	+	−	NA	NA	−
	mEndo	1	+	−	−	−	−	−	−
		2	−	+[d]	−	−	−	+	+
		3	+	−	−	−	±	−	−
		4	−	±	−	−	−	−	−
		5	−	±	−	−	−	+	−
	mFC	1	+	−	−	−	NA	NA	−
		2	−	++[d]	+	−	NA	NA	−
		3	−	−	−	±	NA	NA	−
		4	−	±	±	−	NA	NA	−
		5	−	+[d]	+	−	NA	NA	−
	mHPC	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	−	−	−	±	NA	NA	−
		4	−	+[d]	+	−	NA	NA	−
		5	−	++[d]	±	−	NA	NA	−
	R2A	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	++	−	−	NA	NA	−
		5	−	++	−	−	NA	NA	−
Enterobacter cloacae	TSA	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	−	−	−	+[d]	NA	NA	−
		4	−	++	±	−	NA	NA	−
		5	−	++	+	+	NA	NA	−

Continued on following page

TABLE 2—*Continued*

Organism	Medium	MF[a]	Colony and MF irregularities observed[b]: None	Grid line inhibition: Complete	Grid line inhibition: Partial	Abnormal spreading	Poor sheen	MF sheen	Stamping
	mEndo	1	+	−	−	−	±	−	−
		2	−	±	−	−	−	−	++
		3	−	+	−	+	+	−	−
		4	+	−	−	−	−	−	−
		5	−	+	+	−	−	−	+
	mHPC	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	+[d]	+	−	NA	NA	−
		5	−	++[d]	±	−	NA	NA	±
	R2A	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	++	±	−	NA	NA	−
		5	−	++	−	−	NA	NA	−
C. freundii	TSA	1	+	−	−	−	NA	NA	−
		2	−	++	+	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	+	+	−	NA	NA	−
		5	−	++	+	−	NA	NA	±
	mEndo	1	+	−	−	−	−	−	−
		2	−	±	+	−	−	−	++
		3	−	−	−	+	−	−	−
		4	+	−	−	−	−	−	−
		5	−	−	±	−	−	+	++
	mHPC	1	+	−	−	−	NA	NA	−
		2	−	++[d]	+	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	+	+	−	NA	NA	−
		5	−	++[d]	+	−	NA	NA	−
	R2A	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	+	+	−	NA	NA	−
		5	−	++	+	−	NA	NA	+
K. pneumoniae EPA 207	TSA	1	+	−	−	−	NA	NA	−
		2	−	++	±	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	±	−	−	NA	NA	−
		5	−	++[d]	−	−	NA	NA	−
	mEndo	1	+	−	−	−	−	−	−
		2	−	+[d]	−	−	−	+	−
		3	+	−	−	−	−	−	−
		4	+	−	−	−	−	−	−
		5	−	++[d]	−	−	−	+	−
	mHPC	1[e,f]	+	−	−	−	NA	NA	−
		2[e,f]	−	±	−	−	NA	NA	−
		3[f]	+	−	−	−	NA	NA	−
		4[f]	+	−	−	−	NA	NA	−
		5	−	±	+	−	NA	NA	−

Continued on following page

TABLE 2—*Continued*

Organism	Medium	MF[a]	Colony and MF irregularities observed[b]:						
			None	Grid line inhibition		Abnormal spreading	Poor sheen	MF sheen	Stamping
				Complete	Partial				
	R2A	1	+	−	−	−	NA	NA	−
		2	−	++	−	−	NA	NA	−
		3	+	−	−	−	NA	NA	−
		4	−	++	±	−	NA	NA	−
		5	−	++[d]	−	−	NA	NA	−

[a] MF 1, Millipore lot H1K 66090 C (reference); MF 2, Millipore lot H4H 74099 A; MF 3, Millipore lot H1E 65371 B; MF 4, Gelman lot 4104084; MF 5, Millipore HCWG (0.7 μm) lot H2P 69551 A.
[b] Symbols: −, no defects observed; ±, rare or occasional colony showing defect; +, slight defects visible; ++, moderate to severe defects, very obvious.
[c] NA, Not applicable to any medium except mEndo agar.
[d] Defects are concentration dependent, i.e., more obvious with a large number of organisms on the filter and less obvious or absent with a smaller number.
[e] Bubble formation on the grid line.
[f] Bubble formation between the filter and the agar.

