APPLIED MICROBIOLOGY, Apr. 1975, p. 506-509
Vol. 29, No. 4

Printed in U.S.A.

Inexpensive Device for the Aerobic and Anaerobic Sampling of Microorganisms in Lake and Shallow Ocean Waters

R. M. EDWARDS

Department of Microbiology, University of Otago, Dunedin, New Zealand

Received for publication 15 October 1974

An inexpensive device suitable for sampling microorganisms in water and easily constructed from readily available laboratory equipment is described. The need to transfer subsamples to culturing flasks after collection is eliminated by partly filling the sampling vessels with growth medium prior to sampling. The device is readily adapted for sampling different volumes, is simple and quick to operate, and is suitable for use with prereduced media. Contamination from layers other than that being sampled is insignificant.

A number of devices for sampling water have been developed for chemical studies and with suitable modifications have been used in bacteriological work. These samplers must be capable of preventing contamination of the sample by water from layers other than that sampled and must be able to be triggered by remote control. Before they can be used for microbiological work, however, they must be modified to enable them to withstand sterilization procedures and to enable subsamples to be removed aseptically in the field if the sample cannot be stored in the sample vessel. Existing samplers are complex, expensive to construct, and, because of their bulk, inconvenient for field work (4). Many are slow to operate and usually require the water collected to be transferred to culture vessels as soon as the sample is recovered. It is often difficult to establish aseptic conditions in the field and extremely difficult to provide oxygen-free conditions for the transfer of anaerobes.

The sampler described in this paper was developed to overcome these problems and was used subsequently to collect samples of photosynthetic bacteria occurring at depths of up to 30 m in Lake Hayes, Central Otago, New Zealand. Many of these bacteria are anaerobic, and therefore a system that was convenient, quick, and capable of maintaining anaerobic conditions in the field was required.

MATERIALS AND METHODS

The sampler. The sampler used commercially available Vacutainer (Becton Dickinson & Co.)-evacuated glass tubes, needles, and needle mounts. A 6-inch (15.24 cm) length of pvc pipe (Fig. 1A) whose internal diameter equals the external diameter of the Vacutainer tube was slotted and a rubber stopper was glued a short way up from one end. A length of nylon fishing line was passed through the slots and, together with a suitable length of lead pipe, was cemented to the needle mount with resin glue. The lead sleeve of the prototype was made from a lead vial and the slotted tube from two polythene hose-connectors glued together. Care should be taken that the lead sleeve does not cover the guide line on the needle mount; otherwise the 'Vacutainer' is difficult to insert into the needle mount without prematurely breaking the vacuum seal. The hydrowire was passed down the pvc tube, out one slot, and tied to the center of the loop of nylon line. To complete the sampler, the knot was drawn back into the bore of the tube by pulling on the hydrowire. The hydrowire was marked at 1-m intervals with sleeves of white tape and the aperture of the messenger flared at one end to prevent these markers impeding its motion. An exploded diagram and a cutaway diagram of the completed sampler is given in Fig. 1.

A sterile needle was screwed into the needle mount and a Vacutainer was inserted and pushed up to the mark on this mount. A hematological Vacutainer (16 by 100 mm) with a 10-ml draw, together with a 22-gauge needle, was found satisfactory for this purpose. The slotted tube was slipped over part of the exposed end of the Vacutainer as shown (Fig. 1), and the assembly was lowered to the desired depth. A messenger dropped down the hydrowire strikes the slotted tube, which together with the rubber bung acts as a shock absorber preventing the Vacutainer from shattering under the impact. It is therefore important that the slotted tube fits snugly. The impact forces the Vacutainer down into the needle mount, the double ended needle breaks through the stopper, and a sample is drawn in by the vacuum. A 500-g messenger was used to ensure that the impact was sufficient to force the Vacutainer onto the needle when sampling a short distance below the surface. The assembly was drawn to the surface, the slotted tube was slipped off, and the Vacutainer was removed. The sample may then be

