RAPID COLIFORM ORGANISM DETERMINATION WITH C^{14}

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The radioisotope test seeks to shorten the time required for coliform organism determination to several hours. It does so by incorporating C^{14} into an appropriate medium, inoculating in normal fashion, and detecting any gas evolved by its radioactivity rather than by waiting for visual evidence.

Since the method was last reported (1), changes in apparatus and procedure have been made. The test may currently be described as follows:

Method

Apparatus

The apparatus includes: 1-in. diameter membrane filter assembly (Figure 1) and 1-in. diameter filters, 1-in. diameter paper absorbent pads, pipets, vacuum pump, shaker, 1-in. diameter by ¼-in. deep aluminum planchets with flat lip ⅛ in. wide (Figure 1), 35 mm by 50 mm cover glasses, hot plate or heat lamp, gas flow geiger counter, and scaler.

Medium

The medium consists of British MF MacConkey broth (2) plus C^{14} formate: 3-per cent lactose, 1-per cent peptone, 1-per cent bile salts, 0.5-per cent NaCl, 0.0012-per cent brom cresol purple with 0.002-per cent C^{14} sodium formate (8mc/mM). Following preparation, the medium may be sterilized by autoclaving for 15 min at 15 psi or by membrane filtration. The medium, in a flask plugged with cotton, is then placed on the shaker and shaken overnight in order to reduce by atmospheric exchange small amounts of $C^{14}O_2$ generated in the sterile medium.

Procedure

An aliquot of the water sample, or an appropriate dilution, is filtered through a membrane filter using the one-inch filter and assembly in conventional fashion. The filter membrane is then placed in a sterile planchet which had been covered with a sterile cover glass. Five-tenths ml of sterile medium are immediately dispensed into the planchet by bulb pipet (Figure 1), immersing the filter, and the cover glass is again seated over the planchet. The planchets and cover slips are arranged on a small flat board for easy carrying as a group (Figure 2). After several samples have been prepared in this fashion, over a span of not more than 5 min, the group is placed in an incubator maintained at 44°C. Several sterile controls are also prepared as a check on sterility of the medium, and to measure the level of non-metabolic $C^{14}O_2$ evolved. After incubating all samples for three and one-half hours, the planchets are removed from the incubator and placed near an equal number of identical planchets, each containing an absorbent pad held in position at the bottom of the planchet merely by the snugness of the fit. Each pad is then moistened with five drops of a settled, saturated solution of barium

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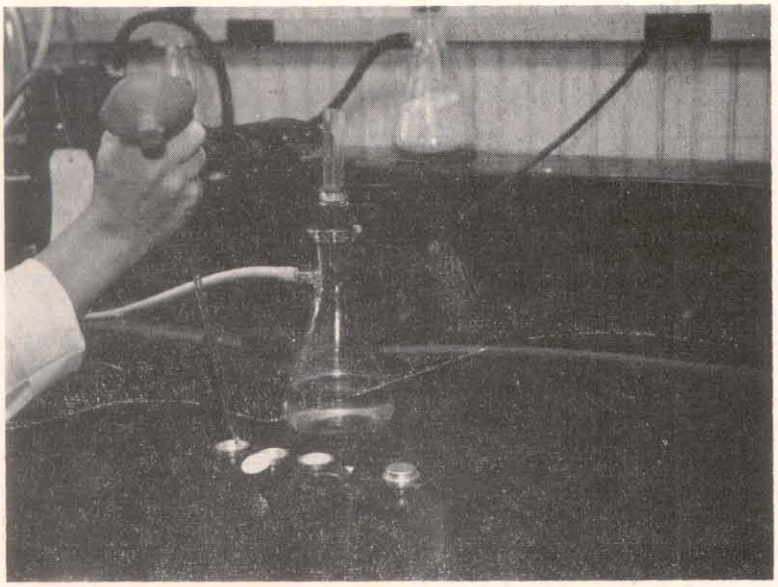

FIGURE 1.—Apparatus for radioisotope test. Labeled medium is added by bulb pipet to culture planchet containing membrane filter. The planchets, pads, and cover glasses are shown in various stages of assembly. The one-inch membrane filter apparatus is immediately behind the row of planchets.

hydroxide delivered from a five-ml volumetric pipet (Figure 3). One such planchet is inverted over the cover glass of each culture planchet (Figure 3). The cover glass is quickly removed and the planchet with the moistened pad

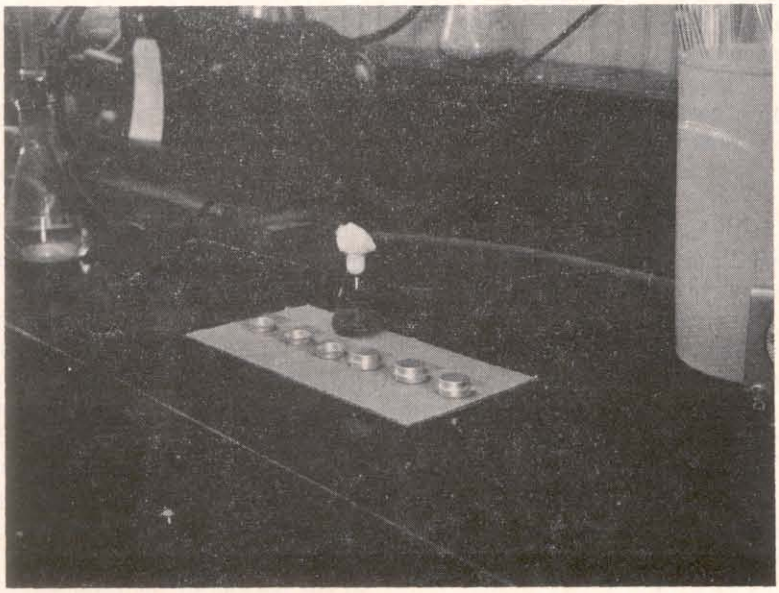

FIGURE 2.—Group of six coliform organism tests on carrying board. Three of the culture planchets have collection planchets positioned, but cover glasses have not yet been removed. Flask of labeled medium is displayed on carrying board for easy viewing.

