July 12, 1949. A. A. HIRSCH 2,476,093

BACTERIOLOGICAL TEST BOTTLE

Filed Sept. 25, 1943.

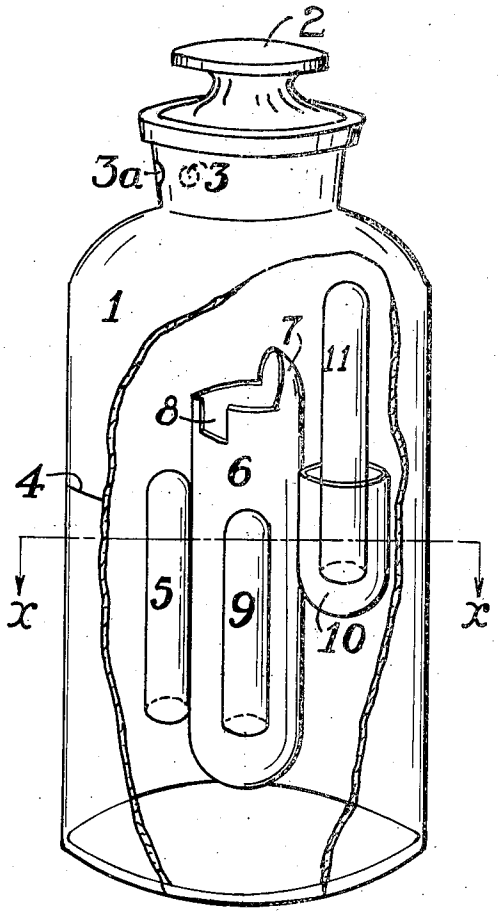


Fig. 1

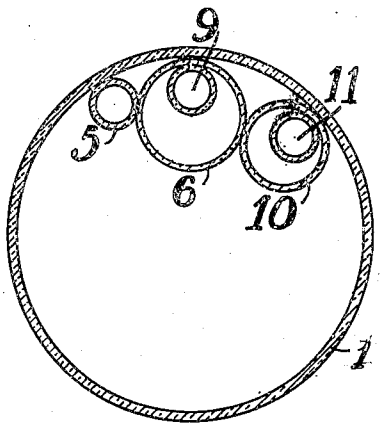


Fig. 2

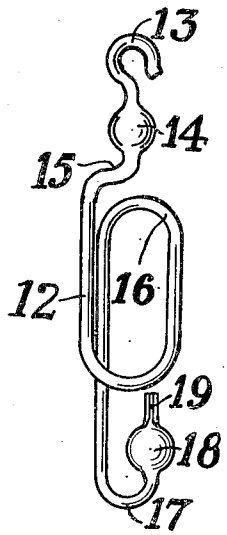


Fig. 3

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Patented July 12, 1949 2,476,093

UNITED STATES PATENT OFFICE

2,476,093

BACTERIOLOGICAL TEST BOTTLE

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Application September 25, 1943, Serial No. 503,786

4 Claims. (Cl. 195—140)

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The invention relates to improvements in bacteriological sampling and testing containers with special application to testing natural and treated waters and other potable materials for the presence of gas-forming bacteria, particularly the coliform group. The objects of my improvement are first, to provide a sampling bottle containing fermentation tubes attached to its interior walls; second, to present a series of containers of various volumes to allow estimation of bacterial density from the size or sizes of sample portions showing gas formation; third, to offer a device and method whereby for water supplies or other liquid products the operations of sampling, inoculation, and culture may be performed simultaneously in a single vessel; and fourth, to provide a means for subsequent confirmatory culture by means of a secondary broth tube which is lowered into the same bottle. These improvements are designed particularly to facilitate performance of tests for the presence of coliform organisms in liquids according to the descriptions and explanations detailed below.

The combination sampling-inoculation-culture bottle is illustrated in the drawing, in which Figure 1 is a cut-away perspective of the assembled bottle and primary fermentation tubes; Figure 2 a section through the bottle along the line x—x; and Figure 3 a vertical view of the supplementary culture tube for confirmatory tests.

Referring to Figures 1 and 2: 1 is a bacterial sample bottle, which may be of conventional shape, covered by a ground glass stopper 2 through which an air hole 3 has been perforated to coincide with a corresponding port 3a in the neck of the sample bottle. A mark 4 is lined on the sample bottle to indicate the proper level for filling.

