AN APPARATUS FOR OBTAINING WATER FROM DIFFERENT DEPTHS FOR BACTERIOLOGICAL EXAMINATION

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(With 3 Figures in the Text)

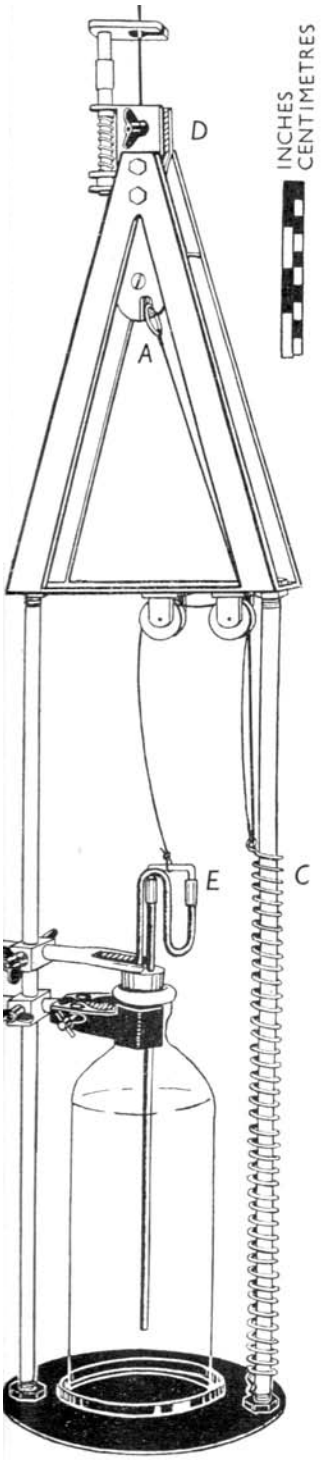
The study of the bacteria of natural waters is dependent on a simple and effective sampling technique. Any routine sampling procedure designed to obtain a vertical series of samples at one station must not only exclude any possibility of contamination of the sampling vessel and its contents while it is lowered to, and raised from, the depth at which the sample is taken, but must also be capable of straightforward operation on a boat or ship under rough conditions. The sampling vessels should be of a standard, interchangeable and robust pattern, and their preparation and sterilization should be a matter of simple routine. It should be possible to use them repeatedly and they should be of sufficient capacity to permit physical and chemical as well as a bacteriological examination of the sample.

Various types of apparatus which have been used for obtaining samples at different depths in rivers, wells and lakes, are described by Thresh, Beale & Suckling (1933), Ohlmüller & Spitta (1931) and by Angerer (1936). Benecke (1933) gives references to apparatus used by various investigators in the sea. Two main types of apparatus can be recognized. In the first type the sampling vessel is an evacuated, sterile bulb or tube, the neck of which is drawn out and fused. Differences between samplers of this type are confined to variations in the size and shape of the evacuated vessel and the means by which its neck is broken under water at the required depth. A convenient pattern often employed by continental workers was described by Ruttner (1924). American workers have frequently used modifications of Wilson's (1920) design. In the second type of sampler the sterile vessel is provided with a stopper carrying a short tube and a long one reaching nearly to the bottom of the vessel. Before sampling, these tubes, which are sterilized with the vessel, are closed by various devices. The vessel, filled with air, is lowered to the required depth, and the tubes are opened. Water enters the vessel through the long tube and the air escapes through the short one. A device described by Thresh *et al.* (1933, p. 197) represents one of the simplest examples of this type. The sampling vessel is a bottle enclosed in a lead casing and the tubes are closed by a loop of rubber tubing which is pulled off at the required depth by jerking

the cable. On p. 198 the same authors describe an apparatus which is often used in this country for obtaining samples from depths of "300 ft. or more" in wells. The bottle is enclosed in a pressure casing and the tubes are closed by long-handled taps which are opened at the required depth by sliding a messenger weight down the cable. The main disadvantage of this apparatus for routine work appears to be that the casing and bottle must be sterilized before taking each sample. Schach (1938) has recognized the disadvantages of most of the types of apparatus hitherto employed for routine work in the sea and has designed a sampler which takes standard glass tubes of 300 c.c. capacity. The inlet and outlet tubes are each closed with a 20 cm. length of rubber tubing plugged at the other end with short lengths of fused glass tubing. These are smashed under water at the required depth by a messenger weight. The elasticity of the rubber tubing swings the inlet and outlet tubes well away from the rest of the apparatus to avoid contamination.

None of the samplers so far described can be considered to have fulfilled all the conditions set out in the first paragraph. The preparation and use of evacuated vessels is tedious and the vessel can rarely be used to take more than one sample. In these respects the air-filled sampling vessels offer definite advantages, but our experience has shown (see below) that the assumption that there is no mixing of the sample with upper water, as the sampler is raised to the surface with the outlet and inlet tubes open, is unjustified. In many cases such mixing would be negligible but it is better to exclude it entirely if possible. The limits imposed by water pressure on the use of vessels, evacuated or air-filled, without pressure protection, may be important if samples are required from considerable depths. Waksman, Reuszer, Carey, Hotchkiss & Renn (1933) have sampled with unprotected evacuated tubes from depths of 330 m. in the sea; Issatschenko (1929) has employed similar apparatus at depths of 600 m. Schach (1938) has successfully used his apparatus to depths of 50 m. in the North Sea, and litre bottles have been used without protection in Windermere (see below) at depths of 50–60 m. By using vessels of thick wall and small diameter it should be possible to obtain samples at considerable depths without the added complication of a water-tight pressure casing.