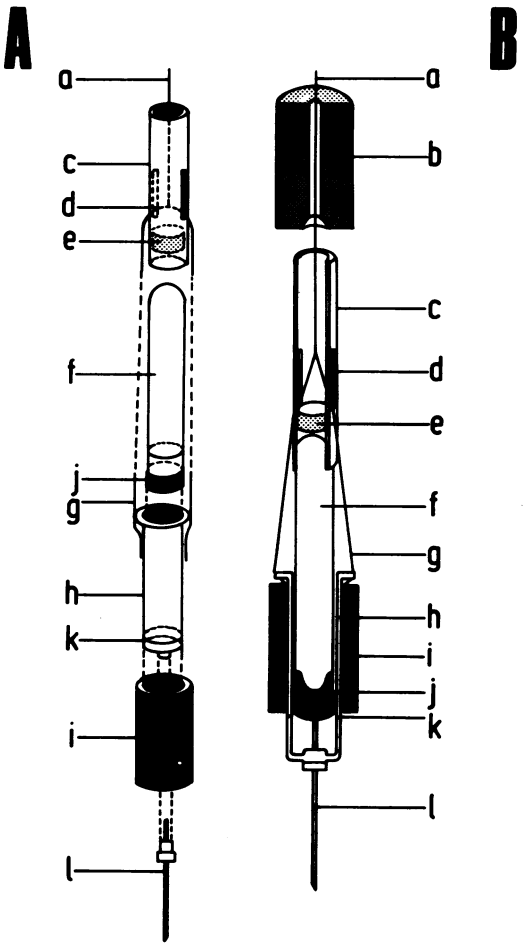

FIG. 1. *Bacteriological water sampler. A, Exploded view; B, view in section. (a) Hydrowire, (b) messenger, (c) slotted tube, (d) slot, (e) rubber bung, (f) Vacutainer, (g) nylon line, (h) needle mount, (i) lead sleeve, (j) Vacutainer stopper, (k) guide line on needle mount, (l) Vacutainer needle.*

stored or incubated without subsequent transfer and returned to the laboratory for examination.

Two assumptions made about the performance of the device during sampling were tested experimentally. The first was that organisms carried down on the outside of the sampler would cause little or no contamination of the sample being taken. The second was that, due to the pressure differential between the surface and the depth to be sampled, some of the sample would flow out of the Vacutainer as the sampler was drawn to the surface, preventing contamination due to the entry of material from the overlying water layers.

Extent of contamination during lowering of the sampler. A culture of *Serratia marcescens* (NCTC 1377) in nutrient broth (Difco) was used to contaminate the needles on the sampler just before it was lowered into the water. A needle and Vacutainer were mounted in the sampler and the needle was dipped into a culture of *Serratia* containing approximately 10^4 bacteria per ml for 30 s. Ten replica samples were taken at a depth of 5 m and, from these, 0.1-ml amounts were spread onto nutrient agar plates and incubated 48 h at 28 C. A second set of samples was taken at a depth of 25 m in which the needles were contaminated with a culture of *Serratia marcescens* in nutrient broth (Difco) containing approximately 10^9 bacteria per ml. Logarithmic dilutions were prepared and 0.1-ml amounts were spread in triplicate onto soil extract agar (3) to which 1.0 g of yeast extract (Difco) per liter had been added. These were incubated at 28 C so that an estimate of total aerobic heterotrophic bacteria in the lake water could be obtained. Triplicate spread plates were also prepared on nutrient agar (Difco) and incubated at 28 C to detect any *Serratia* contamination of the samples.

Serratia marcescens produces the red pigment prodigiosin at 28 C and not at 35 C (1). Red colonies on the nutrient agar plates were picked off and inoculated onto two nutrient agar plates (Difco); one was incubated at 28 C and the other at 35 C. Only those colonies that were red at 28 C and colorless at 35 C were considered to be the marker strain. As an additional check, all isolates were tested for motility, were Gram stained, and were examined microscopically.

Labeling of bacteria with tritium. A tritium-labeled culture of *Pseudomonas aeruginosa* OT15 was prepared by the following method. A 0.5-ml inoculum of an overnight culture of *P. aeruginosa* OT15 in brain heart infusion (Difco) containing 0.4% potassium nitrate was introduced into 10 ml of brain heart infusion/nitrate broth and incubated at 37 C. Once the bacteria had reached the logarithmic growth phase, 100 μCi of a mixture of [5-^{3}H]uracil and [6-^{3}H]uracil was added and the cells were incubated a further 4 h.