Permanently fused or otherwise attached as with stainless steel clips or by other suitable means of fastening to the interior of the sample bottle are an inverted vial 5 and two glass Durham tube assemblies contained in tubes 6 and 10. 6 is a test tube, inside of which and towards whose bottom is fused the inverted vial 9. A segment of a hood 7 and a notched lip 8 provide liquid volume control at the top of tube 6. Inside tube 10 is fused the inverted vial 11 as shown.

Figure 3 shows an auxiliary looped tube 12 for secondary fermentation tests; 13 is a hooked end to provide lifting facility for a wire hook; 14 is a bulb to provide wastage volume; 15 is an offset bend to allow nearly true vertical suspension during transfer and also to minimize overall width; 16 is the looped portion for retaining gas bubbles

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formed in secondary fermentation; 17 is a bend to block entry of externally formed gas; 18 is a bulb in which the interface is formed between primary and secondary broths; and 19 is a capillary nozzle to minimize wastage of secondary broth during insertion of auxiliary fermentation tube 12 into the sample bottle 1. Overall length of the secondary fermentation tube 12 must be somewhat greater than the diameter of sample bottle 1 so that an oblique position of tube 12 is assured when submerged into bottle 1. The length of that portion of tube 12 from the bottom of bend 17 to the top of loop 16 must be somewhat less than the height of liquid in fermentation tube 6 in order to keep the loop full of secondary broth. To permit insertion, the greatest width across tube 12 must be kept slightly less than the diameter of fermentation tube 6.

Typical procedure for using this bacteriological test bottle for detection, confirmation and enumeration of coliform bacteria is explained in stepwise fashion below:

1. Clean test bottle 1 and interior glassware with washing compound, rinse with water and drain, not necessarily dry.

2. Introduce one gram of dehydrated lactose broth powder into the bottle, add about 5 milliliters of distilled water to dissolve the media and prevent darkening during sterilization. Stopper the sample bottle with air vent open, and wrap tin foil, or any heat resistant sheeting, loosely around the stopper and mouth of the bottle.

3. Sterilize in an autoclave at 15 pounds per square inch steam pressure for 15 minutes.

4. After cooling off the bottle, twist the stopper slightly to close the air vent.

5. Sterilize sampling fixture and flush according to accepted practice.

6. Remove stopper from sample bottle without contaminating stopper or mouth of bottle, and fill the sample bottle with water or liquid up to mark 4 as shown in Figure 1. To provide sufficient sample for the distribution as described below, the required volume of water or liquid will be 160 milliliters. Mark 4 therefore is made at a level to contain 165 milliliters, since 5 milliliters of distilled water had been added to dissolve the media.

7. Stopper the bottle with air vent closed, and re-fit foil wrapper over stopper and mouth of bottle.

8. Swirl bottle, while holding it vertically, to mix the media uniformly throughout the sample.

9. Invert the sample bottle to displace air from all the internal tubes and fill them with sample-media mixture. Several inversions may be needed

to remove trapped air completely. Air displacement is made complete if the group of tubes 5, 6 and 10 are finally laid flat under the water level before setting the bottle upright. This operation distributes water sample into portions as follows: 103 milliliters of water-media mixture, corresponding to 100 milliliters of water sample, remains in the bottom of the sample bottle 1; 52 milliliters of water-media mixture, corresponding to 50 milliliters of water sample stands in tubes 6 and 9; and 10 milliliters of water-media mixture, corresponding to a like amount of water sample, is retained in tubes 10 and 11. In this manner a series of 100, 50 and 10 milliliter water inocula are obtained automatically in the sample bottle and furnished with the standard quantity of nutrient enrichment media. Splashing of liquid during later handling from tube 6 into tube 10 is prevented by the deflecting hood 7, and by having the overflow notch 8 open on the side away from tube 10. Liquid levels in tubes 6 and 10 will drop sufficiently on turning bottle 1 upright to prevent spill or splash over during ordinary handling.

10. Incubate the assembly at 37° centigrade or place it in a warm spot near this temperature, with air vent open under the loosened foil cover to maintain aerobic conditions in the culture.

11. Observe for gas formation in inverted vials 5, 9 and 11 at the end of any desired incubation periods, such as 18, 24 and 48 hours. If no gas has formed the test is usually considered negative for members of the coliform group of bacteria, and the test is terminated. If gas has been formed, that portion causing gas is presumed to contain coliform bacteria. This positive presumptive test should be confirmed by the secondary test below under steps 12 to 14, inclusive. Due to the relative size of inocula only rarely will gas form in the 10 milliliter portion in tube 10 without the concurrent appearance of gas in the 50 and/or 100 milliliter portions.

