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Rapid, Radioactive Test for Coliform Organisms

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As previously reported (*1, 2*) the use of radioisotopes for the detection of coliform organisms has been directed principally toward the development of a rapid presumptive test. Recent reports (*3, 4*) mentioned the possibility of achieving a direct, confirmed test by the radioactive method. During the past year, efforts have been concentrated to this end. This is a report on the status of the work.

Medium

The logical confirmatory medium was brilliant-green lactose bile broth utilizing the 1-C^{14} lactose used in the presumptive-test experiments. This labeled compound became unavailable, however, making it necessary to investigate the use of some other, preferably cheaper, labeled material. The use of formate was investigated because it has been used in a *Standard Methods* (*5*) coliform confirmatory medium (formate ricinoleate broth) and C^{14} formate is readily available at low cost. Radioactive sodium formate, in various concentrations, was incorporated into specimens of standard brilliant-green lactose bile broth (BGB), which were inoculated with known concentrations of coliform organisms. As a result, a sodium formate concentration of 0.01 per cent was selected for use. Higher concentrations than this were found to be toxic to the organisms, whereas concentrations below this rendered the detection of evolved $C^{14}O_2$ more difficult. Another change in method was the use of 2-oz ointment jars (Fig. 1) for incubation vessels rather than the combination aerating, culturing, and gas-trapping device previously used.

Procedure

The details of the test procedure being used are as follows. The BGB containing 0.01 per cent C^{14} formate is mechanically shaken for several hours prior to use. Shaking has been found to reduce nonmetabolic $C^{14}O_2$ to a reasonably consistent level. The shaken medium is apportioned into paraffin-coated planchets. Membrane filters, on which the bacterial content of the water sample has been concentrated, are placed in the planchets. The planchets are then put in individual ointment jars, the lids are screwed on, and the jars are incubated at 37°C for 3 hr. At that time, a

planchet containing several drops of a saturated barium hydroxide solution is added to each jar, and incubation is continued for an additional hour. During this time, the $C^{14}O_2$ evolved from the culture planchet is carried by diffusion to the barium hydroxide planchet, where it is precipitated as the carbonate.

This method has been found extremely efficient in collecting the $C^{14}O_2$, and it makes the operation very simple. At the end of the collection hour, the barium hydroxide planchet is removed, dried over a heat lamp, and counted in an internal-flow Geiger counter. For purposes of comparison, bacterial cell numbers are determined by colony counts from triplicate nutrient agar plates when pure strains are run, and by the standard presumptive and confirmed coliform tests when unknown samples are run.

In order to consider this method for use as a direct, confirmed test, it must first be established that the medium containing formate compared favorably with the standard confirmatory medium. Accordingly, a lengthy series of tests has been undertaken to compare the two media. A routine has been instituted whereby raw water from the Potomac River and samples from various points in the District of Columbia treatment plants are taken daily. These samples are run by the *Standard Methods* presumptive test for coliform organisms. All portions are subsequently inoculate into BGB confirmatory medium and, simultaneously, into BGB to which 0.01 per cent nonradioactive sodium formate has been added. Both sets of tubes are read in conformance with *Standard Methods*. To date, 1,158 pairs of tubes have been compared. There was

