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The Membrane Filter in Sanitary Bacteriology

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Removal of bacteria from liquids by filtration through a membrane that has been prepared from a derivative of cellulose is not a new process. Reviews by Bigelow and Gemberling (*1*), Ferry (*2*), or Pierce (*3*) credit Fick in 1855 with the application of collodion membranes in biological investigations. Numerous improvements and modifications were suggested by various investigators.

A major problem of bacteriology has been the development of rapid, accurate techniques of isolating, identifying, and counting organisms in water, air, and food. The authors of this paper have undertaken what is probably the first systematic series of controlled experiments in the United States to develop and apply techniques employing a membrane filter for this purpose with water. The results reported promise a substantial reduction in the time, labor, and space required for specified phases of bacteriological analysis and, at the same time, they indicate a likelihood that these techniques will be more certain and precise in results than methods now in use. These experiments have a particular significance for the hygienic aspects of waterworks operations. They also imply economies and technical advantages, such as ease of transportation, which would permit bacteriologists to extend their services to small water plants and to rural areas.———M. D. HOLLIS, *Assistant Surgeon General.*

Zsigmondy and Bachman (*4*) developed a method for the preparation of a membrane that could readily be adapted to commercial production. A United States patent was issued to Zsigmondy (*5*) in 1922 on an application made in 1919. Membranes of this type have been available in Germany for a number of years.

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The trade names appearing in this study are carried as a means of identifying the products under discussion and do not represent endorsement of the product by the Public Health Service.

Filtration procedures using the Zsigmondy membrane were suggested for the determination of bacterial counts, coliform determinations, and isolation of pathogenic strains of bacteria from water and other fluids. Such methods were especially favored in Germany. It appeared to be the consensus of most investigators that the membrane filter method had advantages of speed, accuracy, and simplicity plus a marked saving in manpower, material, and equipment. The most important factors in sanitary bacteriology were the increased volume of sample for examination and reduced incubation periods.

Dr. Alexander Goetz (*6*, *7*), after a detailed investigation of the membrane filter in Europe, has published a complete report of his findings. In his review he described the characteristic properties and the use of the filter. Goetz (*8*), through his cooperative investigations, designed the filtration equipment for holding the membranes during filtration and developed the method of preparation for an improved membrane from domestic materials. His report included technique and methods of application. Dr. Goetz believes the membrane filtration method has definite bacteriological advantages over the usual cultivation procedures on liquids or gels, including the absence of lateral diffusion from neighboring colonies. In the case of coliforms, and probably all other organisms, growth appears to be stimulated on the surface of the membrane.

Apparatus

The filtration equipment used in this laboratory was designed and supplied by Dr. Goetz. The membranes used in this investigation were prepared in his laboratory at the California Institute of Technology, Pasadena, Calif.

Although the Goetz filtering apparatus is constructed of stainless steel, it can be made of glass, porcelain, or any noncorrosive metal. It consists (fig. 1) of a stainless steel funnel, A, connected by a bayonet locking nut, B, to the receptacle, C. The receptacle has a porous plate that supports the filter membrane and allows free passage of liquids. When the membrane is locked in the funnel, all of the fluid must pass through it. The filtrate is collected in a filter flask. Filtration may be accomplished by gravity where a source of vacuum is not available but reduced pressure in the flask greatly increases the rate of flow. Sufficient vacuum can be produced by a hand aspirator or filter pump on water pressure, but an electric vacuum pump is more satisfactory.

The flask was attached to a one-liter equalizing bottle which served both as a water-trap and vessel to equalize the vacuum. Before reaching the electric vacuum pump, the air in this vessel was drawn through an oil trap and a drying tube filled with "Drierite." When the appa-

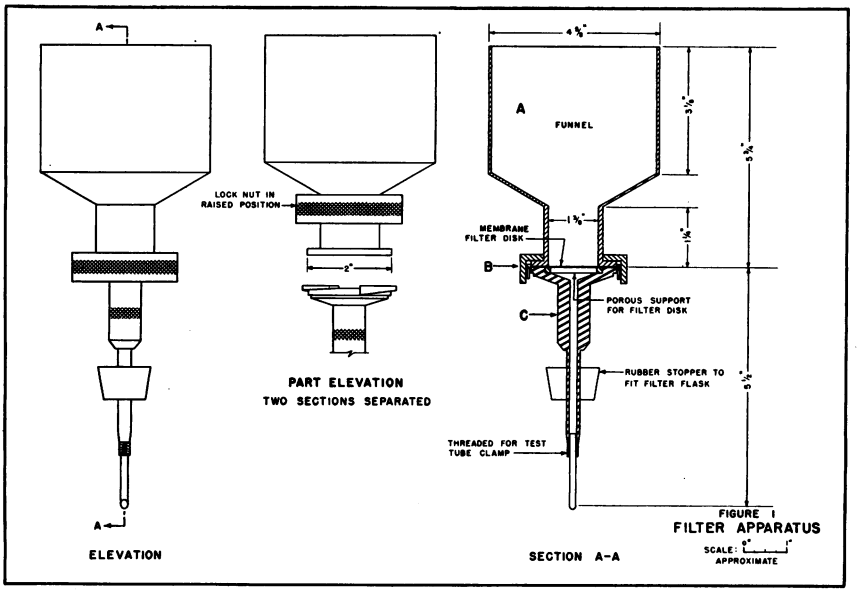

Figure 1. Diagram of Goetz filter apparatus.

ratus was used in research investigations, a mercury manometer was inserted in the vacuum line for measuring the pressure in the system and for testing wet membranes.

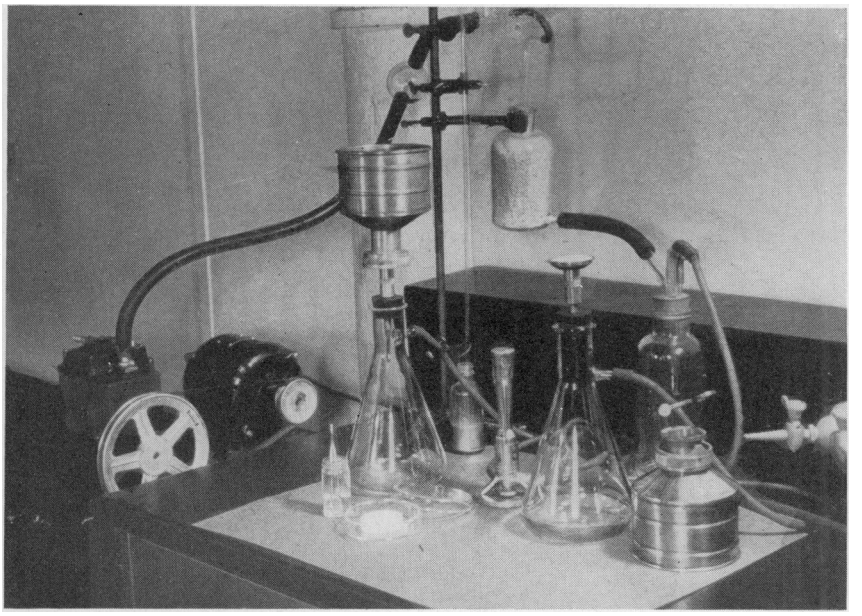

Figure 2. Filtration assembly.

Sterilization Procedure

In all investigations in this laboratory, the funnel was sterilized at 15 pounds steam pressure for 15 minutes. The two parts of the funnel were separately wrapped in kraft paper before autoclaving. It is not necessary to sterilize the funnel between each filtration; the inside surface can be washed down satisfactorily with sterile water. The funnel may be immersed in boiling water for 15 minutes, or even scrubbed in hot soapy water and rinsed in hot water in the absence of autoclaving facilities.

The filter membrane is a circular disk approximately 48 mm. in diameter and 0.1 mm. thick, composed of a cellulose derivative. These membranes were made from American materials by Dr. Goetz (*6*). The membranes are extremely porous. The tubular holes through the disk are parallel to the direction of flow of the liquid, with slightly smaller diameter at the top. The pore size may be controlled in the manufacturing process. Membranes used in this investigation were "low Z."

According to Dr. Goetz (*6*) the "pore size is measured by the determination of a factor Z (Zeit=time) which is necessary to pass 100 cc. of distilled water at room temperature through a filter area of 100 cm. at a differential pressure of one atmosphere (15 lbs.)." The value of Z may range from 5 to 6,000 seconds.

