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Comparison of a New Inorganic Membrane Filter (Anopore) with a Track-Etched Polycarbonate Membrane Filter (Nuclepore) for Direct Counting of Bacteria

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Bacterial counts obtained by using a new Anopore inorganic membrane filter were 21 to 33% higher than those obtained by using a Nuclepore polycarbonate membrane filter. In addition, the inorganic filter had higher flow rates, permitting lower vacuum pressures to be used, while the intrinsically flat, rigid surface resulted in easier focusing and sharp definition of bacteria across the whole field of view.

The use of polycarbonate Nuclepore filters for direct counting of aquatic bacteria has been widely adopted (2, 3) following the publication of modifications of the technique (1). The present study evaluated the use of a new inorganic aluminum oxide filter (Anopore; Anotec Separations Ltd., Banbury, Oxon, United Kingdom) in the acridine orange direct-count procedure and compared it with a track-etched polycarbonate filter (Nuclepore). Polycarbonate membranes (0.2-μm pore size) possess a pore density of 3×10^8 pores cm^{-2} (manufacturer's technical information) and a uniform pore size (Fig. 1 and 2). The 0.2-μm-pore-size Anopore filter has a cross-sectional homogeneous capillarylike structure, with a pore density of 3×10^9 cm^{-2} (manufacturer's technical information) and a rigid, flat surface with a uniform pore size (Fig. 1 and 2).

The water sample used throughout the work was pond water preserved with formaldehyde (final concentration, 2% [vol/vol]). Just before staining, portions of the bulk sample were diluted 1/25 with filter-sterilized 0.05 M phosphate buffer (pH 7.6) and processed within 0.5 h to give 30 to 80 cells per field of view. In preliminary trials, dilution in phosphate buffer gave enhanced cell fluorescence and less background cloudiness than dilution in filter-sterilized pond water. The two membrane filter types used for this study were as follows: (i) irgalan black-stained polycarbonate filters with a 0.2-μm pore size and 25 mm in diameter (Nuclepore 110656); (ii) inorganic membrane filters with a 0.2-μm pore size and 25 mm in diameter (Anopore PTC 1005), prestained with filter-sterilized 0.2% irgalan black in 2% (vol/vol) acetic acid for 45 min, followed by rinsing in sterile distilled water and air drying in sterile petri dishes (1).

The filtration procedure was designed to treat both filter types identically. Although flow rates through the Anopore filters were markedly faster (38% at 10 lb/in^2 and 22 to 25°C) than through Nuclepore filters, stain and destain contact times were maintained for equal durations. Filtration was carried out using a single glass microanalysis assembly with 25-mm glass frit support (Millipore) and 0.45-μm-pore-size, mixed cellulose ester backing filters to give a good distribution of vacuum (Millipore, type HA). Each filter was prewetted in situ with 2 ml of filter-sterilized water and dried under gentle vacuum to facilitate good filter-support contact. A 5-ml volume of freshly diluted sample was pipetted onto the filter surface, followed by 1 ml of 0.06% acridine orange (final concentration of 0.01%), using a swirling motion for mixing, and left to stain in the dark for 5 min. The acridine orange was removed under gentle vacuum, and the filter was destained with 25% isopropanol. The contact time was standardized for both filter types (2.5 to 3 min). The filter was then mounted on a microscope slide with immersion oil

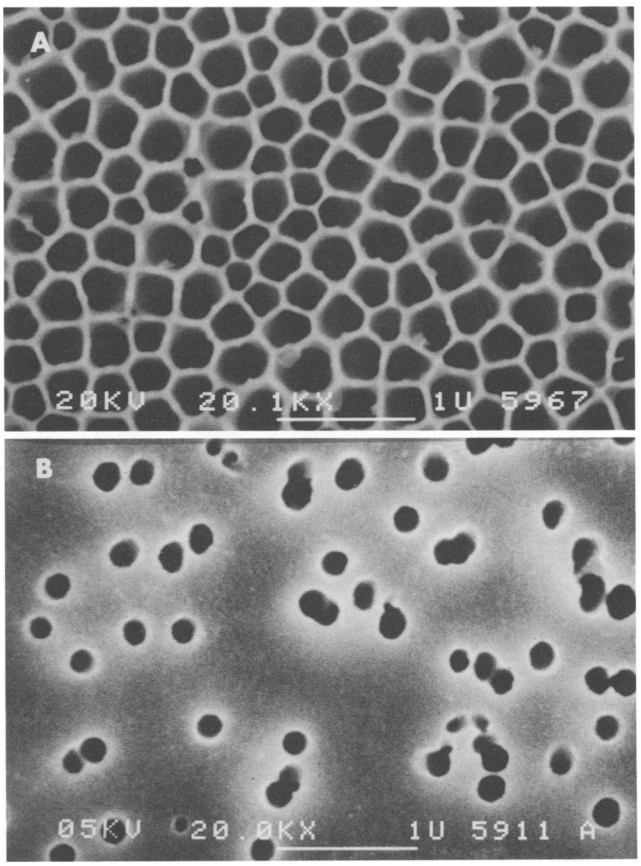

FIG. 1. Scanning electron micrograph of an Anopore membrane (A) and a Nuclepore membrane (B).

