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Quantitative Large-Volume Sampling Technique

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A filtration technique for high-volume sampling of water has been developed that, when combined with the most probable number procedure, permits the quantitation of microorganisms present at very low densities.

Dutka and Bell (3) recently pointed out the need for quantitative techniques for the enumeration of small numbers of enteric pathogens and indicator organisms in large volumes of wastewater and receiving waters. Various qualitative techniques for the isolation of bacteria present in very low densities have been described (2, 4, 6, 8), but quantitative methods for their enumeration under such conditions have not been reported. This note describes a high-volume sampling (HVS) technique that can be used in conjunction with the most probable number (MPN) procedure to permit quantification of bacteria in those instances where an MPN procedure is available.

The HVS technique utilizes a Balston (Lexington, Mass.) type AA cartridge filter (2.5 by 6.4 cm) in conjunction with a type 90 filter holder. The filter is composed of borosilicate glass microfibers bonded with epoxy resin. The retention efficiency for liquids is 98% for 0.3-μm particles. The maximum recommended pressure differential for these filters is 69 kN/m^2 (outside to inside flow). The filters, filter holders, and associated vacuum tubing are sterilized by autoclaving at 121 C for 15 min.

The apparatus as used is represented in Fig. 1. The water sample was collected in or transferred to a sterile calibrated container, and a portion thereof was passed through the filter under vacuum. A manifold was used to provide the capability of filtering five portions simultaneously. A trap was inserted between the manifold and the vacuum pump to collect the filtered water. When the desired quantity of water had been filtered, the filter was aseptically removed from the holder and placed directly into a suitable culture medium for incubation. Another sterile filter was placed in the filter holder, and the procedure was repeated. Resterilization of the apparatus was required only when a new sample was examined.

The amount of time required to filter large volumes of water varied with the turbidity of the sample. With most samples, 10 liters could be filtered within 60 min (Fig. 2). If this is impossible due to excessive turbidity, several filters may be used successively on a single portion and placed in the same container of culture medium.

The HVS procedure was used to obtain MPN estimates of *Salmonella* densities in samples collected from marine waters in the vicinity of New York City (Table 1). All samples were assayed within 12 h of collection. Dulcitol-Selenite medium, incubated for 72 h at 41 C (5), was used for primary enrichment. The tubes were examined daily, and each positive tube (brick red in color) was streaked for isolation onto brilliant green agar (Difco Laboratories, Detroit, Mich.) and Hektoen enteric agar (Chas. Pfizer & Co., Inc., New York, N.Y.) plates. The plates were incubated at 37 C for 24 h. Up to 10 suspect colonies from the brilliant green and Hektoen enteric plates referable to each enrichment tube were examined using triple-sugar iron agar (Difco), the lysine decarboxylase test (Difco), and fluorescent-antibody staining (Clinical Sciences, Inc., Whippany, N.J.). The

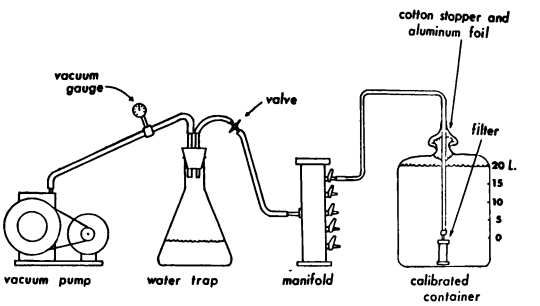

FIG. 1. *Sampling apparatus. Vacuum pump operates at 69 kN/m^2 to draw water through the filter into the trap. A calibrated container is used to permit direct measurement of the volume sampled.*

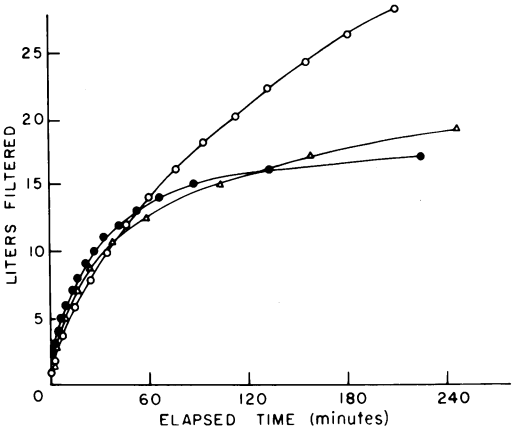

FIG. 2. *Filtration capacity of type AA Balston filters (320-mm Hg vacuum) using water samples of various turbidities. Symbols: Δ, Providence River, 7.5 Jackson turbidity units (JTUs); ●, sewage outfall area, 8.0 JTUs; ○, Narragansett Bay, 4.5 JTUs.*