Each membrane must be sterilized before using for cultivation work. Heat is not recommended for routine sterilization of these membranes, although under certain experimental conditions they have been sterilized by repeated boiling in water. A number of methods of sterilization have been suggested. Exposure to ultraviolet light has been used in some laboratories. Sterilization of membranes in this laboratory is obtained by exposure to ethylene oxide vapor, as recommended by Phillips (*9*), Kaye and Phillips (*10*), Phillips and Kaye (*11*), and Kaye (*12*, *13*). Briefly, the sterilization procedure consists of placing glassine envelopes, each containing 10 membranes, in a vacuum jar. The air is then evacuated and replaced with a mixture containing one gram of ethylene oxide per liter of air at atmospheric pressure. After an exposure period of 2 hours, all traces of ethylene oxide are removed from the membranes by flushing the jar with air by alternate evacuation and replacement. Since ethylene oxide and air mixtures in certain proportions are explosive, care must be used to avoid fire hazards in the sterilization process. Should traces of ethylene oxide be left in the membrane due to insufficient flushing, it will be lost on exposure to the air in 24 to 48 hours. When received, the membranes contain a small amount of moisture which is necessary for the sterilization process. On the other hand, because of the

difficulty of removing the ethylene oxide from wet membranes, they should not be wet.

Each membrane is protected by a disk of material resembling a good quality white blotting paper. During shipment and storage, the blotting paper protects the membrane from injury. Afterwards it serves as a reservoir for nutritional medium. Some European investigators have used unsterile absorbent disks in their procedure, but it is recommended that these blotting pads be sterilized to eliminate growth of contaminating organisms in the pad with possible diffusion of metabolic products through the membrane. The following procedure is used in this laboratory:

1. Boil pads for 10 minutes each in four successive changes of distilled water, or continue until the distilled water remains colorless (to remove all impurities).
2. Dry at room temperature or in an oven at 103° C. or less.
3. Autoclave for 15 minutes at 121° C. in a suitable container (a Petri dish may contain 20 disks).
4. Dry pads in sterile container.

The remaining equipment, such as pipettes, Petri dishes, etc., is found in the average water laboratory equipped to make a total bacterial count, with the possible exception of an incubator capable of maintaining a saturated humidity at 35°–38° C. The standard electric water bath with a thermostatic temperature control and metal cover is ideal. The water level is maintained at about an inch below the incubation shelf. Small Petri dishes 60 mm. × 15 mm. are most convenient for incubation but the standard Petri dish (100 mm. × 15 mm.) may be used.

Bacterial Counts

Standard Methods for the Examination of Water and Sewage recommends the standard plate count for the estimation of bacterial density in water. In routine procedures, one ml. or less of sample is planted in each Petri dish although in research investigations, samples of 10 ml. have been planted in large Petri dishes (200 mm. diameter). After the solidification of the nutrient agar, the plates are incubated at 35°–38° C. for 24 hours. The colonies are counted. If there are less than 30 colonies or more than 300 colonies on the plate, the colony count is not statistically accurate and such counts are discarded. As treated waters commonly have bacterial densities of less than 30 organisms per ml., the standard plate count loses much of its value. The membrane filter technique has a unique advantage for such samples. The membrane will retain all the bacteria contained

in any quantity of water that can be forced through the filter and, after cultivation for a period of hours, the colonies may be counted for the quantitative estimate of bacterial density.

The problem of applying the filter to determinations of total bacterial count resolves into a choice of a suitable culture medium, proper incubation, and counting colonies. It is recognized at the outset that not all the living bacteria from a mixed population in a sample of water can be cultivated on a single medium. To quote Prescott, Winslow, and McCrady (*14*): "The customary methods employed to determine the number of bacteria in water do not reveal the total bacterial content, but only a small fraction of it; this becomes apparent when we consider the large number of organisms, nitrifying bacteria, strict anaerobes, etc., which refuse to grow, or grow only very slowly in ordinary culture media, and which, therefore, escape detection. On the one hand, certain obligate parasites cannot thrive in the absence of the rich fluids of the animal body; on the other hand, the prototrophic bacteria, adapted to the task of wrenching energy from nitrates and ammonium compounds, are unable to develop in the presence of the considerable concentration of organic matter contained in ordinary laboratory media."

The following criteria are used as a basis for consideration of media for total counts:

1. The medium should produce a maximum number of colonies from all types of water. The colony yield should compare favorably, where comparisons are possible, with the bacterial counts determined by other methods.
2. The colonies should develop rapidly to a sufficient size to be counted after a minimum incubation period. The present report is concerned with studies of bacterial counts after 18 hours incubation.
3. The medium should be one which is reproducible and is routinely available in laboratories.

In this investigation, bacteria from a number of sources were used in the evaluation of the media. Typical examples were raw Ohio River water, water taken during various stages of treatment processes, or from the distribution system, domestic sewage, and in special instances, a pure culture of *Escherichia coli* suspended in sterile diluting fluid. The pour plate method was used for comparison counts in most procedures where the expected bacterial count was 30 organisms or more per ml. An estimate of the bacterial density in samples containing less than 3,000 organisms per 100 ml. was made by planting five tubes each of at least three decimal dilutions in lactose broth and calculating the most probable number (M.P.N.) based on the presence or absence of growth in each tube. The M.P.N. method was used in all coliform examinations where good laboratory practice would indicate it was applicable.

Samples containing a large number of bacteria were diluted with sterile dilution water to reduce the counts to approximately one organism per ml. Eight liters of bacterial test suspension were prepared for each comparison of media. One hundred ml. of this suspension could be filtered through each membrane with the expectation of a reasonable colony count after incubation. In the comparison of growth-supporting characteristics of media, it is desirable to have between 50 and 150 colonies and not exceeding 300 on each membrane.

The filtration procedure was as follows:

1. A Petri dish (60 mm.×15 mm.) containing a sterile absorbent pad saturated with approximately 2.2 ml. of the medium was placed beside the funnel.

2. One hundred ml. of test suspension were filtered through the sterile membrane in the funnel.

3. The membrane was removed from the funnel with sterile forceps and placed, grid side up, on the absorbent pad, with care so that no air bubbles were trapped between the medium pad and the filter membrane.

4. The Petri dish in an inverted position was incubated for 18 hours at 37° C. in an atmosphere saturated with water vapor.

5. The colonies on the membrane were counted at a magnification of 15 diameters with the ray of light forming an angle of approximately 20° with the membrane surface.

Ten combinations of five different liquid media were compared for

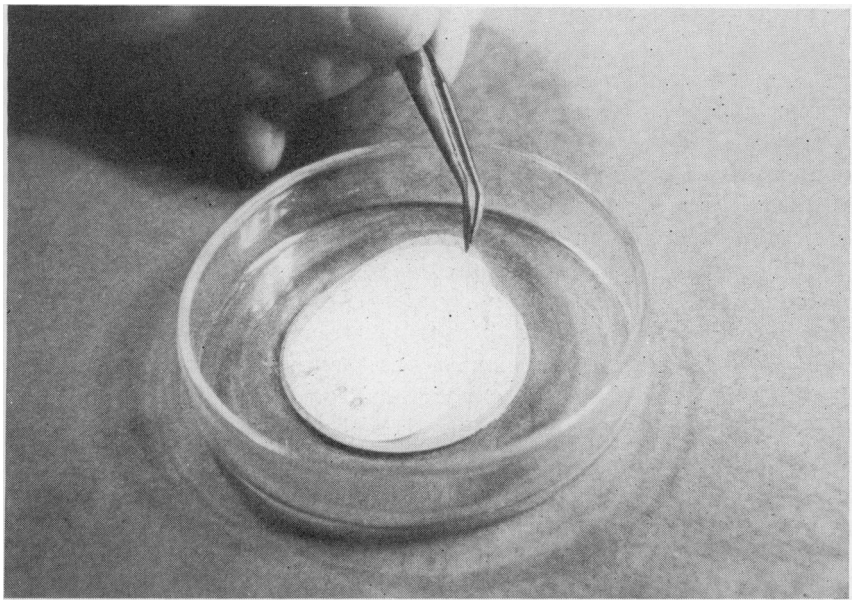

Figure 3. Placing membrane on culture medium.

growth characteristics on the membranes. These data were obtained by filtering the same quantity of a test suspension through each membrane, making duplicate preparations, incubating for 18 hours at 37° C. in an atmosphere of saturated humidity, and counting the colonies. Table 1 represents the average of three separate evaluations.

Table 1. *Total counts on media by the membrane filter method*

Medium [1]	Average colonies per membrane	Size of colony
Nutrient broth, triple strength	151	Small.[2]
Nutrient broth, double strength	136	Do.[2]
Nutrient broth, single strength	136	Do.[2]
Lactose broth, triple strength	130	Larger.[3]
Lactose broth, double strength	102	Do.[3]
Lactose broth, single strength	110	Do.[3]
Medium A, single strength	91	Do.[3]
Albimi M dextrose, single strength	88	Do.[3]
Albimi M lactose, single strength	85	Do.[3]
M2 Medium, single strength	82	Do.[3]

[1] For compositions of media, see appendix.
[2] Colonies on these plates were typically small. The overwhelming number of the colonies were of pinpoint size.
[3] Colonies on these plates were typically larger. While pinpoint colonies were present, their number was smaller than on the other plates which showed such colonies.