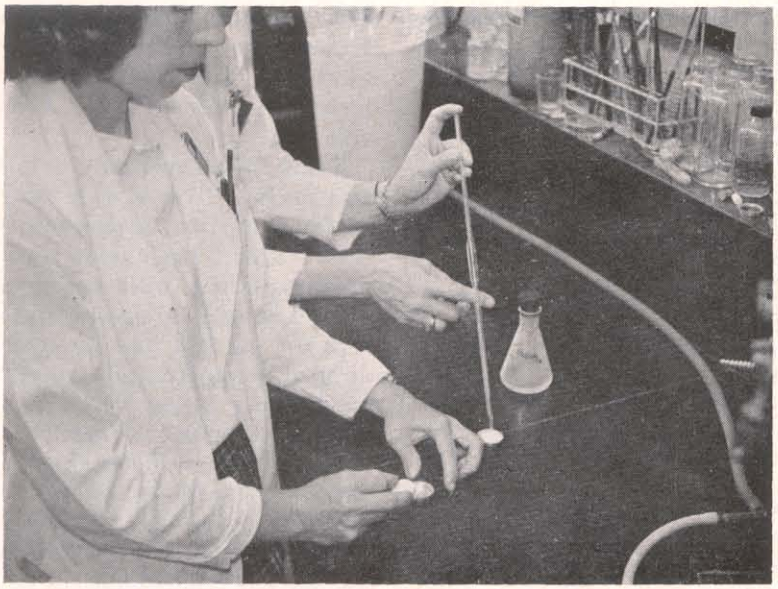

FIGURE 3.—Placing of $C^{14}O_2$ collection planchet. Solution of $Ba(OH)_2$ is added to pad in collection planchet in rear. Collection planchet is being inverted over culture planchet in foreground. Flask contains saturated solution of $Ba(OH)_2$.

placed over the culture planchet. The two planchets thus form a single volume and any $C^{14}O_2$ evolved from the culture planchet diffuses to the absorbent pad in the top planchet where it precipitates as barium carbonate. The paired planchets are then immediately returned to the incubator for 30 min, providing a total incubation period of 4 hr. The planchets are then removed

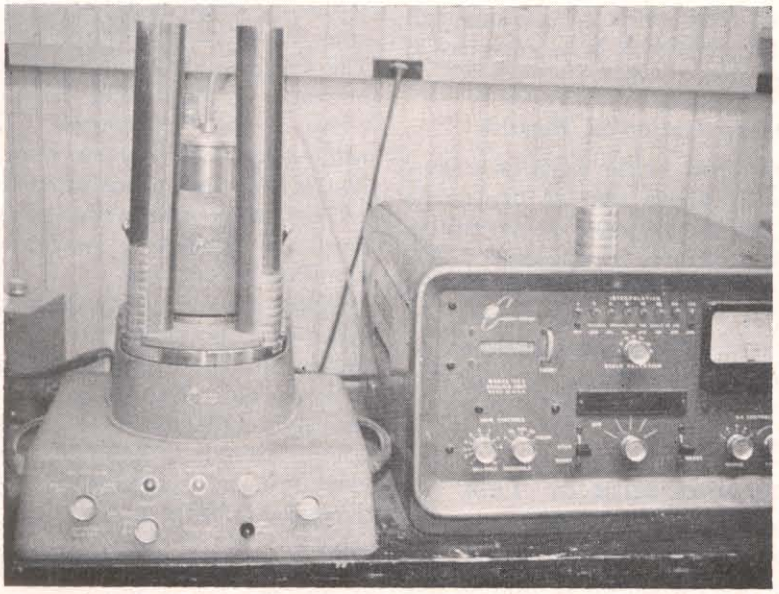

FIGURE 4.—Radioactivity counting equipment. Automatic gas flow counter is to left of scaler.

TABLE I.—Results of Four-Hour Radioisotope Test on *E. Coli* ATCC 8739 Using Nonfiltered Inocula and MF MacConkey Broth

Inoculum (No. Cells)	Avg cpm	cpm/Cell*
12	57	4.75
28	263	9.40
77	625	8.12
83	807	9.73
85	391	4.60
975	7,120	7.30
1,170	5,540	4.73
2,460	16,600	6.75
9,820	70,600	7.19
41,600	211,000	5.08

* Avg cpm/cell = 6.77.

from the incubator and separated. The pads, still in the planchet, are dried for several minutes on a hot plate or under a heat lamp. They are then counted for radioactivity, expressed in counts per minute (cpm), in the gas flow counter (Figure 4). Several drops of disinfectant are added to each culture planchet in preparation for discard. The total activity in each test portion is 1.15 μc and, while it is possible to dispose of the spent medium down the sink, rinse the planchets, and incinerate the pads, preference in this laboratory has been to package all wastes for shipment to a commercial disposer.

Interpretation of Results

Radioactivity in the pads at a level significantly above that of the sterile control pad is evidence of a positive test for coliform organisms. The amount of activity evolved by the organisms is related to the number of cells present. The current phase of the research seeks to define further the specificity of the test and the quantitative aspects.

Characteristics of Test

The radioisotope test is remarkably sensitive. Inocula of only a few cells make themselves known in four to five hours. When labeled formate was first incorporated into MacConkey broth, tube strength broth (3) was used. Later * the British MF MacConkey broth (2) was used. This proved to increase the sensitivity of the test several fold.

Table I presents results obtained with this medium over a wide range of inocula taken from suspensions of *E. coli* ATCC 8739. Each value is an average of 5 replicates with background and sterile control levels subtracted. Except for the fact that the inocula were applied by pipetting 0.1 ml rather than by filtering larger volumes, the tests were performed as described above. Cell counts were determined by nutrient agar pour plates. Here, as throughout this report, cpm/cell are based on the number of cells in the inoculum, not on the cell population at the end of the test period. It is, of course, the number of cells initially present that the test is designed to detect. The data show that the test easily detected 12 cells in 4 hr. This was the smallest inoculum applied in the series. The cpm/cell for inocula ranging from 12 to 41,600 cells showed random differences ranging up to two-fold. Much of this variation might be due to sampling errors incurred in pipetting small volumes of relatively dilute suspensions. The average cpm/cell of 6.77 could be increased by increasing the specific activity of the C^{14} formate. However, while some such increase may be feasible, it is desired to maintain the radioactivity associated with the test at a level low enough to preclude special handling precautions.

While the sensitivity data indicate a significant potential for the test, a number of important problems remain to be solved. Replicates run simultaneously seldom produce results whose spread exceeds a factor of two. However, tests performed with suspensions prepared at different times can produce

* At the suggestion of Dr. E. Windle Taylor, Director of Water Examination of the Metropolitan Water Board of London, England.

more widely ranging results, on occasion approaching an order of magnitude. Moreover, some strains of coliform organisms produce differing cpm/cell. The full extent of these differences and the importance of the strains producing them are not yet known. The specificity of the test has not yet been established and it is known that at least *Pseudomonas* and *Alcaligenes* interfere to some extent. Recently, this interference has been much reduced and, as can be seen from data herein, in the order of 1,000 cells of these species are required to equal the response from one cell of *E. coli* ATCC 8739. Finally, a problem exists relating to the use of the membrane filter to concentrate the organisms for inoculation. Because of the importance of the membrane filter to the development and ultimate use of the radioisotope test, this has been studied in some detail.

For reasons which will be discussed presently, it is believed that some of these difficulties also operate against currently accepted standard coliform determination procedures.