12. By means of a sterile wire hooked under bend 13 transfer aseptically tube 12, filled with sterile brilliant green lactose bile broth (2%) or other acceptable inhibitory broth, to the bottom of sample bottle 1, or lower it carefully into tube 6, should only the latter show gas formation. If only the 10 milliliter inocula is presumptively positive, a relatively rare but not impossible case, tilt the sample bottle to contaminate the 100 milliliter portion with spill-over from tube 10, allow time for gas collection in tube 5, and then introduce the brilliant green lactose bile broth tube 12 into the bottom of bottle 1.

13. Incubate the assembly containing the introduced brilliant green bile broth tube 12 at 37° centigrade for 48 hours.

14. Observe the brilliant green bile broth tube 12 for gas formation. A show of gas in loop 16 constitutes a confirmed test for the presence of coliform bacteria. Failure to show gas indicates absence of these bacteria and demonstrates that the primary gas formation was due to interfering organisms.

15. Bacterial density may be enumerated by noting the sizes of volumes positive or negative for primary gas formation, and noting whether confirmed or not. The most probable number method also may be used. These principles in step 15 are not new but are included to show their applicability to the bacteriological test bottle described herein.

Obvious alternatives to the materials, construction and usages described above are likewise contemplated and embraced in these specifications as

follows: first, thermoduric transparent materials other than glass may be employed; second, another type of stopper or closure or vent may be adopted without departing from the principles of this sample-culture bottle; third, the arrangement of interior Durham fermentation tubes and vials may be varied for manufacturing convenience or other purposes without affecting the principles of this apparatus; fourth, the primary gas tubes may be of the Cowles looped type instead of the Durham vial type; fifth, volumes other than those described may be selected; sixth, other numbers of internal fermentation tubes, of any volumes desired, may be provided; seventh, the volume of the sample bottle and of the portion of sample retained in its bottom may differ from the above description; eighth, other media than that specified in the above procedure may be used; ninth, other materials than potable water may be tested in this apparatus; tenth, other bacteria than the coliform group may be searched for and cultured; eleventh, other methods for confirming the presence of the coliform group than the device described here may be used; twelfth, other means for trapping entry or exit of gas from the secondary fermentation tube may be provided; and thirteenth, alternative methods for attachment of tubes other than by fusion, as illustrated in the drawings, may be provided by such devices as springs, clips, screws, or dowels of stainless steel or other suitable material.

Prior developments, particularly in the field of water bacteriology, which form the elements for the bacteriological test bottle described in these specifications are not claimed as new; these previous items include the Durham fermentation tube and vial, the Cowles fermentation loop, and the use of primary media introduced into the water sample bottle. These basic devices are modified and combined as described herein to enable procedures, conveniences, economies in time and equipment, speed in execution and simplicity in use not foreseen in the separate use of any of the component elements.

I do claim as new and therefore desire to secure rights under Letters Patent to the following features:

1. In combination with a bacteriological test container, a fermentation tube affixed therein, said tube having an open upper end provided with an overflow notch, and a hood partially covering said open end, said notch and said hood being diametrically opposite each other with said hood facing said notch whereby the two tend to prevent spillage of the tube contents in a direction opposite to that in which the hood faces.

2. In combination with a bacteriological test container, a closure for said container, fermentation tubes of various volumes attached securely therein on the same side thereof, and inverted vials affixed in said tubes, respectively, for indicating gas formation in individual tubes.

3. In combination with a bacteriological test container, a closure for said container, fermentation tubes of various volumes affixed in said container on the same side thereof, inverted vials attached securely within said tubes, respectively, for indicating gas formation in individual tubes, and a looped tube having a capillary bottom tip, a hooked top end and a narrow profile to facilitate insertion of said looped tube into said container or said fermentation tubes when closure is removed temporarily, for purpose of making secondary fermentation tests.

4. In a sterile test container having fermenta-

tion reservoirs and inverted vials attached therein, a method of performing fermentation tests which consists in introducing sterile broth, placing a closure having an air vent in the container with air vent open, again sterilizing and cooling the broth charged container if so desired, closing the air vent until such time as ready for sampling, introducing the sample to be tested into said container, stoppering the container with air vent closed, swirling the container to mix uniformly the broth and the sample, inverting the container to displace air from the fermentation tubes and inverted vials, initiating a confirmatory test by inserting into fermentation tubes and reservoir inside original test container a looped tube previously filled and sterilized with secondary broth, incubating the assembly in an upright position with the air vent partly open, and at the end of desired incubation periods observing for gas formation appearing in inverted vials or collected within looped tube.

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