Testing the extent of sample loss during sampler retrieval. Samples (500 μl) of tritium-labeled cells were added to Vacutainers giving a final count of 184,160 dpm. Samples of lake water were then collected into some Vacutainers and the radioactivity remaining was compared with that in Vacutainers not used for sampling. The sample volume varied from 9.0 to 11.0 ml and this was simulated in the controls by adding 9.5 ml of diluent to the input radioactivity. Subsamples (500 μl) of both test and control specimens were then filtered through 25-mm membrane filters (Millipore Corp.; 0.45-μm average pore diameter) which were washed, placed in scintillation vials, and dried in a hot air oven. Scintillation fluid (5 ml of 0.8% butyl 2-phenyl-5-(4-diphenyl)-1,3,4-oxadiazole [Ciba] in toluene) was added to each vial and the radioactivity was measured in a Packard Tri-Carb 2002 liquid scintillation spectrometer. The efficiency of counting was estimated by the external standard channels ratio method (2).

RESULTS

Determination of sampler contamination. The estimate of viable bacteria in the inocula.

used to contaminate the needles was 2.9×10^4 and 1.2×10^9 bacteria per ml for the first and second experiments, respectively. The estimated number of aerobic heterotrophic bacteria in the lake water was 5.4×10^4 bacteria per ml. After contaminating the needles with *Serratia* at a concentration of 1.2×10^9 bacteria per ml, 180 viable cells of *Serratia* per ml were detected in the lake water samples as determined by the viable count method. When the needles were contaminated with cells at a concentration approximating that of the number of bacteria in the lake water (10^4 bacteria per ml), no colonies of *Serratia* were found on any of the plates prepared from the 10 water samples. Therefore, under normal sampling conditions, there is no significant contamination of the samples due to bacteria being carried down on the sampler.

Estimation of material lost during sampler retrieval. To detect loss of sample during retrieval, total radioactivity in the Vacutainers was calculated from the radioactivity (disintegrations per minute) in the 0.5-ml subsamples and from the volume of liquid (estimated by weighing) in each Vacutainer.

Table 1 shows that the radioactivity remaining in the Vacutainers after sampling is considerably lower than the controls. When the variances of the test samples and controls were compared using the F distribution, the calculated value of F was 29.92, where $F_{1,7} = 29.25$ at the 0.1% significance level. Therefore, the null hypothesis postulating that there is no significant loss of material from the sampler during its recovery is rejected at the 0.1% significance level.

TABLE 1. *Loss of ^3H radioactivity from Vacutainers during sampler retrieval*

Sample no.	Total radioactivity in Vacutainer[a]		Percent loss of radioactivity[b] $\left(\frac{\bar{x} - y}{\bar{x}} \times 100\right)$
	Input radioactivity (controls) (x)	Radioactivity remaining after sampling (y)	
1	151,629	50,189	71.59
2	132,241	51,389	70.91
3	177,346	72,787	58.80
4	245,500	46,643	73.60
5		27,215	
Mean	$\bar{x}$ 176,679	$\bar{y}$ 49,645	$\frac{\bar{x} - \bar{y}}{\bar{x}} \times 100 = 71.90$
Standard deviation	49,460	16,209	

[a] Disintegrations per minute per milliliter times final volume (milliliters in Vacutainer).

[b] Loss of radioactivity in each sample as a percentage of the mean radioactivity for the controls.

DISCUSSION

Samplers developed for microbiological work have a number of requirements in common with those used for chemical studies. It should be possible to trigger the device by remote control and to recover it without contaminating the sample with organisms from other water layers (4). An additional requirement of microbiological samplers is that they should be sterile prior to use, and since it is not always possible to satisfactorily sterilize materials that can easily be rendered chemically clean this factor has to be considered when designing these devices. The device described satisfies these requirements as it utilizes readily available and easily sterilized or presterilized components, is triggered by remote control, and eliminates contamination by organisms from other water layers (experiments 1 and 2). In addition, it has the advantage of enabling the bacteria to be cultured in the collecting vessels, thus eliminating the need to provide sterile or, in the case of anaerobic work, oxygen-free conditions in the field for transferring organisms to culture flasks. This is achieved by partly filling the Vacutainers with a suitable growth medium in the laboratory and sampling directly into that medium. This direct sampling is an important consideration when handling anaerobic bacteria because of the difficulty of providing oxygen-free conditions in the field.