The apparatus described here was constructed as the result of a series of experiments carried out at the suggestion of Dr C. B. Taylor and with his co-operation. It has proved itself satisfactory in use for routine sampling over a period of nearly two years in connexion with his investigation of the bacteria of the English Lakes (Taylor, 1939, 1940). Its construction is simple (see Figs. 1 and 2, which are scale drawings) and its operation straightforward. The sampling bottle is held by an adjustable clamp in a framework heavy enough to sink the bottle filled with air. Standard 1 l. Winchester sampling bottles have been used, but the apparatus is designed to take bottles of a larger or smaller size. The bottle is provided with a rubber stopper carrying two tubes, one long and one short. The latter is bent into a double loop for



The apparatus ready to take a sample.

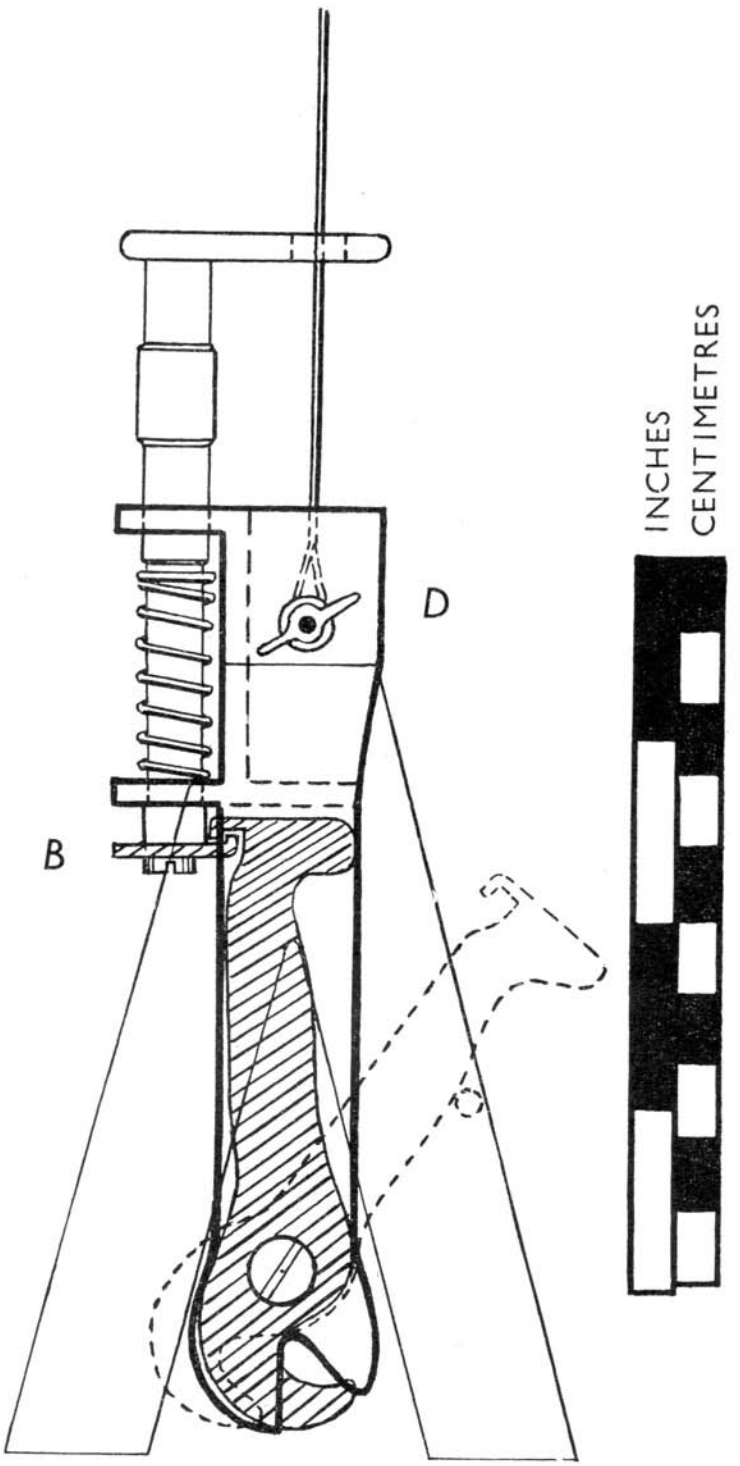


Fig. 2. A diagrammatic side view of the release mechanism.

https://doi.org/10.1017/S0022172400028138 Published online by Cambridge University Press

a reason to be explained later. These tubes are made of copper to avoid breakage. Bending the tubes is facilitated if they are packed with sand. The upper ends of both tubes are at the same level and are closed by a link of glass rod or other suitable material, attached to the tubes by short connexions of rubber tubing. The sampling operation involves the removal of this link under water by means of a spring which is released at the desired depth.