Calibrations

A frustrating need in the current phase of the research is the lack of an absolute coliform organism determination method against which to calibrate the radioisotope test. In order to establish the relationship between the quantity of $C^{14}O_2$ collected and the number of organisms initially present in the sample, that number must be known accurately. The great sensitivity of the radioisotope method makes it imperative to calibrate the test against a highly accurate cell count.

With pure cultures, this seems to offer little difficulty when the pour plate technique is used. However, the actual calibration must be carried out in mixed cultures of random composition encountered in surface waters. It then becomes necessary to determine the number of coliform organisms present in the unknown sample in order to check the specificity and quantitative aspects of the radioisotope test.

Thought was at first given to use of the Standard Methods MPN test (4). However, the statistical errors inherent in the method, as cited by McCarthy *et al.* (5), are too great to permit using the MPN test as a standard.

Use of the membrane filter technique seemed the most plausible way to obtain the information needed. When an aliquot of the sample was filtered for the radioisotope test, another portion could be analyzed by the membrane filter test. The results of the membrane filter test would become available the following day and the number of coliform organisms that had been present in the radioisotope test could be ascertained.

There was additional logic in standardizing against the membrane filter test. In the early stages of developing the rapid test, the test medium was inoculated by pipetting aliquots from prepared suspensions of coliform organisms. As research on the method progressed, it became desirable to test samples of water with relatively few coliform organisms. Portions taken directly by pipet or other means from the water to be tested could not provide a representative sample. It thus became necessary to concentrate the bacteria from such waters before inoculating the radioactive medium. The membrane filter seemed to provide a readily available means for collecting all organisms present in a sample of sufficient volume to insure statistical reliability.

The radioisotope procedure was modified accordingly, and the water sample was filtered through a membrane filter which was placed directly in the culture chamber and immersed in the labeled medium. Equal nonfiltered inocula were put through the procedure initially for comparison. It was immediately apparent that those cultures which had been filtered invariably evolved much less $C^{14}O_2$ on a per cell

basis than did the cultures which had not been collected on the filter membrane.

Investigation of Membrane Filter Effect

The following set of experiments was performed to investigate the difference between filtered and nonfiltered inocula in greater detail.

Filtered Inocula

Pure cultures of the test organisms cited below were grown for 24 hr in nutrient broth. A suspension of the culture used was made and serial dilutions prepared. Ten-ml portions of the appropriate dilution, based on experience, were filtered through 25-mm diam membrane filters using commercially available filter assemblies. In this manner, groups of six replicates each, with sterile controls accompanying each test group, were prepared. Five such groups were prepared for each culture. Using tube-strength MacConkey broth, because this work began before the use of MF MacConkey broth was introduced, the radioisotope procedure described earlier was then carried out with the following exception. At 1½ hr, 1 group of 6 tests and 3

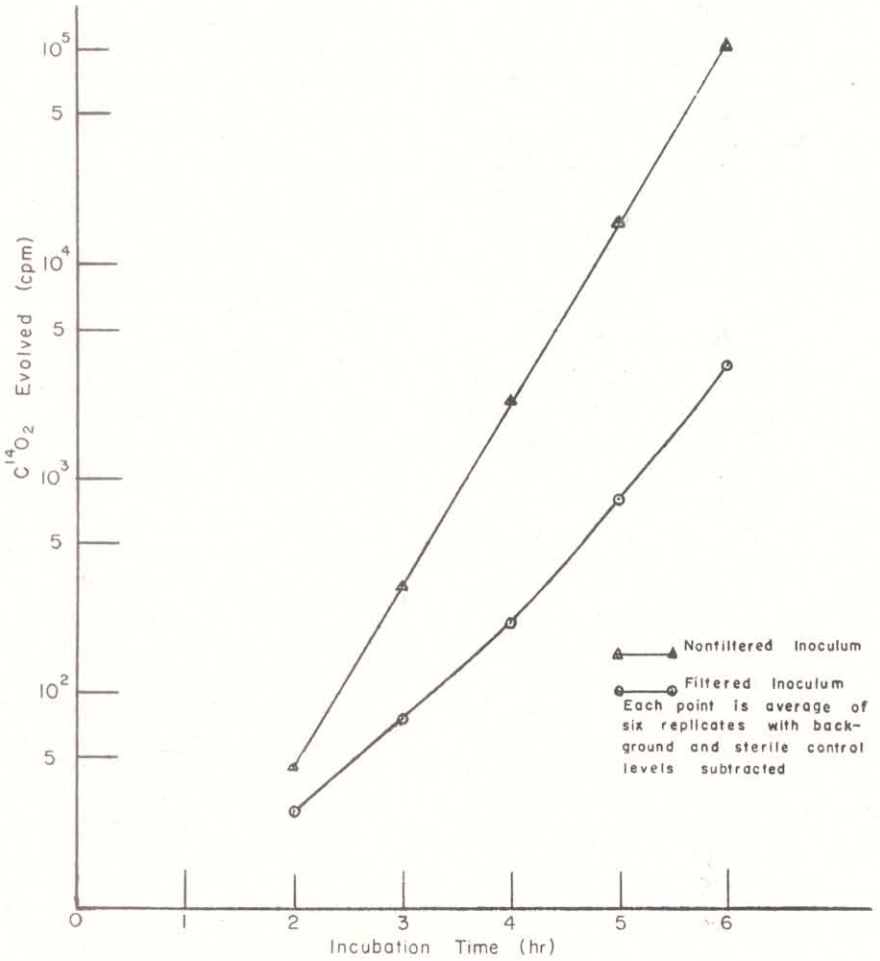

FIGURE 5.—Effect of membrane filter on *E. coli* ATCC 8739. $C^{14}O_2$ evolved as a function of time for filtered and nonfiltered equal inocula.