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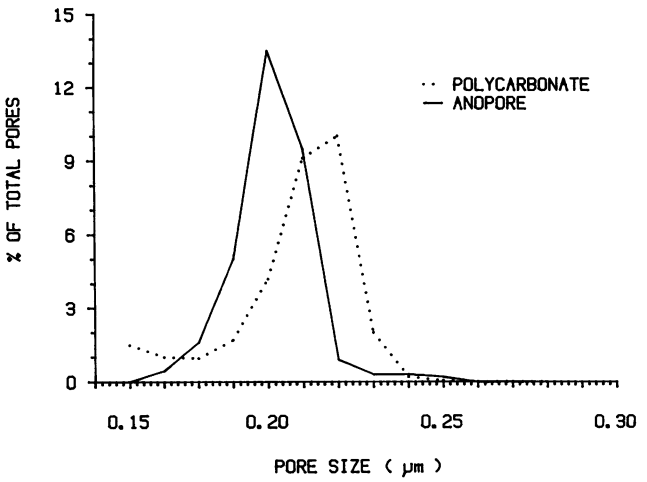

FIG. 2. Distribution of pore size of the Anopore membrane and the Nuclepore membrane.

(Lenzol, Gurr) and left to air dry for 15 to 20 min in the dark. A drop of immersion oil was gently placed onto the dried filter, a cover slip was applied, and the filter was viewed under oil immersion (magnification, ×1,000) using an epifluorescence microscope (Leitz Ortholux, BG 12 filter, 450 to 490 nm). An eyepiece micrometer was used to delineate a portion of the field of view (88 by 88 μm).

Three individuals, with various levels of counting experience, counted 10 filters of each type, 10 fields per filter, to give a total of 600 counts. Counting was carried out over the entire filter, using a balanced design format to counteract any differences in cell deposition. Count data were normalized by log transformation, and analyses were performed using Statgraphics software. The residuals were found to satisfy the analysis-of-variance assumptions of normality (Kolmogorov-Smirnov test, $P > 0.10$) and homogeneity of variance (Bartlett's test, $P > 0.10$). Table 1 lists summary statistics of sources of count variation. Counts on each filter type were not the same ($P < 0.0005$); i.e., counts from Anopore filters produced consistently higher densities (6.3×10^6 to 7.26×10^6 cells ml^{-1} on Anopore filters and 5.2×10^6 to 5.46×10^6 cells ml^{-1} on Nuclepore filters). The differences between the subjects were not significant ($P > 0.25$), and there was no interaction between subject and filter type ($P \gg 0.25$).

TABLE 1. Analysis of variance for log-transformed bacterial count data

Source of variation	Sum of squares	df	Mean square	F ratio	P
Subject	0.1919	2	0.0959	1.272	NS[a]
Filter type	1.6205	1	1.6205	21.489	<0.0005
Subject by filter type	0.0409	2	0.0205	0.271	NS
Between filters	4.0722	54	0.0754	5.809	<0.0005
Within filters	7.0106	540	0.0130		

[a] NS, Not significant.

TABLE 2. Bacterial counts on two filter types[a]

Subject	Nuclepore	Anopore
1	42.08 (36.55–48.42)	55.89 (46.35–67.36)
2	41.98 (35.91–49.05)	54.31 (48.90–60.30)
3	40.03 (34.02–47.07)	48.50 (43.48–54.09)

[a] Sample statistics are the mean, with the 95% confidence interval in parentheses. One hundred fields were involved in each count.

Summary statistics are given in Table 2 for the two filter types and three subjects. These values were calculated using the log-transformed count data and then untransformed to give representative count values (thus, the confidence intervals are not symmetrical about the mean). Bacterial counts were 21% (subject 3) to 33% (subject 1) higher on the Anopore filter type.

Since the staining procedure was identical throughout the study, higher counting efficiencies on the inorganic filter would seem to be due to filter configuration—the rigid, planar surface of the Anopore membrane allows for easier focusing under the microscope and produces a very sharp definition of stained bacteria. In contrast, flexible organic membranes tend to conform to any undulations of the surface on which they are placed, in this instance, a mixed ester backing membrane on a glass frit support. However, we acknowledge that this assessment is of necessity subjective and that surface charge effects associated with the aluminum oxide may also have played a part. In addition, the fine sieve-like morphology of the Anopore membrane permits the use of gentle vacuum filtration while maintaining adequate flow rates, thereby reducing damage to other delicate microorganisms trapped on the surface, e.g., microflagellates.

It may be tentatively concluded that Anopore filters offer enhanced bacterial counting characteristics as compared with polycarbonate membrane filters because of their rigid, planar surface and possible surface charge effects. Furthermore, the planar surface may be extremely advantageous for use in image analysis, in which sharp focus across the whole field of view is highly desirable. This application is currently under evaluation in our laboratory.

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