Assuming each membrane had approximately the same density of organisms on the surface, the greatest number of colonies was grown on nutrient broth. The nutrient broth plus lactose resulted in slightly smaller numbers of colonies. The remaining four media were grouped together in third place. Furthermore, triple strength broth, with or without the addition of lactose, appeared superior to the corresponding double or single strength broths. In subsequent experiments, increasing the nutrient broth concentrations to quadruple strength or more failed to increase productivity of the media.

In the evaluation of a medium, the colony size, which depends on speed of growth and accuracy in counting, are practical considerations along with the productivity. The triple strength nutrient broth produced a greater number of colonies, but they were so small that they were difficult to distinguish from sediment or dust particles. On the other hand, colonies on nutrient broth with added lactose were large, easy to count, and apparently grew more rapidly. Therefore, triple strength nutrient broth plus lactose was considered the best general medium in the above group.

A careful examination of the growth on the Albimi M medium indicated that further improvement in this formula was possible. The concentrations of Albimi M peptone and yeast autolysate were

doubled and 0.5 percent lactose was added to the sterile medium. In addition, it had been observed that a large quantity of dipotassium hydrogen phosphate was necessary to secure a final pH 7.0 (after sterilization). This increased the phosphate ion ratio to the other ingredients. This modified medium was made in three separate lots. Each lot was divided in three equal parts. The first part was sterilized without adjusting the hydrogen ion concentration; the second portion was made to a pH 7.0 with potassium hydroxide (10 percent aqueous solution); and the third fraction was adjusted to pH 7.0 with dipotassium hydrogen phosphate solution. All of these media were compared with the standard plate count for productivity, using *E. coli* as a test organism. The average colony count was compared with the average of 10 replicate standard plate counts after using an equal quantity of bacterial suspension on each membrane and making 10 replicate membranes for each medium. The data are summarized in table 2. The standard plate counts were incubated for 24 hours at 37° C. and the membranes were incubated for 18 hours at 35° C. in an atmosphere of saturated humidity.

Table 2. *Comparison of Albimi M medium at pH 6.8 and pH 7.0 with dipotassium hydrogen phosphate and pH 7.0 with potassium hydroxide*

Medium	pH	Colony count	Percent of standard plate count
Nutrient agar plate		93	Reference count in percent:
Albimi M—no pH adjustment	6. 8	84	90.3.
Albimi M—phosphate	7. 0	71	75.9.
Albimi M—potassium hydroxide.	7. 0	86	92.6.

The Albimi M lactose medium at pH 7.0 with potassium hydroxide and the same medium at pH 6.8 without pH adjustment appeared to offer little choice, although the addition of sufficient dipotassium hydrogen phosphate to pH 7.0 resulted in a marked decrease in productivity. Double strength Albimi M medium plus 0.5 percent lactose and adjusted to pH 7.0 with potassium hydroxide has been designated as the medium of choice for total bacterial count procedures in this laboratory. The adjustment to a pH 7.0 with potassium hydroxide solution results in slightly better recovery rates (92.6 percent) and has the added advantage that every lot of medium will have the same hydrogen ion concentration.

Samples from a number of sources were examined following the development of a satisfactory medium for bacterial counts. The same general procedure was used for all samples, but certain changes in the routine method had to be made for each group. For example, waters

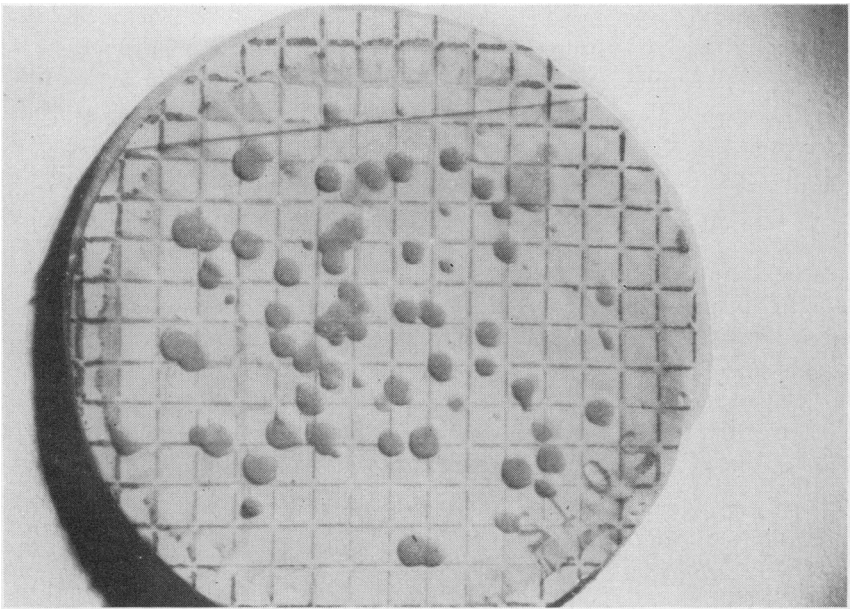

Figure 4. Colonies on Albimi M medium.

with a low bacterial density required samples up to 500 ml. while those with a large bacterial population were diluted with sterile water so that a fraction of an ml. could be examined. The membrane, for most accurate results, should not have in excess of 300 colonies on it. The quantity of each sample was measured to produce an expected colony count of less than 300 and, where an estimate of the bacterial population of the sample was impossible, several membrane counts were prepared by filtering different quantities of the sample. Samples with low bacterial density (less than 30 colonies per ml.) could not be compared with a standard plate count and, in such instances, an M.P.N. method (previously described) was used for estimating total bacterial density. When a series of dilutions were made of the sample (Ohio River water or sewage), an appropriate dilution in the series was planted in five replicate plates for an average standard plate count.

The average results of membrane counts with the corresponding average most probable number of bacteria from sample points in the distribution system of several cities are summarized in table 3. All counts are calculated on a unit of 100 ml. of original sample. Fractions of a bacterium are found in the column on membrane counts because the samples examined were greater than 100 ml. The colony count was calculated, for the purpose of comparison, to 100 ml. of water. Bacterial densities were so low that a standard plate count with 1 ml. of sample was unsatisfactory (less than 30 colonies per plate).

Table 3. *Comparison of membrane counts with the M.P.N. method on city water supplies*

Sample point	Colony count on membrane calculated per 100 ml.	M.P.N. (lactose broth) of bacteria at 37° C.—24 hours
	City A	
1	2. 25	1. 7
2	101. 50	540. 0
3	44. 00	240. 0
4	113. 00	79. 0
5	16. 00	13. 0
6	80. 00	11. 0
	City B	
1	443. 00	920. 0
	City C	
1	14. 00	14. 0
2	9. 00	3. 1
3	2. 50	2. 3
	City D	
1	113. 00	79. 0
2	117. 00	79. 0
	City E	
1	53. 50	350. 0
2	87. 00	140. 0

There is no common basis of comparison between the membrane count and an M.P.N. count for the total number of bacteria, but inspection of the above data reveals both methods produced comparable counts nine times. The membrane count was more than twice the M.P.N. once and the M.P.N. method was more than twice the membrane count four times.

The membrane technique is applicable to water samples from the distribution system in the estimation of total bacterial density. It can be used for total bacterial density in samples of any quantity up to a liter or more. This is a distinct advantage over the standard plate count. It reduces the time for the test by at least 25 percent and makes even greater reductions in labor, space, and materials.

Water taken during the various stages of treatment in water plants did not give satisfactory membrane preparations. A heavy cake of

sediment which formed on the membranes during the filtration caused the bacterial growth to consist of a spreading mat of ill-defined and uncountable colonies. The examination of samples in the treatment process was limited to water with high initial suspended solids treated with coagulants. The process could probably be used with excellent results in waters that were relatively clear.

Thirteen samples of fresh domestic sewage were examined. It was necessary to filter very small quantities of sewage because of their high bacterial density. Serial dilutions (using 99 ml. sterile water blanks) were prepared to contain 0.0001 ml. of sewage in 100 ml. of water, and the last dilution was filtered through the membrane. The total counts were made by the nutrient agar pour-plate method by planting one ml. of appropriate dilutions. During the latter part of the series, six M.P.N. counts for total bacterial density were added to illustrate the relationship with the standard plate count. The data are presented in table 4 with all counts calculated in density per 100 ml. of sample.