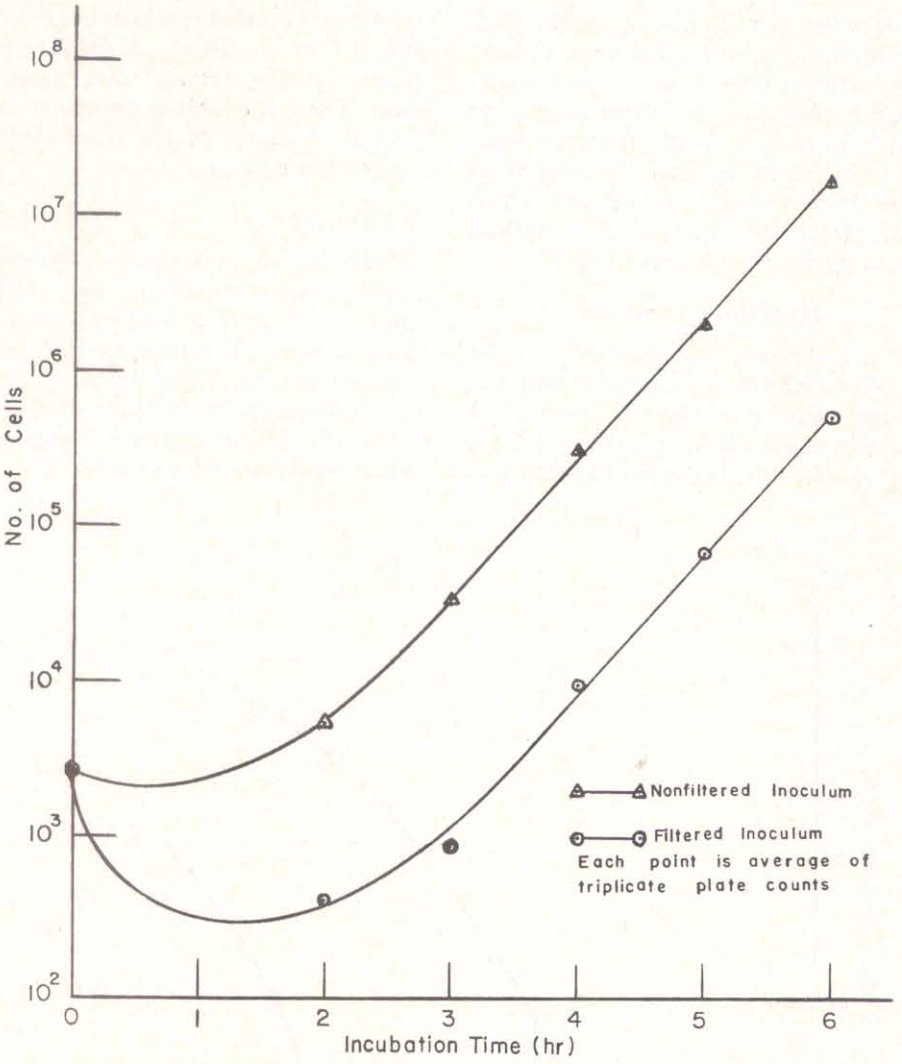

FIGURE 6.—Effect of membrane filter on *E. coli* ATCC 8739. Cell population as a function of time for filtered and nonfiltered equal inocula.

controls were removed from the incubator. Planchets containing the barium hydroxide moistened pads were inverted over each culture planchet and the cover glasses removed. The group was then returned to the incubator. Total time out of the incubator for this operation was approximately two minutes. One-half hour later, after a total elapsed incubation period of two hours, this group was removed from the incubator. The paired planchets were immediately separated and the planchets containing the barium hydroxide impregnated pads were dried on a hot plate. In this manner, the five equally inoculated groups were incubated for two, three, four, five, and six hours, respectively. In each case, collection of the $C^{14}O_2$ was made during the terminal one-half hour of incubation.

As soon as the paired planchets in each group were separated at the termination of the incubation period, the planchets containing the filters and cul-

tures were placed into a single flask containing a measured volume of buffered sterile water and shaken vigorously. Triplicate nutrient agar pour plates were then made directly from the flask and from serial dilutions made from the suspension in the flask. These pour plates were incubated 24 hr and the bacterial colonies counted.

Nonfiltered Inocula

The procedure was the same as that described for filtered inocula except for the method of inoculation. Inoculation was accomplished by pipetting 0.1 ml of the serial dilution 100-fold more concentrated than that dilution used in the filtered runs. This provided equal inocula to the filtered and pipetted tests. The cell counts were determined by triplicate pour plates of serial dilutions of the culture.

Test Organisms

Three test organisms were selected: *E. coli, Pseudomonas sp.,* and *Alcaligenes sp. E. coli* ATCC 8739 was selected as a typical coliform. The *Pseudomonas sp.* and *Alcaligenes sp.* were selected because they were isolated from cultures of Potomac River water and had seriously interfered with the spe-

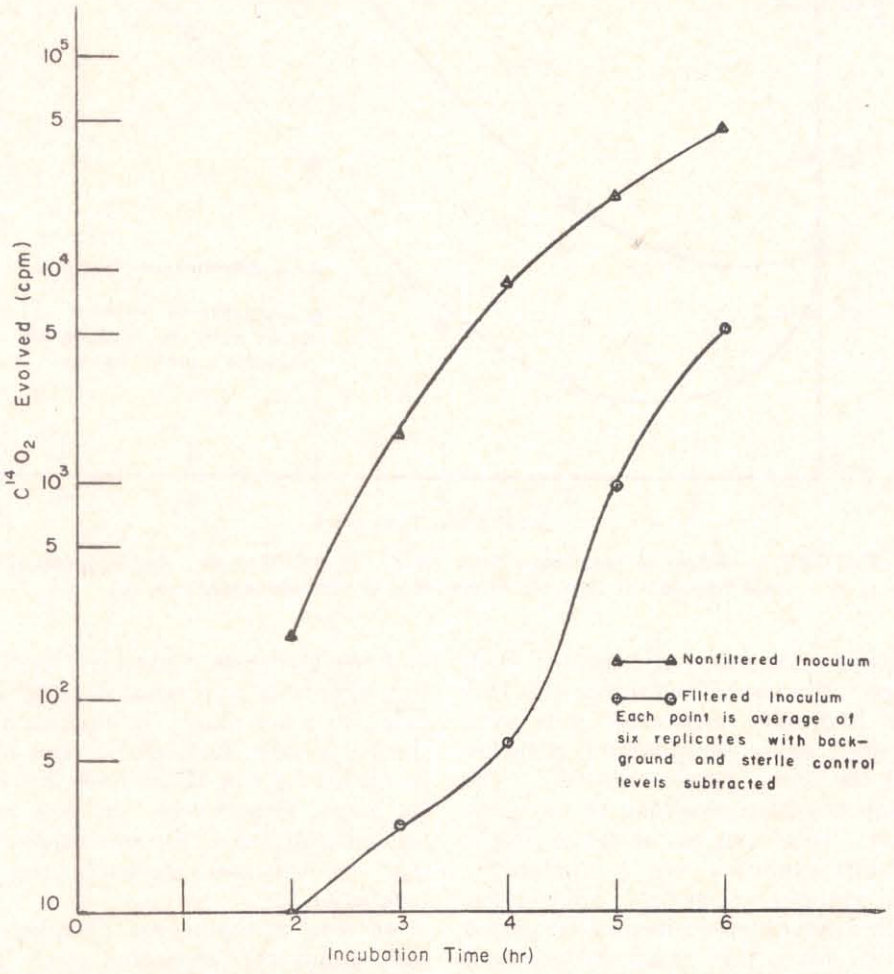

FIGURE 7.—Effect of membrane filter on *Pseudomonas sp.* $C^{14}O_2$ evolved as a function of time for filtered and nonfiltered equal inocula.