TABLE 1. *Salmonella densities in water samples from New York City beaches*

Water source	Date taken	MPN code	Organisms/100 liters[a]
W. 25th Street	6-12-73	510	22.0
W. 25th Street	7-7-73	200	4.0
Riis Park	7-7-73	010	1.8
W. 25th Street	7-14-73	100	2.0
Riis Park	7-14-73	000	<1.8
W. 25th Street	7-15-73	110	4.0
Riis Park	7-15-73	210	6.8
W. 25th Street	7-22-73	200	4.0
Riis Park	7-22-73	000	<1.8

[a] Five-tube MPN values were calculated for volumes filtered. The W. 25th Street sample taken 12 June 1973 was assayed using 15, 1.5, and 0.15 liters for each of three sets of five tubes. All other samples were assayed using 10-, 1.0-, and 0.1-liter volumes.

fluorescent-antibody smears were prepared from cultures on nutrient agar. It would appear that the MPN codes obtained by this procedure occur with a normal frequency, indicating an absence of disturbing influences (7).

This HVS technique can be applied to any situation where a selective-enrichment medium is available for use in conjunction with the MPN procedure. For each application, however, it must be determined whether factors characteristic of large-volume sampling, such as the adsorption of toxic metal ions and overgrowth by background organisms, may impose a bias. The accuracy of the HVS procedure when used to obtain *Salmonella* and coliform densities can be seen in Table 2. For the coliform analysis, potable water sterilized by autoclaving was seeded with predetermined quantities of polluted water; in the *Salmonella* trials, portions of *S. enteritidis* suspension held in sterile estuarine water for 24 h were added to estuarine water that contained less than 1 coliform per 100 ml and less than 1.8 salmonellae per 100 liters. The expected bacterial densities were determined from a five-tube, total coliform MPN (1) on the polluted water and on triplicate spread plates of brain heart infusion agar (Difco) for the stressed *S. enteritidis* suspension. The HVS procedure for coliform enumeration differed from the standard method in that 250 ml of lactose broth was used in each tube and all tubes that exhibited turbidity were transferred to the confirmatory broth. With both organisms, the observed HVS estimates were similar to the expected values (Table 2).

TABLE 2. *Accuracy of HVS technique*

Trial	Water source[a]	Seeded with	Bacterial density/100 liters	
			Expected[b]	Observed[c]
1	Potable (sterilized)	Coliforms	130	130
2	Potable (sterilized)	Coliforms	130	49
3	Potable (sterilized)	Coliforms	32	47
4	Potable	Coliforms	440	240
5	Potable	Coliforms	196	230
6	Potable	Coliforms	9	10
7	Estuary	Salmonella	810	920
8	Estuary	Salmonella	810	350

[a] Potable water for trial 5 was obtained from the Westerly, R.I. water supply. All other water samples are from the Wakefield, R.I. water supply.

[b] The expected coliform count is based on a *Standard Methods* (1) MPN. Samples of polluted water used as the inoculum were obtained from a polluted river. The expected *Salmonella* count is based on three replicate brain heart infusion spread plates from a suspension of *S. enteritidis*.

[c] Trials 1, 2, 3, 4, 7, and 8 are based on sample volumes of 10, 1, and 0.1 liters. Trial 5 is based on 40, 4, and 0.4 liters, and trial 6 is based on 70, 7, and 0.7 liters.

LITERATURE CITED

1. American Public Health Association. 1971. Standard methods for the examination of water and wastewater. American Public Health Association, Inc., New York.
2. Brezenski, F. T., and R. Russomanno. 1969. The detection and use of salmonellae in studying polluted tidal estuaries. J. Water Pollut. Contr. Fed. **41**:725–737.
3. Dutka, B., and J. Bell. 1973. Isolation of salmonellae from moderately polluted waters. J. Water Pollut. Contr. Fed. **45**:316–324.
4. Moore, B., E. L. Perry, and S. T. Chard. 1952. A survey by the sewage swab method of latent enteric infection in the urban area. J. Hyg. **50**:137–141.
5. Raj, H. 1966. Enrichment medium for selection of *Salmonella* from fish homogenate. Appl. Microbiol. **14**:12–20.
6. Spino, D. F. 1966. Elevated-temperature technique for the isolation of salmonellae from streams. Appl. Microbiol. **14**:591–597.
7. Woodward, R. L. 1957. How probable is the most probable number? J. Amer. Water Works Ass. **49**:1060–1068.
8. Yoshpe-Purer, Y. 1971. A convenient method for isolation of salmonellae from sewage and contaminated sea water. Water Res. **4**:113–116.