In the samples of domestic sewage tabulated above, the membrane count was higher than the plate count in 9 of the 13 determinations. The plate count averaged 87 percent of the membrane count. Six M.P.N. determinations were included for comparative values. As the membrane count averaged higher than the plate counts in the series, it would indicate that the filtration method is the better procedure for determining total bacterial densities in sewage.

Bacterial Counts—Summary I

The filtration method has a marked advantage over the standard

Table 4. *Membrane counts and standard plate counts on sewage*

Average filter colony count* in millions	Average nutrient agar count* in millions	Ratio of plate count/ filter count	M.P.N. in millions
116. 2	80. 4	0. 69	----------
121. 6	87. 8	0. 72	----------
110. 0	74. 0	0. 67	----------
141. 6	78. 0	0. 55	----------
44. 2	51. 4	1. 17	----------
50. 8	57. 6	1. 15	----------
183. 6	120. 8	0. 66	240
102. 2	67. 6	0. 66	28
150. 5	105 0	0. 63	35
147. 8	128 8	0. 87	49
178. 3	189. 8	1. 06	350
58. 3	52. 6	0. 90	130
84. 0	85. 4	1. 02	----------

*These average figures are based on five replicate preparations.

plate method for the enumeration of bacteria in samples of low bacterial density. Larger samples of 1,000 ml. or more may be examined by this procedure. The quantity of sample that can be used in a single test is limited to the amount of water that can be passed through the filter and the bacterial density since membranes with more than 300 colonies are not recommended for accuracy. The membrane method offers savings in elapsed time for test, incubator space, equipment, materials, and labor.

Total count data from the distribution systems of municipal water plants demonstrated the value of the membrane method because, in most samples, a one ml. plant on the standard agar plate would have given only a few colonies (less than 30).

The series on domestic sewage furnished a direct comparison between the membrane method and the standard plate count. The filtration procedure gave higher counts than the standard plate count in 9 out of 13 trials (69 percent). It appears that the filtration procedure is probably more productive than the standard plate count and is, at its poorest, equal to it.

The membrane filter is recommended for the counting of bacteria in relatively clear water with high or low bacterial densities and in turbid waters with high bacterial populations. It has not been used successfully so far on samples with extremely high turbidity and very low bacterial density.

Quantitative Estimation of the Coliform Group

The coliform group or one of its members has been used as the indicator of water quality throughout the civilized world. Some disagreements have developed over the significance of the various strains in the coliform group. These same disagreements have resulted in numerous procedures and media for potability tests on water and the investigations of pollution problems. In the United States, the coliform group is used as a pollution indicator, without separation of the fecal and nonfecal strains. Before considering any method for coliform examinations, the aims of an ideal test should be established. The coliform test procedure should:

1. Enumerate all coliforms present in the sample. For example, one organism that is a member of the coliform group should initiate a clear characteristic reaction.
2. The procedure should be specific for members of the coliform group.
3. The method should be rapid. Results should be available in hours rather than the 2 to 5 days now required.
4. The routine test should be simple. Involved methods are avoided or slighted in control procedures and small plants are not equipped to perform such test.

The procedures of Standard Methods are considered to be the nearest approach to the ideal test and, in the evaluation of membrane procedures, this reference test was the standard of comparison.

In the establishment of the membrane procedure for the quantitative estimation of coliforms, numerous types of media were investigated. The major problem in the application of the membrane filter to the identification of groups of organisms such as the coliforms is the development of selective media. Established media quite frequently behave abnormally when they diffuse through a membrane. Comparisons with the agar plate counterpart should be approached with caution.

Preliminary investigations of each medium were made with a water sample containing a member of the coliform group and some non-lactose fermenting organisms. Many unsatisfactory media were discarded after preliminary testing. A modification, presented below, of Endo medium as a broth was found to be most satisfactory.

Endo Broth—E.H.C. Modification

Basal medium

Lactose	20 gm.
Neopeptone (Difco)	20 gm.
K_2HPO_4	7 gm.
Distilled water	1,000 ml.

The constituents were dissolved in the water, using moderate heat if necessary. Medium was adjusted to pH 7.5, using KOH solution. It was tubed in 30 ml. portions, and sterilized in the autoclave for 15 minutes at 121° C.

Sodium sulfite solution

Nine percent aqueous solution of anhydrous Na_2SO_3 was freshly prepared in sterile distilled water.

Basic fuchsin solution

Basic fuchsin	3.0 gm.
Ethyl alcohol	50 ml.

The dye was dissolved in the alcohol, and sufficient distilled water added to make 100 ml. The dye solution was stored in the refrigerator to reduce evaporation of the solvent.

Use of the medium

To one 30 ml. tube of the basal medium was added 1.0 ml. of the sodium sulfite solution and 1.0 ml. of the basic fuchsin solution. It was thoroughly mixed by agitation of the tube, and applied to the blotting pads in 2–2.2 ml. quantity. This medium was used the same day.

Basic fuchsin may differ in dye content from lot to lot and from manufacturer to manufacturer, making it necessary to standardize the proportions of basic fuchsin with each new lot of dye.

The standardization of fuchsin-sulfite proportions by addition of only enough sodium sulfite to decolorize the fuchsin cannot be used with this medium. In standardizing this medium, a number of tubes of the medium with varying amounts of the basic fuchsin were

prepared and one ml. of sulfite solution was added to each tube. The medium was evaluated on the basis of differentiation of coliform organisms and the productivity.

Studies were undertaken with the medium, using pure-culture suspensions of coliform organisms to determine (1) the efficiency of coliform recovery; (2) the optimum incubation period; and (3) the value of preliminary incubation of the inoculated membranes on a non-selective enrichment medium. Following these studies, a series of tests on various polluted waters were undertaken to determine coliform counts in comparison with M.P.N. procedures of Standard Methods. The M.P.N. was based on the "confirmed test" in brilliant green lactose bile broth.

Effect of Preliminary Enrichment on Coliform Productivity

A series of duplicate filtrations of suspensions of *E. coli* were used to compare the productivity of the E.H.C. Endo broth with a preliminary enrichment period. One filter was incubated 16 hours on the Endo medium and the duplicate was cultured for the first 2 hours on Albimi M lactose medium before the filter was transferred to the Endo medium for 14 hours incubation. The counts recorded in table 5 represent the colonies exhibiting the metallic sheen typical of coliforms on the filters.

Table 5. *Growth on membrane filter (E.H.C. Endo broth)—comparison of (a) 2-hour enrichment before transfer, with (b) direct transfer*

Test No.	On Endo medium (no enrichment)	Two-hour enrichment before Endo medium
1	175	225
2	12	45
3	19	62
4	7	64
5	26	53
Average	48	90

The above data demonstrate a significant increase in coliform growth when organisms on the membrane have 2 hours preliminary enrichment on lactose Albimi M medium before being transferred to the Endo medium. Selective media, such as Endo, have a slightly toxic action to all bacteria, which may suppress growth unless the organisms are in an actively growing phase. It is suggested that the increased yield of coliform bacteria (following enrichment) is due to growth being initiated on a rich, nonspecific medium and, after

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growth is started, it survives any unfavorable action of the fuchsin-sulfite combination in the Endo medium.

Preliminary enrichment on Albimi M lactose medium for 2 hours before the membrane is transferred to the Endo medium is essential for careful quantitative determinations of the coliform group.

Productivity of Coliforms on Endo Medium

In this evaluation a pure culture of *E. coli* suspended in sterile dilution water was used as a test organism. Ten replicate filtrations were made and 10 replicate nutrient agar plate control counts were prepared for each of three series. The incubation was 2 hours on Albimi M lactose medium followed by 14 hours on the Endo medium.

Table 6. *Recovery of* E. coli *in suspension by membrane method and standard plate count*

Series	Average coliform count on filter	Average nutrient agar plate count	Index of recovery: $\frac{\text{filter count}}{\text{plate count}}$ = index
1	96. 0	99. 9	0. 963
2	79. 1	71. 7	1. 107
3	80. 2	78. 6	1. 022
Average	85. 1	80	1. 063

Data in table 6 indicate that the growth on the Endo medium (used with the filtration technique) is at least equivalent to coliform growth on nutrient agar plating medium.

The elapsed time for the finished test for coliform examinations has been reduced to a period of hours. Final incubation period for coliform counts on Endo medium by the membrane technique is derived from the data in table 7. Identical samples of water containing a suspension of *E. coli* were filtered through membranes (10 replicate preparations), incubated 2 hours on Albimi M lactose medium and transferred to Endo medium for continued incubation. The total number of colonies and the typical coliform colonies (with metallic sheen) were counted at periods of 10, 13, 16, 19, and 22 hours. This procedure was repeated 5 times.