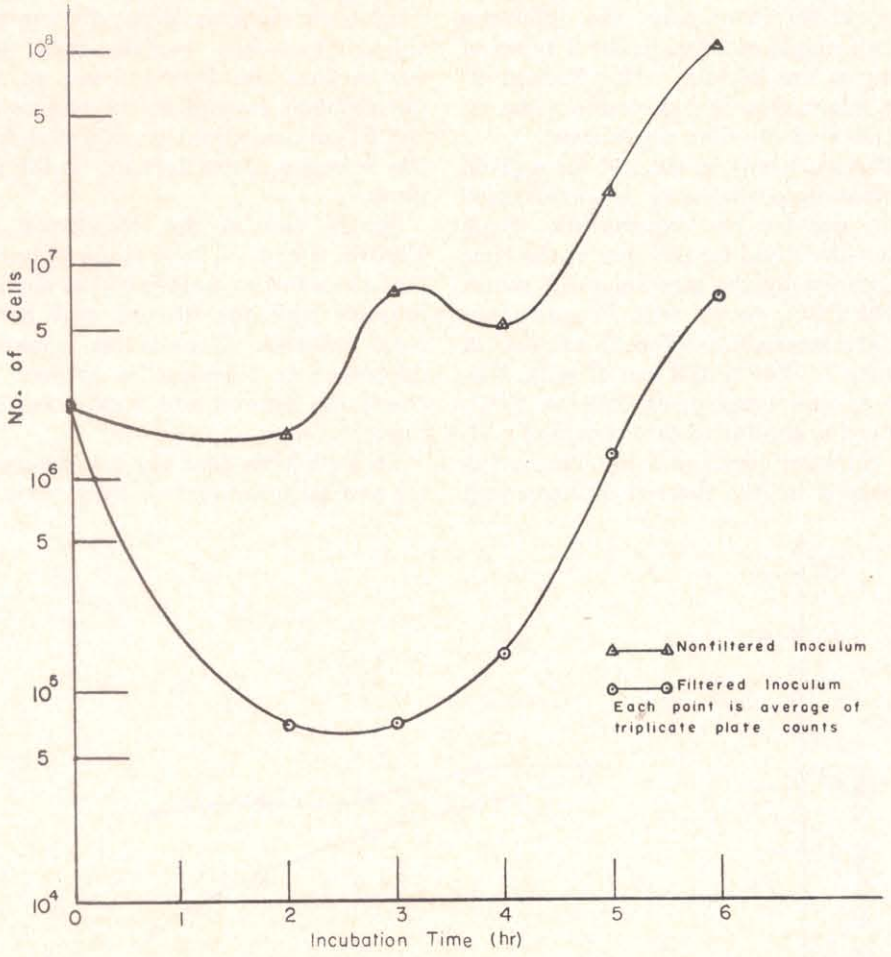

FIGURE 8.—Effect of membrane filter on *Pseudomonas sp.* Cell population as a function of time for filtered and nonfiltered equal inocula.

cificity of the coliform test in earlier work.

Results

The results obtained are presented graphically in Figures 5 through 10. Figure 5 shows that the effect of the filters on *E. coli* was to reduce the amount of carbon dioxide evolved from the cultures. At 4 hr this amounted to almost a 10-fold effect and at 6 hr, the termination of the experiment, the difference between the amount of gas collected from the filtered and nonfiltered cultures exceeded 30-fold. This curve is of great interest in that its exquisiteness demonstrates the inherent precision of the radioisotope technique in monitoring metabolic activity. Figure 6 shows the effect of the filter on cell reproduction as a function of time. When the inoculum had not been filtered, the cells underwent a slight lag and then began reproducing. Before the elapse of two hours, the cultures were in exponential growth. The filtered cultures showed a high rate of mortality during this first two-hour period. The surviving organisms then began reproducing but did not achieve exponential growth until the third hour. When the curves became par-

allel, at the third hour, the difference in cell numbers between the 2 types of cultures was 30-fold. This 30-fold effect was maintained throughout the remainder of the 6-hr experiment.

The sensitivity of the test for a given incubation period may be determined here, and for the experiments which follow, by dividing the cpm at the time of interest by the zero time cell count.

The $C^{14}O_2$ curves with *Pseudomonas sp.* are somewhat different as seen in Figure 7. The initial loss of cells, Figure 8, and consequent loss in $C^{14}O_2$ evolved is similar to that obtained with the coliform organisms, but the curves produced by the filtered cultures and nonfiltered cultures do not diverge with time. It is also evident from both curves that the *Pseudomonas sp.* did not respond as well in the test as did the *E. coli,* as would be expected from the selective characteristic of the medium.

In the case of the *Alcaligenes sp.,* Figures 9 and 10, it is again apparent that the selective nature of the medium inhibits both the filtered and nonfiltered cultures. Nonetheless, a marked difference in response is evident between the filtered and nonfiltered cultures.

This evidence that the membrane filter had an inhibitory or even toxic ef-

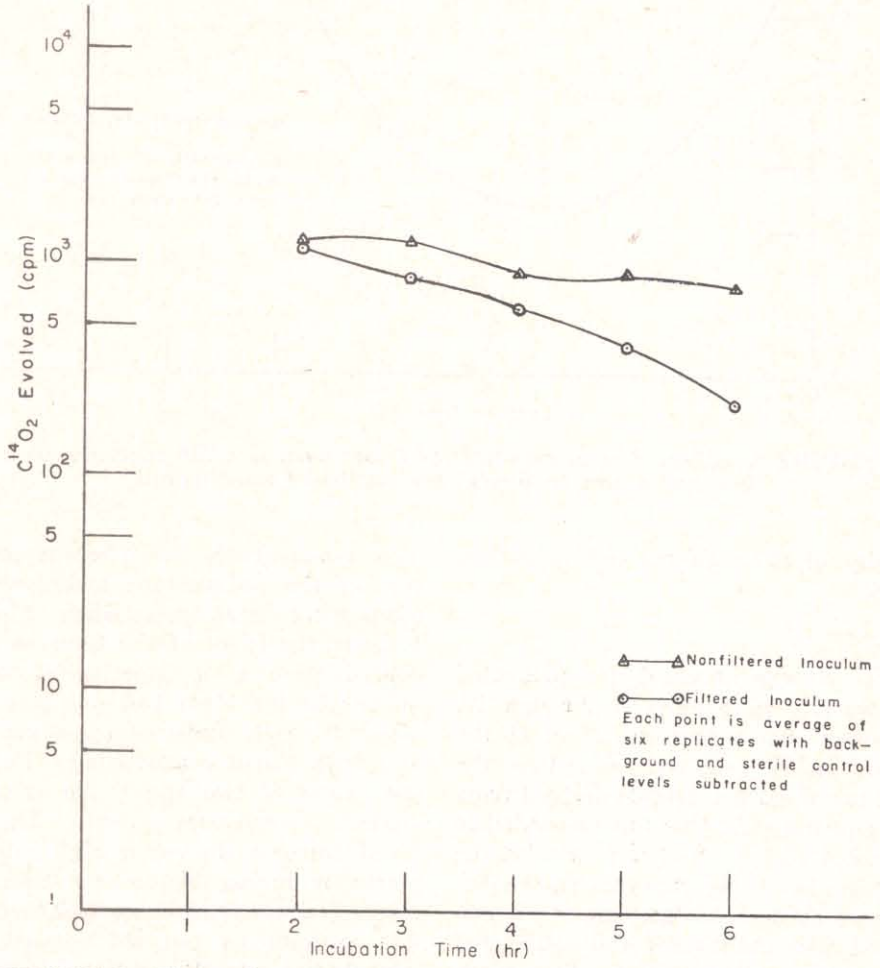

FIGURE 9.—Effect of membrane filter on *Alcaligenes sp.* $C^{14}O_2$ evolved as a function of time for filtered and nonfiltered equal inocula.