ities detected by the *Enterobacter aerogenes* test with those detected by using other microorganisms and media was made to determine whether filter defects were restricted to the coliform methods. The defective membrane filters chosen for this study exhibited either grid line inhibition or abnormal spreading with the pure culture test. The *Staphylococcus aureus*, *E. coli*, *P. aeruginosa*, and *S. faecalis* cultures were used in this study because there are established MF methods for their detection (3, 4, 9, 19–21, 32). *Salmonella typhimurium* was used because this serovar, along with five other *Salmonella* serovars, showed abnormal spreading and/or grid line inhibition on a variety of MF media in our laboratory (T. Covert, personal communication). *Proteus vulgaris* was included to determine whether its extreme motility would affect defect formation on the MF heterotrophic plate count media. A reference filter lot was tested concurrently with all of the organisms and media.

In a comparison (data not shown) of *Enterobacter aerogenes* and *E. coli* EPA 206 with nine media including TSA, the MF heterotrophic plate count media (mHPC and R2A), the standard total and fecal coliform media (mEndo and mFC), and other media (mTEC, mT7, mHAR, and mHARC) for total coliforms and *E. coli* (1, 9, 20, 21, 32; Haines and Rankin, personal communication), *Enterobacter aerogenes* showed defects with all media except mHAR and mHARC (Haines and Rankin, personal communication), whereas *E. coli* showed defects with all but mHAR, mHARC, mFC, and mTEC. Both organisms produced a channel growth effect on mT7 instead of the expected grid line inhibition. The reason for this is not known. *Enterobacter aerogenes* was the more sensitive organism, a fact which was shown previously in the coliform comparison study. Although the *E. coli* EPA 206 used in this study did not demonstrate any defects on mFC agar, the ATCC *E. coli* strain used above and some environmental isolates of *E. coli* have shown both grid line inhibition and abnormal spreading on mFC agar. In those cases, however, the spreading was usually less severe and less frequently observed than with *Enterobacter aerogenes*.

A comparison (data not shown) of the filter defects exhibited by five other organisms (*Staphylococcus aureus*, *Salmonella typhimurium*, *S. faecalis*, *P. aeruginosa*, and *Proteus vulgaris*) with those demonstrated by *Enterobacter aerogenes* on three nonselective media and appropriate selective media showed that no medium or organism was entirely free of MF problems. All of the organisms exhibited grid line inhibition and abnormal spreading with at least one medium, except for *S. faecalis*, which showed only grid line inhibition. Most organisms demonstrated problems with the nonselective media (TSA, mHPC, and R2A), indicating that the mHPC and R2A heterotrophic plate count methods could be seriously affected by the quality of the MFs used. The problems were not restricted to the coliform and heterotroph methods, because all of the organisms, except *Proteus vulgaris*, which was tested only on nonselective media, showed grid line inhibition and/or spreading on their respective selective media. Although *Enterobacter aerogenes* failed to grow on KF, mE, XLD, VJ, and mPA-C agars, the defects exhibited by this organism on TSA correlated well with the occurrence of MF irregularities produced by the other organisms on their respective selective media, indicating that the *Enterobacter aerogenes*-TSA screening test may also predict potential problems with these methods.

Screening 0.45-μm MFs for acceptability. Since the same types of defects observed on filters with the pure culture were also seen with natural samples, the *Enterobacter aerogenes*-TSA and *Enterobacter aerogenes*-mEndo agar tests were used to screen 0.45-μm filters for use in our laboratory for coliform analysis and to determine the prevalence of these problems in filters from different manufacturers. The results of screening tests on 142 lots of MFs from 13 different manufacturers are shown in Table 4. Filters with no defects of any type or only a rare occurrence of a minor defect, such as a small wrinkle or a rare colony demonstrating grid line inhibition, were judged acceptable; those with a slight defect were considered marginal; and those with obvious, severe, or multiple defects were considered unacceptable. Generally, when several boxes of the same lot were tested, there was more variation between boxes than between filters within a box. Similar colony irregularities were usually seen on most, if not all, of the filters of a test lot.