The *E. coli* colonies grow rapidly on the membrane. There were 100 colonies (90.1 percent) on the filter after a 10-hour incubation period, but, of this number, only 20 percent had developed the typical differential characteristics of a coliform colony. The total colony count, 11, increased slightly in the next 12 hours. During

Table 7. *Incubation period and development of sheen by colonies of* E. coli *(elapsed time including preliminary enrichment)*

Hours of incubation	Total colonies	Typical coliform colonies	Percent of typical coliform colonies
10	100	20	20. 0
13	102	78	76. 4
16	103	96	93. 2
19	107	101	94. 4
22	111	102	91. 9

the same period, many of the colorless colonies were developing the typical metallic sheen of the coliform. These data indicate that the minimum elapsed time for a reasonably quantitative coliform density (93 percent) must be at least 16 hours. There is a possibility of using the shorter incubation period of 10 hours for a qualitative test, but this problem has not been investigated. The minimum time for the coliform estimation may be further reduced through the development of new media.

Coliform densities of domestic sewage were determined comparatively by the M.P.N. procedure and the membrane technique on E.H.C. Endo medium (table 8). The inoculated membranes were first enriched for 2 hours on Albimi M lactose media, then transferred to the Endo medium for 14 hours incubation. Colonies were counted with a magnification of 15 diameters. Counting of coliform colonies was limited to those colonies having metallic sheen since many noncoliform organisms also grown on Endo medium. The M.P.N. pro-

Table 8. *Comparison of coliform counts on E.H.C. Endo medium by membrane method with most probable number by confirmed test*

Sample	Coliform per 100 ml. by—		Sample	Coliform per 100 ml. by—	
	Membrane test (in millions)	Confirmed test (in millions)		Membrane test (in millions)	Confirmed test (in millions)
1	13. 0	11. 0	9	13. 5	13. 0
2	9. 1	17. 0	10	7. 3	3. 3
3	6. 0	17. 0	11	6. 1	33. 0
4	7. 9	7. 0	12	18. 5	22. 0
5	8. 9	2. 3	13	17. 0	13. 0
6	12. 5	23. 0	14	35. 0	49. 0
7	9. 8	4. 9	15	26. 0	79. 0
8	13. 5	23. 0	16	16. 9	33. 0

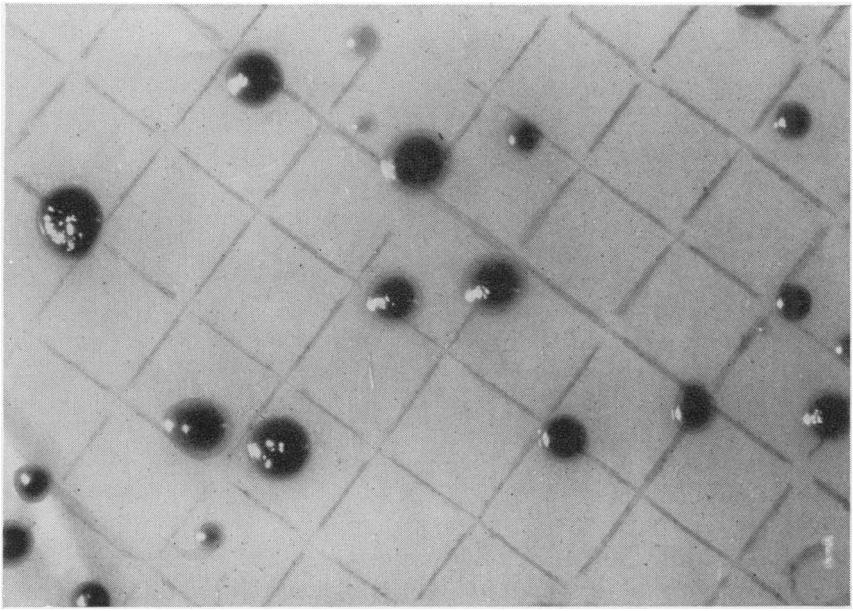

Figure 5. Coliforms on E.H.C. Endo medium.

cedure consisted of planting at least three-decimal dilutions of five tubes each in lactose broth and confirmation of all tubes with gas in brilliant green lactose bile broth. All filter counts were based on an average of five replicate determinations.

Discussion

Out of 16 determinations, the M.P.N. value by confirmed test for the coliform count was higher in 9 cases than the coliform count by the filter technique. In the remaining 7 examinations, the coliform value by the filter technique was higher than that of the M.P.N. determination. The median of the M.P.N. series was 17,000,000 coliforms per 100 ml. of sample, while the median of the membrane filter series was 12,900,000. The membrane filter method with the E.H.C. Endo medium gives coliform densities that are not significantly lower than those obtained by the Standard Method M.P.N. procedure. The M.P.N. values fluctuate over a higher and lower range than do their corresponding values obtained by the membrane procedure. The growth of an organism in a colony on the membrane resembles the development of a colony on a nutrient agar plate. Assuming equivalent productivity of the medium in both methods, the filter procedure would be equal in accuracy to the standard plate count and far superior to the approximation obtained by the M.P.N. method. It would seem that any coliform determination with the membrane filter

technique would have a greater degree of reliability than the M.P.N. coliform determination. Additional advantages favoring the filter procedure are the rapidity of the test and the relative simplicity of the method. While the M.P.N. procedure may require 96 hours to finish the "confirmed test", the filter technique required 16 hours. In the confirmed test, 2 to 4 different observations had to be made and transfers of from 5 to 14 positive lactose tubes to brilliant green lactose bile broth were necessary. On the other hand, in the filter method, after the initial 2-hour period on the enrichment medium, the membranes were transferred to the Endo medium and counted 14 hours later.

The estimation of total bacterial density on waters in various stages of the treatment process was not successful due to the heavy precipitate collected on the membrane. This criticism does not apply to samples examined for coliform density. Table 9 presents data on plant control tests in a municipal plant using sedimentation, iron and lime flocculation, filtration, and chlorination.

Table 9. *Plant control tests by membrane method and confirmed test for coliforms*

Type of sample	Coliforms per 100 ml.							
	Membrane procedure				M.P.N. (confirmed test)			
	1	2	3	4	1	2	3	4
Settled river water	1, 400	1, 210	388	1, 488	2, 000	780	780	700
Filter influent	80	93	10	86	<180	68	45	70
Filter effluent	6	1	12	4	13	2	22	13
Filter effluent (chlorinated)	0	0. 08	0	0. 08	<1. 8	2	<1. 8	<1. 8

All membrane filters in the above series were countable. Apparently the Endo medium suppresses spreader formations that were troublesome on the Albimi M lactose medium. The coliform density determined by the filter method was high twice in four trials on river water, high twice in three tests on filter influent, and generally lower in tests on the filter effluent and chlorinated water. However, in the membrane test, a 200 to 500 ml. portion of water was examined, while the M.P.N. was based on a sample of 55.5 ml. (5–10-ml. tubes, 5–1-ml. tubes, and 5–0.1-ml. tubes).

The membrane filter method suggests an adequate test for coliform density in water-plant control. A final answer will depend on the data obtained after trials in different treatment plants with waters of different degrees of pollution, varying chemical composition, and treatment methods.

Coliform Count—Summary II

The application of the membrane filter method to coliform determinations has been reported. A modified Endo broth (Endo Medium-E.H.C. modification) is recommended for coliform differentiation. The technical procedure of filtration and cultivation has been described. Representative experimental data have been included. Advantages of the membrane filter procedure are: large samples; reasonable accuracy; minimum time for testing (16 to 18 hours); and marked reduction in labor, equipment, space, and material.

Culture of *Salmonella typhosa* From Water Samples on a Membrane Filter

Over the years, much time has been expended on efforts to isolate pathogenic organisms from water and sewage. All the methods that have been developed are tedious and laborious. Furthermore, none have been satisfactory as a quantitative, or even a semiquantitative, procedure. The isolation of pathogenic organisms from water supplies is not a routine method for the determination of water quality. In epidemiological investigations, such a procedure would be of particular value in the direct isolation of pathogens from water supplies when the dangerous organism is not associated with the coliform group or other normal environmental indicator organisms.

According to Prescott, Winslow, and McCrady (*14*) methods for the isolation of pathogenic organisms from water must follow one or a combination of more than one of the following general procedures:

1. Cultivation on a differential medium that inhibits or kills unwanted bacteria.
2. Development of characteristic growth which may be easily recognized by visual inspection.
3. Concentration of organisms by filtration, agglutination, or adsorption on physiologically inert precipitates.

Numerous methods have been described for the isolation of *Salmonella typhosa* from blood, urine, and feces. Many of these methods depend on selective or inhibitory media. Typical media used in the cultivation of *S. typhosa* are tetrathionate broth by Müller (*15*), brilliant green Esbach broth recommended by Ruys (*16*), brilliant green eosin agar suggested by Teague and Clurman (*17*), desoxycholate citrate agar proposed by Leifson (*18*), or the bismuth sulphite medium originated by Wilson and Blair (*19*).