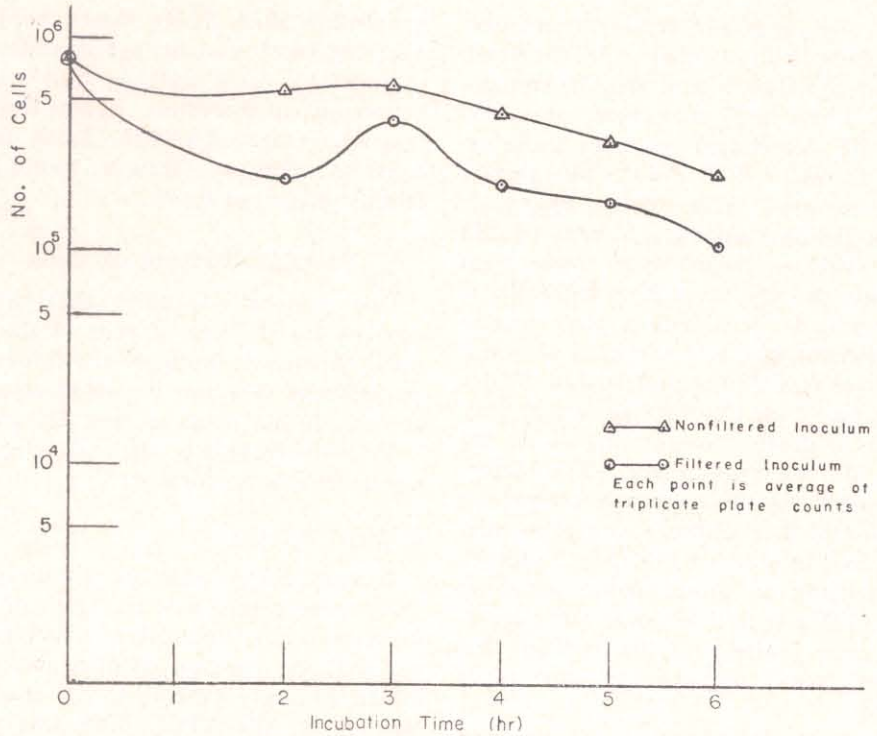

FIGURE 10.—Effect of membrane filter on *Alcaligenes sp.* Cell population as a function of time for filtered and nonfiltered equal inocula.

fect on bacteria constituted an unforseen and unwelcome obstacle to the development of the radioisotope test. Furthermore, the results were difficult to reconcile with the published literature on the membrane filter. Experimental work was continued in this vein. At the same time the literature was watched for evidence of a membrane filter effect.

Wolochow (6) provided the first supporting evidence in his account of erratic results obtained with various organisms in membrane filter determinations. He investigated several possible variables, none of which pinpointed the cause, but he felt the difficulty was due to both the media and the filter membranes. McCarthy *et al.* (5) pointed out that some bacteriologists believe the MF technique gives false positives and inhibits the growth of certain members of the coliform group. While these investigators clearly established the statistical problems in the MPN test, they did not report on the bacteriological reliability of the MF test. Henderson (7) reported differences, approaching 10-fold, between the MF and MPN on replicate coliform determinations with low numbers of cells. The differences tended to increase with increasing cell population. He reduced the discrepancy on the basis of statistical error in the MPN test, but even then the results remained several fold higher than the MF results, and the trend for wider discrepancy with increased cell population remained. Henderson, however, compared replicate MF and total plate counts of coliform organisms and found good agreement. He concluded that the MF is accurate on the basis of the correlation found with the pour plate technique.

Similar comparison was attempted in this work without success in duplicating agreement between the two meth-

ods. Using M-enrichment broth followed by brilliant green fuchsin broth in some instances and the one-step m-endo broth MF in others, standard (3) MF determinations were made using *E. coli* ATCC 8739. The results were compared with nutrient agar pour plate determinations. A total of 113 MF determinations were made with inocula ranging from 22 to 565 cells, as determined by replicate pour plates. Agreement between averages of replicates of test groups fell within 20 per cent, based on the pour plates, in only 6 of the groups, comprising 35 of the 113 MF determinations. The differences for the other groups ranged up to 15-fold in 2 instances and were several fold in others. For only 2 groups, both being of those groups agreeing within 20 per cent with the corresponding pour plates, did the average MF count exceed the average pour plate count.

Recently Malaney *et al.* (8) reported large discrepancies between MF and MPN coliform determinations when the confidence limits of the MPN were based on the Poisson distribution to correct for the statistical error inherent in the MPN test using five tubes in each of three dilutions. In the study reported, the mean MPN result exceeded the MF mean by at least six-fold. Moreover, by the 95-per cent confidence limits established, agreement between the two methods occurred in less than one-third of the cases. Finding the number of false positives in the MPN confirmed test to be greater than expected, Malaney *et al.* (8) compared the MPN confirmed test, the Standard Methods completed test, and the MF. The MF results were found to be roughly four times as high as those of the completed test, and the MPN results approximately nine-fold greater than those of the completed test. They believe the Standard Methods completed test to be the most accurate of the three.

In an attempt to determine the bactericidal factor associated with the membrane filter, filters were placed in nutrient broth and heated in order to extract any toxic material that might be present in the filter. When the filters were removed and the broth used, coliform organisms grew as well as in freshly prepared broth.

Possible Rupture of Cells

One obvious possibility that might account for the loss of cells in the filtered cultures is mechanical rupture of the cells as they are impacted against the membrane pores during filtration.

To investigate this, the following experiment was performed:

Filtered Inocula

A manometer and an air by-pass valve were placed into the train of the membrane filter apparatus in such manner that the vacuum applied could be measured and controlled. A suspension of *E. coli* ATCC 8739 was prepared and served as the inoculum pool. Filtrations of 10-ml portions were made in sextuplicate under vacuums of 5, 10, 20, and 29 in. of mercury.