The sampling bottles, with rubber stopper carrying the tubes fitting loosely in the neck, are sterilized in an autoclave for 20 min. at 15 lb. pressure. The same number of glass stoppers are wrapped separately in paper and sterilized at the same time. After sterilization and cooling the rubber stoppers are fixed firmly in the necks of the bottles, after which they may be transported to the sampling station.

The details of the releasing mechanism are shown in Fig. 2. It is a modification of a standard pattern supplied by Friedinger, Lucerne, for use with closing plankton nets. The pivoted link, which is shaded in Fig. 2, was lengthened to supply the necessary leverage to hold against the fairly powerful spring (Fig. 1 *C*). Before lowering the apparatus, and after fitting the bottle in the frame, the ring *A* (Fig. 1) is placed over the lower hook of the pivoted link which is then set in the position shaded in Fig. 2. It is held in this position by the catch *B* (Fig. 2) which is mounted at the base of a sliding pillar and held up in position by a small compression spring shown in Fig. 2. In this way the ring *A* is securely held and the spring *C* (Fig. 1), which is attached to ring *A* by a thin steel (Bowden) cable, is extended. Also attached to the top of the spring at *C* is a cord which passes over two pulleys. This cord is now attached to the link at *E* (Fig. 1). This is most conveniently done by simply tying it on. The rubber stopper is held down by an adjustable fork so that it shall not be jerked out when the spring is released. The winch cable is passed through the slotted plate mounted on the top of the sliding pillar in the release mechanism and is secured by a wing-nut at *D*. The apparatus is then lowered on a winch (Friedinger pattern, recording depth in metres) to the desired depth, and a messenger weight similar to the one described on p. 198 of Thresh *et al.* (1933) is dispatched down the cable. When it hits the slotted plate the sliding pillar and catch *B* are momentarily depressed, the pivoted link falls to the position indicated by a broken line in Fig. 2, and the ring *A* is released. The contraction of the spring pulls out the link at *E*. If the cord is securely knotted the link can be recovered and used again. Water enters the bottle through the long tube and air escapes through the short one. One minute is allowed for filling and the apparatus is hauled up. The bottle is removed from the frame and a sterile glass stopper quickly substituted for the rubber stopper and tubes.

During preliminary tests both inlet and outlet tubes were straight and of glass. When the bottle and tubes were filled with a coloured solution, closed, lowered to 50 m., opened and hauled up, it was found that water had displaced the solution to the bottom of the long tube. This phenomenon was repeated

in all similar tests and shows that some mixing of the contents of the bottle with water from other layers may occur. This can be prevented by bending the outlet tube as shown in Fig. 1. After the bottle has filled, a bubble of air is left in the top of the loop and this effectively prevents any circulation in the system while it is being hauled up. When removing the stopper and tubes from the full bottle the best practice is to close the top of each tube with a finger until the tubes and the water they contain are lifted out of the bottle.

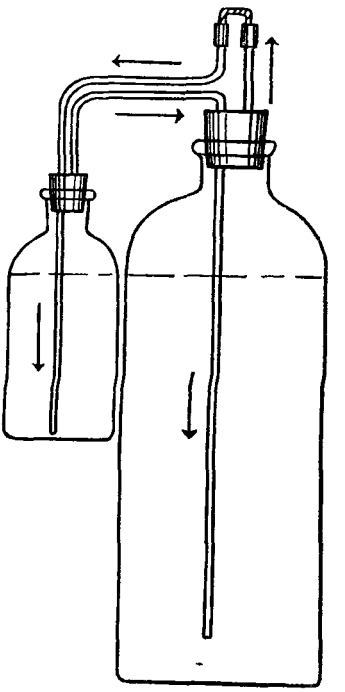


Fig. 3. A suggested arrangement for taking samples for bacteriological examination and dissolved oxygen determination simultaneously. Arrows indicate the direction of flow.

One litre of sample is ample for bacteriological purposes and sufficient for some chemical determinations and microscopic examination as well. It has been found that some bottles liberate appreciable amounts of silica into the water, probably as a result of the sterilization process. If silica determinations are made, the result should be checked on a duplicate sample obtained by other means. Samples obtained in the manner described above clearly cannot be used for dissolved gas determinations. Samples for the dissolved oxygen determinations made during Taylor's (1940) investigation were taken separately with a Friedinger water bottle. In the absence of other means, however, samples for the determination of dissolved oxygen as well as for bacteriological examination could be obtained with the bacteriological water sampler with an arrangement similar to that shown in Fig. 3. The

water entering the large sample bottle must first pass through the smaller bottle. In this way an adequate flushing of the latter is assured. Both bottles and tubes must, of course, be sterilized.

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(*Received for publication* 18. VII. 40—Ed.)