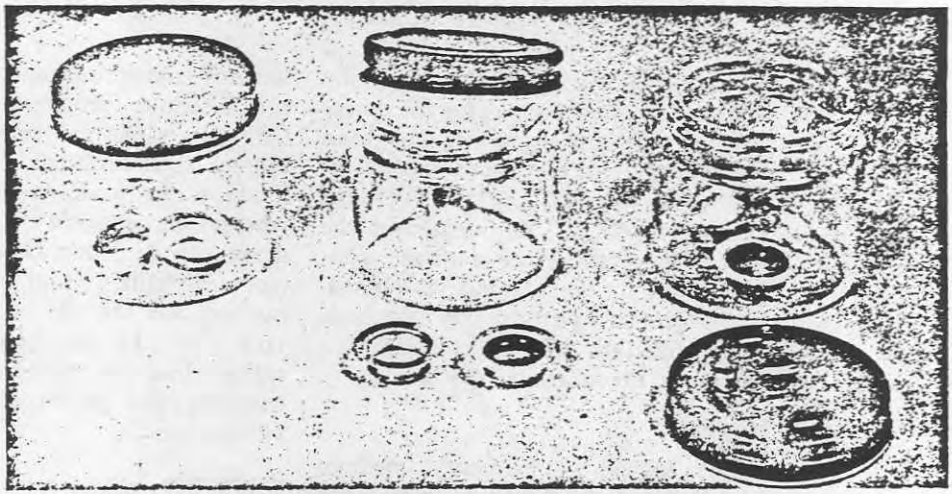

Fig. 1. Ointment Jars With Barium Hydroxide and Culture Planchets

Planchets containing cultures are incubated for 3 hr; barium hydroxide planchets are then added to jars and incubation continues for 1 hr. Evolved $C^{14}O_2$ then diffuses to barium hydroxide planchets and is precipitated as carbonate.

agreement between 1,155 pairs. These data will continue to be collected until the acceptability of the formate medium has been thoroughly investigated.

Proof that the formate medium is satisfactory for use in a standard, confirmed test does not mean that noncoliform organisms will not interfere in a shorter, radioactive test with that medium. Some of the noncoliform groups may live long enough to produce detectable gas in the radioactive test, but not long enough to produce a visible gas bubble in the standard test. This problem is being investigated.

Concurrently with these tests on river and filtration plant water, aliquots of the same samples are being run by the direct, confirmed, radioactive method. As these data are accumulated they will be reviewed and compared for consistency. The data will also be analyzed in an attempt to relate levels of evolved radioactivity with numbers of cells. Table 1 is a summary of quantitative information obtained from pure cultures (chilled to induce lag) of *Esch. coli* in the BGB C^{41} formate medium. The data represent triplicate determinations at each cell population in each test. Approximate cell counts were determined by triplicate plate counts. The activities have been presented for ranges of cell counts and the averages and standard deviations are given. The data show a reasonable degree of correlation between evolved $C^{14}O_2$ and numbers of cells.

Initial work on the river water and filtration plant samples indicates that several organisms do interfere. When

TABLE 1

Correlation Between Esch. coli *Population and Evolved Radioactivity*

Run No.	Evolved Radioactivity*—cpm								
	1–25 Cells	25–50 Cells	50–100 Cells	100–200 Cells	200–400 Cells	400–800 Cells	800–1,600 Cells	1,600–3,200 Cells	3,200–6,400 Cells
1	†	27	64	94	181	400	792	1,625	4,290
2	7		31		106				
3	11	37	89	155	224	449	906	1,820	6,271
4		37	52	135	391	673	1,373	2,283	6,060
5		24	39	93	164	298	768	1,336	
6	16	24	57	96	224	472	858	1,621	
7	15	28	40	105	245	755	1,676	4,000	6,262
8			14	36	109	254	669		
9		15	37	66	167	321	771	1,871	
10					117	247	520		
11					149	285	480		
12					358	488	985		
13						342	762	2,400	5,450
Avg	12	27	47	98	203	415	880	2,119	5,667
Avg deviation	±4	±7	±21	±37	±92	±163	±340	±841	±839

* Average of three replicates without sterile controls.
† Blanks indicate no data available.

isolated, they do not produce visible gas in the standard method, but do produce radioactive gas in the isotope method. The seriousness of the interference and the possibilities of preventing it are still unknown, as the work is in an early stage.

As a double check, organisms are being routinely isolated from Potomac River water taken from a sewage polluted zone and run by the radioactive confirmed test to determine the behavior of the normal range and populations of noncoliform groups as compared to the coliforms groups. Pure strains of organisms found in water have been obtained from the American Type Culture Collection and are being tested in a similar manner. This work is also in its initial phase. Of some 20 different organisms tested, only two have demonstrated interference. One is *Pseudomonas aeruginosa* and the other, isolated from river water, has not yet been identified.

It is planned to attempt to reduce or closely define the interference from noncoliform organisms and to continue the routine collection of the types of data cited above. When this has been accomplished the data will be submitted for consideration of the method as a standard one.

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