Pipetted Inocula

A 0.1-ml portion of a 100-fold less diluted suspension of the same culture preparation from which the inocula for filtration were prepared was pipetted into each of six culture planchets. Sterile membrane filters were merely placed into 6 other planchets and 0.1-ml portions of the coliform organism suspension were placed on top of the membranes by pipet.

Tagged MF MacConkey medium was immediately added to all culture planchets which were then incubated for four hours at 44°C. The cover slips were removed and collection of the evolved gas occurred during the last 30 min of the incubation period. Triplicate sterile controls were run in the presence and absence of the filter membrane. The inocula were determined by triplicate plate counts.

TABLE II.—Effect on *E. Coli* ATCC 8739 of Culturing After Impaction on Membrane Filter vs. Mere Proximity to Membrane Filter

Item	Counts Per Minute							
	Pipetted Inocula		Filtered Inocula				Sterile Controls	
			Vacuum (in. Hg)					
	− filter	+ filter	5	10	20	29	− filter	+ filter
—	11,100	6,110	755	960	648	590	44	50
—	13,800	4,020	614	625	604	590	59	60
—	11,600	3,470	416	1,030	731	445	42	39
—	11,600	3,330	498	1,050	671	435	—	—
—	12,800	4,800	387	590	980	830	—	—
—	14,600	3,870	597	715	875	860	—	—
Avg	12,600	4,600	545	820	750	625	48	50
No. cells in inoc.	2,800	2,800	2,040	2,040	2,040	2,040	—	—
cpm/cell	4.50	1.64	0.27	0.40	0.37	0.31	—	—

Results

Table II presents the results of the experiment. It is shown that the mere presence of the filter in the culture reduced the amount of gas evolved by approximately three-fold. The effect of the filters on the cultures which had been impacted against them was much more drastic and constituted an average reduction in gas evolved of approximately 10-fold. There was no relationship between degree of vacuum applied and gas production, with a vacuum of only 5 in. of mercury having essentially the same effect as one of 29 in. The sterile controls are of interest here in that the same amount of nonmetabolic $C^{14}O_2$ was evolved from the medium when the filter was present as when it was not. This demonstrates that the reduction in gas evolved from the cultures is not due to adsorption and retention of the gas on the filter surface.

These data, in conjunction with the cell survival data cited, leave no doubt that the bacteria suffer a real effect from the presence of the filter membrane. The cell mortality observed is caused by at least two factors. First, damage to cells is incurred when suspended cells are impacted on filter membranes. This damage may result from simple mechanical rupture of the cell wall. Second, however, the mere presence of the membrane in close proximity to or in contact with the cell exerts a pronounced effect. This effect is more difficult to explain.

It was conceivable that the cell survival data were open to question on the grounds that those cells unaccounted for did not die, but adhered to the filter and did not come off during resuspension. If so, they would not have been counted when the resuspended culture was plated. Wolochow (6) has reported on this difficulty in resuspending cells impacted on the membrane filter. However, it is extremely unlikely that a a sufficiently high percentage of new generations of cells would stick to the membrane to produce the results obtained in the later hours of the test runs. Nonetheless, the possibility that cells not impacted on the filter membrane, such as new cells generated from those initially collected by the filter, might be strongly attracted to the filter membrane was investigated. One milliliter of a 10^7 dilution of a 24-hr nutrient broth culture of *E. coli* ATCC 8739 was pipetted onto 47-mm sterile filter membranes placed in sterile petri

TABLE III.—Numbers of Unimpacted *E. Coli* ATCC 8739 Adhering to Membrane Filter

Item	1 ml Inoculum Pipetted onto MF		0.2 ml Inoculum No MF Present (per ml)
	Cells Remaining on MF	Cells Remaining in Petri Dish	
—	9	727	154×5=770
—	48	741	165×5=825
—	29	728	152×5=760
—	22	767	—
—	19	718	—
—	27	755	—
—	18	759	—
—	29	742	—
—	27	737	—
Avg/ml	25	742	785
Total cells/ml	767	767	785

dishes. After standing several minutes, the filter membranes were removed and incubated in m-endo broth MF according to the standard MF procedure. Nutrient agar was poured into these petri dishes and into ones that contained no filter but had received 0.2 ml of the same suspension. The results are presented in Table III. Only about three per cent of the cells adhered to the filter membranes. This was additional evidence that the differences in cell populations reported for filtered and pipetted tests were not due to the incomplete removal of cells from the filter membrane. The probability that the differences were due to inhibitory or toxic effects of the filter membrane was therefore strengthened.

Filter Brands

To determine whether the filter membrane effect might be due to a characteristic of the S and S brand filter being used, the available commercial brands were compared. Replicate testing was performed with S and S, Millipore, Oxoid, and Gelman Type 27A filter membranes. No appreciable differences were found between the effects of the first three brands. The Gelman Type 27A filter, however, exhibited a much less pronounced adverse effect. Typical results for a test of six replicates each with equal inocula of *E. coli* ATCC 8739 showed an average of 650 cpm for the S and S filters versus an average of 1,800 cpm for the Gelman Type 27A filters. Because of this difference, an extended test, similar to those reported in Figures 5 through 10, was made with this strain of *E. coli* to compare results obtained with pipetted and Gelman-filtered inocula. The data are presented in Figures 11 and 12. Correlation is seen to be much better than with the tests made with other filters. The filter effect was reduced to approximately two-fold for cpm evolved. Agreement between cell numbers obtained with and without filters improved similarly. This encouraging and important difference in membranes warrants further investigation.

Discussion

The observations reported herein are at variance with numerous initial reports on the membrane filter method for coliform determination. The salient difference between this work and the standard method for coliform determination by the membrane filter is that here MacConkey broth was used and incubated at 44°C in accordance with the MacConkey method, while the standard MF method uses other lactose-base media with incubation at 35°C. For the purpose reported, the standard MF media will not do in that their specificity relies on color rather than gas production. It may be, therefore, that with the recommended MF media, cell survival and reproduction would have been greater than that reported herein. However, in a comparison between the standard MF test and pour plates, the data obtained did not so indicate. Furthermore, a logical explanation for the fact that the filter membrane would be inhibitory or toxic in one medium and not in another seems difficult to formulate. It has not been learned

why the cells responded differently in the presence of the filter membrane than in its absence.

Future Plans

By utilizing the Gelman Type 27A filter and perhaps extending the length of the test another hour to compensate for this minimized filter effect, it is planned shortly to begin testing raw and finished water samples. The results will be compared to those obtained from the same samples by the standard MF test. For the reasons cited and the cautions expressed by the prescribed Standard Method (4) MF test for coliform organisms, this comparison will not be made without hesitation. However, no more precise method for making the comparison is available.