A number of different methods have been employed in the preparation of media-filled Vacutainers. The manner in which the media are dispensed is dependent upon the nature of the medium, those with heat-labile components requiring filter sterilization rather than autoclaving. Heat-stable media are usually autoclaved in the Vacutainer tubes. However, prereduced media with or without heat-labile components are normally sterilized in bulk under oxygen-free gas and transferred to sterile, gassed Vacutainers using a silicone rubber supply line and steel hub needle. The medium is drawn into the Vacutainer by the vacuum when the needle is pushed through the stopper, and oxidation in the supply line is minimized if the medium is kept dripping from the needle between transfers. Vacutainers are supplied silicone coated; this minimizes oxygen diffusion

into media, or samples, stored in these tubes. Prereduced media have been stored in this laboratory under these conditions for periods of up to 2 months without oxidation being detected. Vacutainers with silicone rubber stoppers may be found to reduce the rate of oxygen diffusion compared with conventional stoppers.

When water samples are required for chemical as well as microbiological studies, larger volumes are required. The device may be readily modified to draw larger samples subject to the availability of different sizes of Vacutainers and their accessories. However, the device described was designed to collect small samples from which microorganisms could be isolated and, as such, is unsuitable for collecting large volumes. It should be noted that, in essence, the device comprises a small entry orifice coupled to a vacuum source and may therefore give rise to shear forces at the orifice. This may preclude the use of this device when collecting vacuolated or fragile microorganisms, although further testing will be required to establish this. If shear force damage does occur, it may be reduced or eliminated by using wide bore needles or a reduced vacuum source.

Despite these problems, the device has proved very useful for collecting water samples. Vacuolated bacteria have been cultured from the samples, suggesting that shear force damage may be minimal. The likelihood of the sample being contaminated by bacteria carried down on a sampler has been found to be a chance event and not, therefore, a significant source of contamination (experiment 1). It has also been found that there is a significant loss of radioactivity, and therefore sample, from the device as it is drawn to the surface (Table 1). Contamination of the sample by material from the upper water layers therefore is rendered extremely unlikely, as the backflow of sample out of the entry orifice prevents the entry of material from those layers.

The sampler described is inexpensive, easily constructed, and versatile. It is particularly useful when several groups of microorganisms with different metabolic requirements are being studied concurrently. The Vacutainers are prepacked with media suitable for the growth of each group of organisms studied, and because the Vacutainers are readily interchangeable the device has been found to be particularly convenient for this kind of study. Also, the rate of sampling is readily increased by mounting the device on a fishing rod with a conventional fishing reel. The quick retrieval of the device facilitated by this modification enables samples to be collected in as little as 1 to 2 min at depths of up to 25 m.

ACKNOWLEDGMENTS

I am indebted to S. L. Bilimoria for his comments and suggestions on the design of the radioactivity experiments and during the preparation of the manuscript; Carolyn Burns for her assistance in the collection of samples from Lake Hayes; D. R. Musgrave for the preparation of the tritium-labeled bacteria; and Y. Joel for the preparation of the figure.

LITERATURE CITED

1. Breed, R. S., E. G. Murray, and N. R. Smith (ed.). 1957. Bergey's manual of determinative bacteriology, 7th ed., p. 359–361. Ballière, Tindall, and Cox, Ltd., London.
2. Kamp, A. J., and F. A. Blanchard. 1971. Quench correction in Cerenkov counting: channels ratio and external source channels ratio methods. Anal. Biochem. **44:** 369–380
3. Löhnis, F. 1920. Landwirtschaftlich-bakteriologisches Praktikum, 2nd ed. Gebrüder Borntraeger, Berlin.
4. Sorokin, Y. I., and H. W. Jannasch. 1972. Sampling techniques, p. 40–44. *In* Y. I. Sorokin and H. Kadota (ed.), Techniques for the assessment of microbial production and decomposition in fresh waters, 1st ed. Blackwell Scientific Publications, Oxford.