The results of this study showed that of the 142 lots tested, 42 (29.6%) lots were acceptable, 14 (9.9%) were marginally acceptable, and 86 (60.6%) were unacceptable. No manufacturer had filters that were defect-free, although Sartorius had a combination of acceptable and marginal lots only. However, the total number of lots tested was small in this case. Of the 86 unacceptable lots, 50 (58.1%) had multiple (i.e., two or more) defects, while the remaining 36 (41.9%) lots showed only one type of irregularity. Percentages of the four single defects observed, i.e., grid line inhibition, abnormal spreading, MF sheen, and decreased recovery, were 20.9, 12.8, 1.2, and 7.0%, respectively. Of the defects found on the 50 lots of membranes with more than one type of irregular-

TABLE 3. Comparison of MF irregularities observed with the pure-culture screening test and natural water samples

Sample	Medium	MF[a]	MF irregularities observed[b]:				
			None	Grid line inhibition	Spreading	Confluent squares	Other
Pure culture (*Enterobacter aerogenes*)	TSA	1	+	−	−	−	−
		2	−	+	+	+	Wrinkles
		3	−	+	+	−	−
	mEndo	1	+	−	−	−	−
		2[c]	−	+	+	+	Stamping pressure, MF sheen, wrinkles
		3	−	+	+	−	Stamping pressure, MF sheen
Tylersville Artesian Well	TSA	1	+	−	−	−	−
		2[c]	−	++	++	+	−
		3	−	+	+	−	−
	mEndo	1	+	−	−	−	−
		2	−	++	+	+[d]	Nonwetting areas, wrinkles
		3	−	−	+	−	Stamping pressure
	mHPC	1	+	−	−	−	−
		2	−	+	++	+	Wrinkles
		3	−	±	±	−	−
	R2A	1	+	−	−	−	−
		2	−	+	+	−[e]	−
		3	−	++	±	−	−
	mFC	1	+	−	−	−	−
		2	−	±	+	−[d]	−
		3	+	−	−	−	−
Burnet Woods Lake	TSA	1	+	−	−	−	−
		2	−	+	+	+	−
		3	−	+	+	−	−
	mEndo	1	+	−	−	−	−
		2[c]	−	+	+	+[d]	−
		3	−	+	+	−	Stamping pressure
	mHPC	1	+	−	−	−	−
		2	−	+	+	+	Nonwetting areas, wrinkles
		3	−	+	++	−	Stamping pressure
	R2A	1	+	−	−	−	−
		2[c]	−	+	+	+	−
		3[c]	−	+	−	−	−
	mFC	1	+	−	−	−	−
		2	−	+	+	+	−
		3[c]	−	+	−	−	Stamping pressure, channel growth

[a] MF 1, Millipore lot H1K 66090 C (reference); MF 2, Oxoid Nuflow lot 3706; MF 3, Millipore HCWG (0.7 μm) lot H2P 69551 A.
[b] Symbols: −, no defects observed; ±, rare or occasional colony showing defect; +, defect plainly visible; ++, severe defect, very obvious.
[c] Recovery was less than 85% of the reference lot recovery.
[d] The non-target/background colonies formed confluent squares.
[e] This plate had a confluent background.

ity, 30.8% were grid line inhibition, either partial or complete, 25.6% were abnormal spreading, 11.5% were caused by the stamping effect, 12.1% resulted from sheen problems on mEndo agar, and 7.1% were due to reduced recovery. The other 11 types of defects totaled 12.8%, with individual percentages of less than 4.0%. The average number of defects per MF lot ranged from 2.3 to 4.5, depending on the manufacturer, with an overall average defect rate of 3.1 per MF lot.