Direct cultivation of *S. typhosa* from water is difficult even on one or more of the excellent specialized media because of the small numbers of the pathogen in the limited quantity of sample to be examined. A number of investigators have attempted to overcome this difficulty

by concentration of the bacteria in a large sample of water by filtration through membrane filters. Mueller (*20*) was successful with this type of procedure in her investigation of an epidemic of typhoid fever occurring in Hamburg. She filtered her samples through a nitrocellulose membrane filter and cultivated the typhoid organisms on a modified bismuth-sulfite medium. She reported that all other methods failed.

The method developed in this study consists of a combination of the filtration procedure which removes the organisms from a large sample of water and the subsequent cultivation of the pathogenic organism on bismuth-sulfite broth of Wilson and Blair. A characteristic type of colony growth of *S. typhosa* is obtained on the medium.

The membrane filter technique requires a liquid medium. It was necessary to examine a number of modifications of differential typhoid media for their suitability as to growth-promoting ability, quantitative recovery rates, and differential characteristics of the typhoid colonies. Best results were obtained with a double-strength bismuth-sulfite broth.

The medium used in this procedure was commercial dehydrated bismuth-sulfite broth [1] (without agar). Pure cultures of *S. typhosa* and *E. coli* were used to determine the recovery rates in dilution water dosed with approximately 100 organisms per sample. The *E. coli* strain used in this experiment was found to grow on the membrane impregnated with bismuth-sulfite broth as a black colony 2–3 mm. in diameter with no zoning and less than 25 percent recovery. This dosed-dilution water was filtered through a sterile membrane filter, which was then carefully placed on a sterile absorbent pad saturated with approximately 2 ml. of culture medium. The sterile dish containing the absorbent pad and the membrane filter was incubated at 37° C. in an atmosphere of saturated humidity for 30 hours. Growth was then ready for examination. The results are given in table 10.

After incubation for 30 hours, colonies of *S. typhosa* on the single-strength bismuth-sulfite broth were only 0.5 mm. to 1 mm. in diameter

Table 10. *Recovery of* S. typhosa *on single- and double-strength bismuth-sulfite broth*

Medium	Percent recovery	
	S. typhosa	*E. coli*
Bismuth-sulfite broth (single strength)	57. 2	3. 1
Bismuth-sulfite broth (double strength)	65. 1	12. 3

[1] Difco Laboratories, Detroit, Mich.

and after 48 hours many of the colonies still had not developed the jet-black center or any degree of zoning around the colonies.

The typhoid colonies on the double-strength bismuth-sulfite broth, after 30 hours incubation, appeared as smooth glistening colonies 2–3 mm. in diameter with jet black centers surrounded by a thin clear white border. These colonies were generally surrounded by a black or brownish zone which may be several times the size of the colony. This zoning by reflected light exhibited a distinctly characteristic metallic sheen.

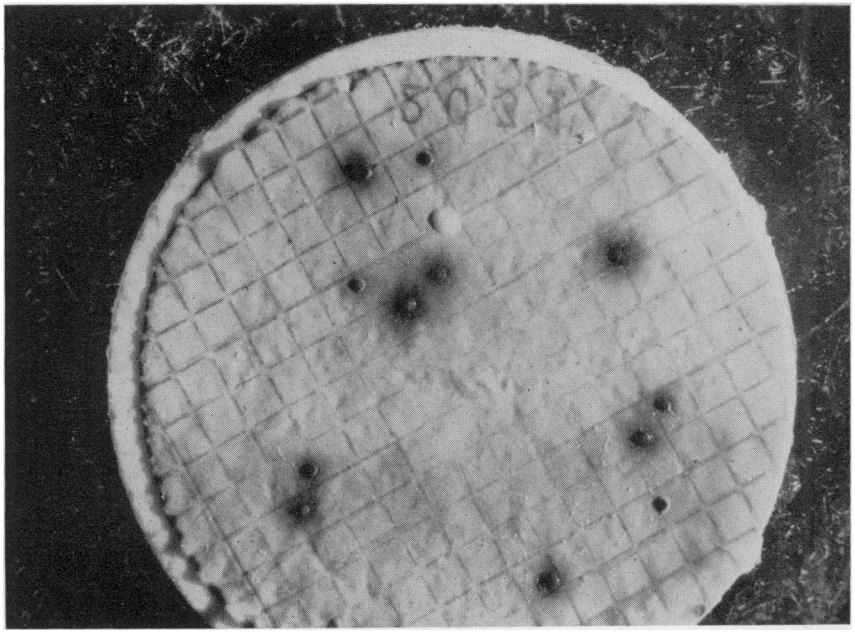

Figure 6. ***Salmonella typhosa*** **on bismuth-sulfite broth.**

As a result of this comparative study, the bismuth-sulfite broth was adopted for routine use. It was prepared by dissolving 6.4 gm. (for double strength) of the dehydrated bismuth-sulfite broth in 100 ml. of distilled water and then heating to the boiling point. While heating, the broth was swirled occasionally to aid the formation of the characteristic precipitation. At the first indication of boiling, it was removed from the heat to prevent the selectivity of the medium from being destroyed. The medium was used within 5 hours.

Following the development of a satisfactory medium, a series of nine separate recovery rate studies were made on *S. typhosa* polluted tap water. The lowest density of *S. typhosa* used was 29 per liter and the highest 147. The other seven suspensions had typhoid densities between those two extremes. Albimi M medium was used

Table 11. *Recovery of* S. typhosa *from tap water*

[*S. typhosa* dosed tap water—500 ml. quantities filtered]

Albimi M total count	*S. typhosa* dosage	Percent recovery rate		Percent typhosa of total count	
		Enrichment	No enrichment	Enrichment	No enrichment
425	73. 6	44. 8	47. 6	94. 3	97. 2
85	59. 2	43. 9	54. 1	83. 9	94. 1
115	51. 2	29. 3	44. 9	75. 0	95. 8
45	42. 0	31. 0	57. 1	86. 7	96. 0
45	28. 4	49. 3	38. 7	63. 6	91. 7
10	28. 4	38. 7	49. 2	73. 3	93. 3
5	22. 8	----------	52. 6	----------	92. 3
580	14. 6	41. 1	34. 2	80. 0	100. 0
65	14. 4	55. 6	41. 7	75. 0	83. 3

to determine the total bacterial density; that is, the organisms that grow on the usual laboratory medium. The formula and use of this medium have been described elsewhere in this report.

The enrichment procedure consisted of incubation for 2 hours on the Albimi M medium before the filter membrane was transferred to the selective medium. The data have been summarized in table 11.

Based on nine replicate experiments, the recovery of *S. typhosa* on double-strength bismuth-sulfite broth without enrichment had a median value of 47 percent of the total typhoid density. Of these, a median of 94 percent of the colonies were *S. typhosa*. With 2-hour enrichment on Albimi M medium prior to transferring the membranes to double-strength bismuth-sulfite broth for the remainder of the 30-hour incubation, the median of the total typhoid density dropped to 43 percent. The typhoid colonies were 78 percent of the total number.

A representative number of typical typhoid colonies were cultured on Krumwiede triple sugar agar and lead acetate agar. Of these, 278 colonies showed typical characteristics in both media and one colony appeared to be a member of the coliform group. Seventy-two typical colonies were checked serologically with anti-typhoid agglutination sera, with 69 positive and 3 doubtful.

Occasionally a few colonies from river and sewage samples produced a blackening on double-strength bismuth-sulfite broth, but they failed to be agglutinated by *S. typhosa* antiserum, and gave a typical triple sugar reaction characteristic of the coliform group. Growth of *E. coli* on this medium is described in the Difco Manual (*21*) as follows:

> Coli is usually completely inhibited. Occasionally a strain will be encountered that will develop small black, brown, or greenish glistening surface colonies.

This color is confined entirely to the colony itself and shows no metallic sheen. Likewise a few strains of aerogenes may develop on this medium forming raised, mucoid colonies. These may exhibit a silvery sheen, appreciably lighter in color than that produced by typhoid. . . . There are some members of the coliform group capable of producing hydrogen sulfide that may develop on this medium, giving colonies similar in appearance to typhoid.

It therefore seemed advisable to isolate and identify such organisms as well as to determine their recovery rate on double-strength bismuth-sulfite broth. Five coliforms which produce H_2S were used in this experiment. Each of five 99-ml. samples of sterile water were dosed with approximately 100 organisms. Each dosed dilution was filtered through a membrane and placed on a pad containing 2 ml. of double-strength bismuth-sulfite medium. The pads were then placed in an incubator of saturated humidity at 37° C. Poured nutrient agar plates of each culture were made to determine the amount of suspension filtered onto a membrane. The filtration results plus the Imvic. reactions are summarized in table 12.