It is encouraging that other investigators are now working on the radioisotope test. Among the laboratories conducting research on the method are those of the Illinois State Health Department (9), the Metropolitan Water Board of London, and The Liverpool College of Technology, and L. Lyons and Company, Ltd., of London (10). Further work based on the method is being done in the Department of Microbiology of the Georgetown University, School of Medicine and Dentistry (11) in an attempt to develop a rapid test

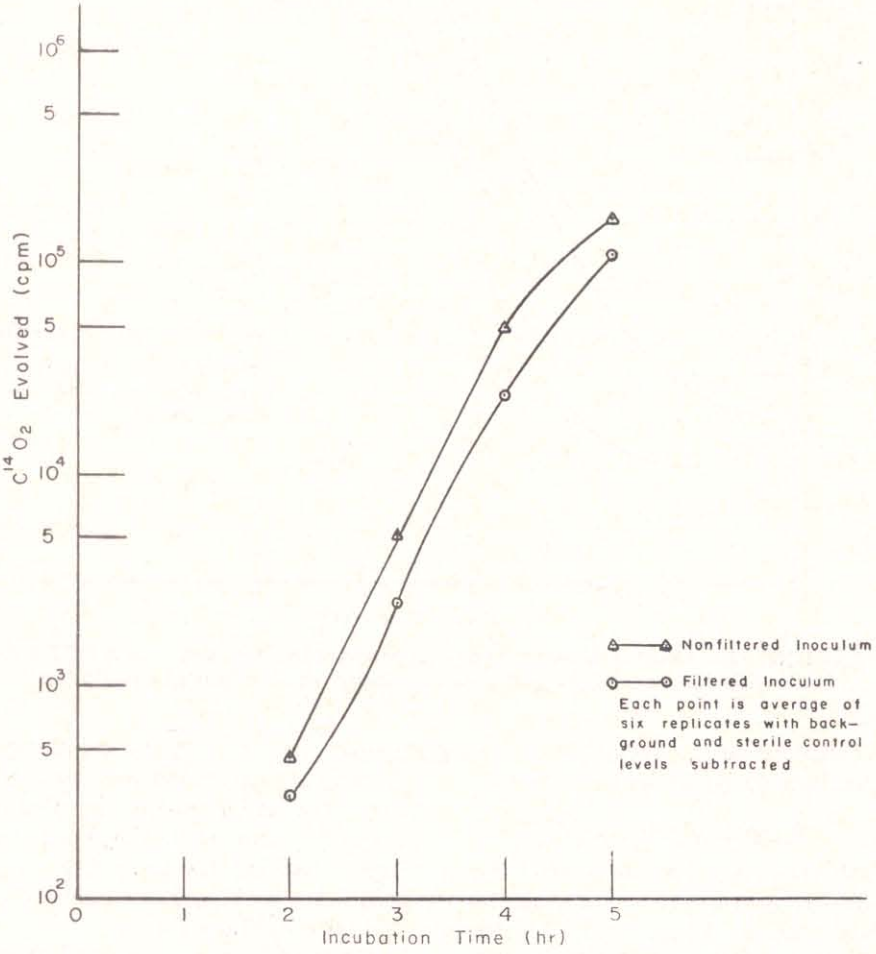

FIGURE 11.—Effect of Gelman Type 27A membrane filter on *E. coli* ATCC 8739. $C^{14}O_2$ evolved as a function of time for filtered and nonfiltered equal inocula.

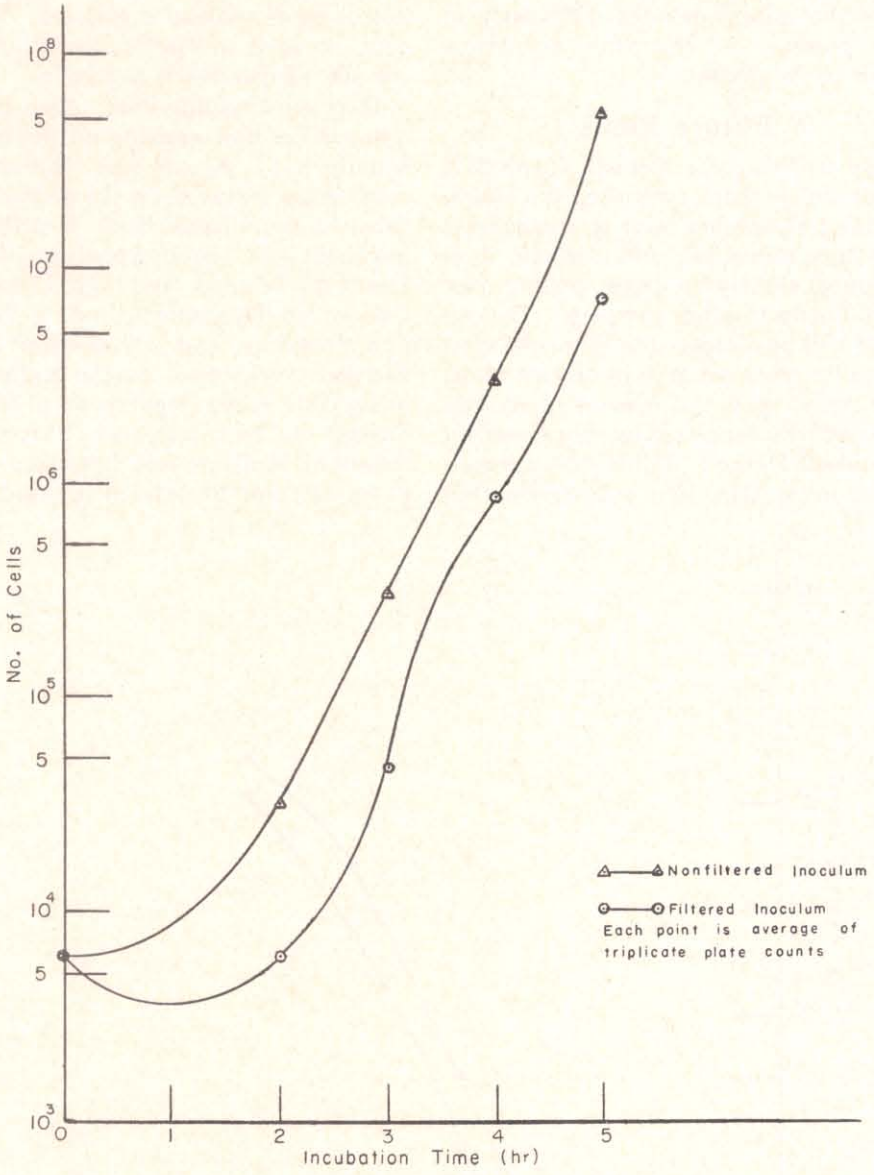

FIGURE 12.—Effect of Gelman Type 27A membrane filter on *E. coli* ATCC 8739. Cell population as a function of time for filtered and nonfiltered equal inocula.

for determining the antibiotic of choice in combatting bacterial infections.

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