DISCUSSION

Problems associated with the quality control of filter manufacturing are not new. As early as 1955, Gaspar and Liese (13) showed that grid line ink was inhibitory to the growth of *Pasteurella tularensis* (currently *Francisella tularensis* [29]). In 1975, a symposium on the recovery of indicator organisms by using MFs was held in Fort Lauderdale, Fla. Technical and quality control problems with

TABLE 4. Acceptability of 0.45-μm MF lots from several manufacturers by the pure-culture screening test

Manufacturer	No. of MF lots that were:		
	Acceptable	Marginal	Unacceptable
AMF Cuno	0	0	1
Amicon	0	0	4
Domnick Hunter	0	0	4
Gelman Sciences	8	5	24
Micro Filtration Systems	2	0	3
Millipore Corp.	26	2	31
Micron Separations, Inc.	1	0	5
Nalge Co.	1	0	1
Nuclepore Corp.	0	2	6
Oxoid Ltd.	0	0	2
Schleicher & Schuell, Inc.	0	1	4
Sartorius	1	2	0
Whatman, Inc.	3	2	1

filters, including various types of defects observed such as grid line inhibition, nonwetting areas, wrinkling, brittleness, and warping, were discussed (6, 14). Today, almost 15 years later, many of the same problems still exist, as indicated by this study.

Some investigators (5, 10, 12, 23, 31) have noticed similar types of irregularities, whereas others (6, 7, 16, 17, 22, 26, 30) have reported that, despite manufacturer claims, brands of filters vary in their ability to recover both total and fecal coliforms. In addition, decreased germination of *Clostridium perfringens* spores has been observed with some filter lots (M. Hesford, personal communication), and the growth of tissue culture cells and chemical analyses have been adversely affected by materials leaching from the filters (8, 11, 18, 24, 25).

In this study, we observed a large number of different MF defects, varying from minor to severe, with pure cultures and natural samples. The *Enterobacter aerogenes*-TSA and the *Enterobacter aerogenes*-mEndo agar tests, used in combination, were shown to be a useful screening tool for determining the number and types of MF defects and, hence, the acceptability of 0.45-μm MFs for coliform analysis. In addition, the *Enterobacter aerogenes*-TSA test proved to be useful for predicting problems with other MF methods.

The results of the MF filter lot screening study showed that no manufacturer produced defect-free filters. Although the number of lots tested from individual manufacturers was small in most cases and may or may not be representative of their product, the total number of unacceptable filter lots from all manufacturers indicated the generally poor quality of the membrane filters and the need for better overall quality control during manufacturing. Some of the differences in recovery observed between filters from different manufacturers (6, 10, 16, 22, 23, 26, 30) or between the MF method and other methods, such as the presence-absence or most-probable-number tests (27), may have resulted because defective filters were used. Although the calculated MF rejection rate was 48% for the first 100 filter lots, the current rate has increased to 61%. Although this may be due, in part, to increased experience in observing defects, we believe it is due to a downward trend in the quality of filters produced, perhaps because of worn or malfunctioning equipment; failure to control production conditions such as temperature and relative humidity; or the use of newer inks, surfactants, and wetting agents.

Although this screening test can serve as an indicator of potential problems in enumerating a variety of organisms in natural samples, there may be some instances in which the test is not accurate. For example, if susceptible organisms are reduced in number or if the defects present are concentration dependent (i.e., the effect was obvious when a large number of bacteria were present on the filter, but was less noticeable or absent at lower concentrations), it is possible to have defective filters with no apparent colony irregularities. On the other hand, acceptable filters may occasionally show colony spreading as a result of chemicals, dish and laundry detergents, or other substances in the sample itself. In this situation, laboratories using only acceptable MFs, as determined by this screening test, would be able to determine the true cause of the difficulties.

One advantage of the screening test is its ability to detect poor or absent sheen formation by coliforms on mEndo agar. Normally, decreased or absent colony sheen would go undetected with natural samples unless the sample was concurrently analyzed by using an acceptable filter lot or unless the filters were checked for this defect prior to use.

With effluent, surface water, or recreational water samples, which usually contain a high concentration of microorganisms, MF defects such as those described in this paper may affect counting and recovery and, hence, compliance with federal regulations. However, since the concentrations of organisms in drinking-water samples are usually very low or zero, the use of defective filters would be less likely to affect the actual counts. Nevertheless, drinking-water laboratories may decide to perform this test to protect the consumers by erring on the side of safety.

In conclusion, we recommend that all laboratories using MFs test all lots for irregularities and adequate recovery to ensure the accuracy of their data and that researchers developing new MF methods determine whether their tests are adversely affected by poor-quality filters.

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