Table 12. *Coliform H_2S producers on double-strength bismuth-sulfite broth*

Culture No.	Percent recovery	Indole	M.R.	V.P.	Citrate	Lactose	Colony appearance
Intermediates:							
1–3	00.0	−	±	+	+	+	No growth.
1–10	62.1	+	+	−	+	+	Dark brown center with narrow white border, 0.5–2 mm. in diameter, no zoning.
1–48	33.2	−	+	+	+	+	Dark brown center with narrow white border, 0.5–3 mm. in diameter, no zoning.
Aerogenes: A–37	00.0	−	−	+	+	+	No growth.
Escherichia: C–30	89.1	+	+	−	−	+	Dark brown, 1–3 mm. in diameter, no zoning.

The effect of crowding by nontyphoid organisms was investigated. A known suspension of *S. typhosa* was added to different dilutions of sewage containing a known number of coliforms as determined by Endo medium counts for coliforms.

The results in table 13 indicate that the amount of zoning falls off rapidly as the coliform density increases in the water sample.

Salmonella typhosa Culture—Summary III[2]

A method for the rapid isolation of *Salmonella typhosa* from large samples of water has been described. The quantity of sample for

[2] For the convenience of the reader separate summaries are inserted at the conclusion of the sections on bacterial counts and coliform counts.

Table 13. *Recovery of* S. typhosa *from dosed sewage—500 ml. quantities*

Endo medium coliforms	*S. typhosa* dosage	Percent recovery 2x B. S.[1] broth	Zoning of *S. typhosa*
14, 750	54. 0	22. 4	No zoning.
5, 875	42. 0	17. 0	No zoning.
1, 285	46. 0	15. 1	No zoning.
750	61. 0	26. 3	50 percent zoning.
210	72. 0	38. 9	90 percent zoning.

[1] Double-strength bismuth sulfite.

examination will be restricted only by the amount that will pass through the filter. Precipitate or sediment in the water will clog the filter, but most waters of drinking quality will permit filtration of a liter or more. The procedure has been satisfactory when the minimum density of *S. typhosa* was approximately 10 organisms in the sample, as one-half the bacteria grow on the membrane. Excessive numbers of *S. typhosa* colonies on the membrane are not recommended. It is suggested that the density of *S. typhosa* be less than 100 organisms per sample. *S. typhosa* grew as a characteristic black colony and many other species of bacteria were inhibited. Sulfite-reducing organisms have been found to produce brownish or black colonies. Such colonies may be disregarded and the *S. typhosa* colonies confirmed by serological agglutination or cultural methods.

EDITORIAL NOTE: The experiments reported here, although they indicate at points that counts on the filter have statistical superiority to counts by other methods, are not primarily concerned with these statistical advantages. Experiments concerned primarily with the statistical advantages will be reported in later papers. The present studies suggest that the filter offers great operating advantages while achieving statistical results at least as good as those of other methods.

APPENDIX

Media:

Nutrient broth

Single strength..... 8 gm. dehydrated broth per liter.
Double strength..... 16 gm. dehydrated broth per liter.
Triple strength..... 24 gm. dehydrated broth per liter.

Lactose broths

Nutrient broth in various strengths, plus addition of 0.5 percent lactose.

Media—Continued

Albimi M (single strength)

Albimi M peptone	20 gm.
Yeast autolysate (Albimi)	3 gm.
Dipotassium hydrogen phosphate	1.5 gm.
Sodium chloride	2.5 gm.
Distilled water	1,000 ml.

(Double or triple strengths obtained by increasing dry ingredients.) Sugars may be added in concentration of 0.5 percent.

Medium A

Tryptose (Difco)	40 gm.
Yeast extract (Difco)	10 gm.
Dipotassium hydrogen phosphate	7 gm.
Distilled water	1,000 ml.

M–2 Medium

Albimi M peptone	40 gm.
Yeast autolysate (Albimi)	6 gm.
Dipotassium hydrogen phosphate	3 gm.
Sodium chloride	5 gm
Distilled water	1,000 ml.

Adjust to pH 7 with dipotassium hydrogen phosphate.

Bismuth-sulfite broth

Beef extract	1 gm.
Peptone	2 gm.
Dextrose	1 gm.
Disodium hydrogen phosphate	0.8 gm.
Ferrous sulfate	0.06 gm.
Bismuth-sulfite indicator	1.6 gm.
Brilliant green	0.005 gm.
Water	100 ml.

(Heat to boiling—do not autoclave.)

E.H.C. Endo broth

(See text.)

Sterilization: Autoclave all media for 15 minutes at 121° C., with the exception of the bismuth-sulfite broth.

REFERENCES

(*1*) Bigelow, S., and Gemberling, A.: Collodion membranes. J. Am. Chem. Soc. **29:** 1576–1589 (1907).

(*2*) Ferry, J. D.: Ultrafilter membranes and ultrafiltration. Chem. Rev. **18:** 373–455 (1936).

(*3*) Pierce, H. F.: Nitrocellulose membranes of graded porosities. J. Biol. Chem. **75:** 795–815 (1927).

(*4*) Zsigmondy, R., und Bachman, W.: Ueber neue Filter. Ztschr. F. anorg. chemie. **103:** 119–128 (1918).

(*5*) Zsigmondy, R.: U. S. Patent No. 1,421,341 (June 27, 1922).

(*6*) Goetz, A.: Fiat final report 1312, Joint Intelligence Objective Agency. U. S. Department of Commerce, Washington, D. C. (1947).

(*7*) Goetz, A.: Aerosol filtration with molecular filter membranes. Chem. and Engin. News. **29:** 193 (1951).

(*8*) Goetz, A.: Personal communication.

(*9*) Phillips, C. R.: The sterilizing action of gaseous ethylene oxide. II. Sterilization of contaminated objects with ethylene oxide and related compounds: Time, concentration and temperature relationships. Am. J. Hyg. **50:** 280–288 (1949).

(*10*) Kaye, S., and Phillips, C. R.: The sterilizing action of gaseous ethylene oxide. IV. The effect of moisture. Am. J. Hyg. **50:** 296–306 (1949).
(*11*) Phillips, C. R., and Kaye, S.: The sterilizing action of gaseous ethylene oxide. I. Review. Am. J. Hyg. **50:** 270–279 (1949).
(*12*) Kaye, S.: The sterilizing action of gaseous ethylene oxide. III. The effect of ethylene oxide and related compounds upon bacterial aerosols. Am. J. Hyg. **50:** 289–295 (1949).
(*13*) Kaye, S.: The use of ethylene oxide for the sterilization of hospital equipment. J. Lab. and Clin. Med. **35:** 823–828 (1950).
(*14*) Prescott, S. C., Winslow, C.-E. A. and McCrady, M. C.: Water Bacteriology, ed. 6. John Wiley and Sons, Inc., 1945, p. 35.
(*15*) Müller, L.: Milieux pour la recherche des bacteries du groupe typhodysentérique. Compt. rend. Soc. de biol. **93:** 433–436 (1925).
(*16*) Ruys, A. C.: Ein brilliantgrün-Medium für die Isolierung von Paratyphus B Bakterien aus Stuhl und Urine. Zentral. Bakt. I. Abt. Orig. **132:** 349–351 (1934).
(*17*) Teague, O., and Clurman, A. W.: An improved brilliant green culture medium for the isolation of typhoid bacilli from stools. J. Infect. Dis. **18:** 647–652 (1916).
(*18*) Leifson, E.: New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Path. and Bact. **40:** 581–599 (1935).
(*19*) Wilson, W. J., and Blair, E. M. McV.: Use of a glucose bismuth sulfite iron medium for the isolation of *B. typhosus* and *B. proteus*. J. Hyg. (London) **26:** 374–391 (1927).
(*20*) Mueller, G.: Eine trinkwassergebundene Ruhrepidemie. Zentralbl. Bakt. Abt. I. Orig. **152:** 133–135 (1947).
(*21*) Difco Manual of Dehydrated Culture and Media Reagents, ed. 8, p. 103.

Incidence of Disease

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

Reports From States for Week Ending July 7, 1951

Poliomyelitis

For the current week, a total of 407 cases of poliomyelitis was reported which represents an increase of 19 percent over the previous week's figure of 341. For the same week last year, 478 cases were reported. The cumulative total since the seasonal low week is 2,026 as compared with 2,525 for the same period last year, and the cumulative total for the calendar year is 3,239 as compared with 3,656 last year. No section of the country showed a marked increase in cases for the current week as compared with the previous week. In fact, three sections—New England, South Atlantic, and Pacific regions—reported fewer cases.

The two States reporting large numbers last week, namely, Texas with 62 and California with 48, reported fewer cases as shown in the table. Up to and including the week ended June 30, the incidence in Texas has been highest in Nueces and San Patricio Counties, 30 percent of the cases reported in the State. Nearly one-half of the cases during this period occurred in Bexar, Harris, and Tarrant Counties, all of which have large urban populations. In California, there has been no great concentration of cases in any one or group of counties.

In Louisiana, where 30 cases were reported for the current week as compared with 22 for the previous week, 12 occurred in Shreveport. Prior to the current week, about one-third of the cases reported in the State were in the five counties in the extreme northwest part. Additional personnel and equipment have been supplied to Charity Hospital where most of the cases of the area are being treated.

In Oklahoma, poliomyelitis cases reported in June were concentrated in two counties—Oklahoma and Garvin. In Alabama, nearly half of the cases have been in Jefferson County. Colorado showed a sharp increase in incidence from 2 cases reported for the week ended June 30 to 21 for the current week. Information is not available at this writing to indicate where most of these cases were located.

Epidemiological Reports

Tularemia

Dr. D. W. McEnery, Wyoming Department of Public Health, states that of the eight cases of tularemia reported so far this year, one case

gave a history of handling rabbits, one was a person who had been shearing sheep, and one may have become infected while skinning mink. The source of infection was not determined in the remaining five cases.

Human Rabies

Dr. R. H. Hutcheson, Tennessee Commissioner of Health, has reported a tentatively diagnosed case of human rabies in a 4-year-old boy. The child, whose residence was reported to be in Leflore County, Mississippi, was admitted to a hospital in Memphis about May 31 after an illness of about 10 days. A diagnosis of encephalitis was made. He died, and an autopsy was performed on June 3, but sections of the brain were not examined until June 27 when rabies was reported to be the cause of death. The laboratory of the Memphis and Shelby County Health Department reported a tentative diagnosis of rabies on examination of sections of the brain. The family of the child stated they did not know the child had been bitten.

Relapsing Fever

Dr. W. L. Halverson, California Director of Public Health, reports that the first cases of relapsing fever for 1951 were discovered this week. One case was reported by the Long Beach Health Department, while the second, a child 10 years of age, contracted the disease in Eldorado County in the Sierra Nevada Mountains where chipmunks and tamarack squirrels are known reservoirs and the *Ornithodorus hermsi* tick is a known vector.

Comparative Data for Cases of Specified Reportable Diseases: United States

[Numbers after diseases are International List numbers, 1948 revision]

Disease	Total for week ended—		5-year median 1946–50	Seasonal low week	Cumulative total since seasonal low week		5-year median 1945–46 through 1949–50	Cumulative total for calendar year—		5-year median 1946–50
	July 7, 1951	July 8, 1950			1950–51	1949–50		1951	1950	
Anthrax (062)	1	1	------	(1)	(1)	(1)	(1)	40	25	29
Diphtheria (055)	37	61	96	27th	4,915	7,399	10,968	2,008	3,128	4,610
Encephalitis, acute infectious (082)	17	15	9	(1)	(1)	(1)	(1)	464	381	243
Influenza (480–483)	272	257	274	30th	129,960	148,719	148,719	115,418	138,135	127,880
Measles (085)	7,042	5,961	5,961	35th	472,162	290,499	561,860	443,461	271,370	526,914
Meningitis, meningococcal (057.0)	52	66	54	37th	3,460	3,258	3,130	2,499	2,345	2,158
Pneumonia (490–493)	730	913	(2)	(1)	(1)	(1)	(1)	43,042	56,222	(2)
Poliomyelitis, acute (080)	407	478	478	11th	2,026	2,525	2,525	3,239	3,656	2,878
Rocky Mountain spotted fever (104)	13	24	20	(1)	(1)	(1)	(1)	[3] 144	192	193
Scarlet fever (050) [4]	493	413	604	32d	67,143	55,169	78,970	51,452	38,730	56,426
Smallpox (084)	------	------	------	35th	15	43	66	7	23	45
Tularemia (059)	10	19	19	(1)	(1)	(1)	(1)	358	530	539
Typhoid and paratyphoid fever (040,041) [5]	44	84	90	11th	729	998	1,027	1,164	1,506	1,512
Whooping cough (056)	1,083	2,534	1,648	39th	62,031	93,245	82,489	40,429	71,739	51,223

[1] Not computed. [2] Data not available. [3] Addition: Virginia, week ended June 30, 1 case.
[4] Including cases reported as streptococcal sore throat. [5] Including cases reported as salmonellosis.

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 7, 1951

[Numbers under diseases are International List numbers, 1948 revision]

Area	Diphtheria (055)	Encephalitis, infectious (082)	Influenza (480–483)	Measles (085)	Meningitis, meningococcal (057.0)	Pneumonia (490–493)	Poliomyelitis (080)
United States	**37**	**17**	**272**	**7,042**	**52**	**730**	**407**
New England	**2**	**1**		**584**	**3**	**18**	**11**
Maine				101		1	
New Hampshire				4		2	
Vermont				26			1
Massachusetts	2	1		351	1		5
Rhode Island				24			
Connecticut				78	2	15	5
Middle Atlantic	**3**	**7**	**1**	**1,748**	**6**	**52**	**29**
New York	2	4	(1)	970	4		17
New Jersey		3	1	439	1	21	5
Pennsylvania	1			339	1	31	7
East North Central		**5**	**3**	**1,756**	**10**	**61**	**59**
Ohio				392	3		7
Indiana				34	1	5	3
Illinois			2	278	3	40	16
Michigan		5	1	142	2	16	21
Wisconsin				910	1		12
West North Central	**2**		**8**	**369**	**8**	**122**	**24**
Minnesota			1	26	2	6	4
Iowa				82		3	
Missouri	2		1	101	4		6
North Dakota			4	58		106	1
South Dakota				10			1
Nebraska				15	1		7
Kansas			2	77	1	7	5
South Atlantic	**3**		**146**	**795**	**15**	**191**	**41**
Delaware			1	20			1
Maryland			2	297	1	23	1
District of Columbia				17		9	3
Virginia	1		78	273	1	27	2
West Virginia				31	1		1
North Carolina	2			24	1		6
South Carolina			3	28	1	4	3
Georgia			62	67	7	128	14
Florida				38	3		10
East South Central	**5**			**130**	**2**	**56**	**43**
Kentucky	1			38		3	
Tennessee	2			48	1		4
Alabama	2			42	1	44	22
Mississippi				2		9	17
West South Central	**11**	**3**	**59**	**403**	**6**	**146**	**124**
Arkansas			47	46		21	15
Louisiana				7		7	30
Oklahoma		1	12	21	3	10	21
Texas	11	2		329	3	108	58
Mountain	**2**		**50**	**267**		**32**	**33**
Montana	1			53			
Idaho				40			
Wyoming				17			1
Colorado	1		11	23		23	21
New Mexico				17		2	2
Arizona			39	62		7	7
Utah				55			2
Nevada							
Pacific	**9**	**1**	**5**	**990**	**2**	**52**	**43**
Washington	2		3	83		2	2
Oregon	3		1	213		16	2
California	4	1	1	694	2	34	39
Alaska							
Hawaii				30		1	

[1] New York City only.
Anthrax: Pennsylvania, I case.

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 7, 1951—Continued

[Numbers under disease are International List numbers, 1948 revision]

Area	Rocky Mountain spotted fever (104)	Scarlet fever [1] (050)	Smallpox (084)	Tularemia (059)	Typhoid and paratyphoid fever [2] (040, 041)	Whooping cough (056)	Rabies in animals
United States	**13**	**493**		**10**	**44**	**1,083**	**126**
New England		**52**			**2**	**35**	
Maine						8	
New Hampshire		3					
Vermont		1				9	
Massachusetts		46				16	
Rhode Island		1					
Connecticut		1			2	2	
Middle Atlantic	**1**	**108**		**1**	**5**	**96**	**9**
New York	1	63		1	1	33	5
New Jersey		13				29	
Pennsylvania		32			4	34	4
East North Central		**126**		**2**	**5**	**155**	**14**
Ohio		30			1	31	3
Indiana		7			2	19	6
Illinois		19		2		24	4
Michigan		50			1	33	1
Wisconsin		20			1	48	
West North Central		**21**			**2**	**64**	**16**
Minnesota		4				1	3
Iowa		2			1	13	5
Missouri		2			1	13	7
North Dakota		4				2	
South Dakota		1				1	
Nebraska		3				4	
Kansas		5				30	1
South Atlantic	**6**	**30**		**1**	**8**	**131**	**18**
Delaware							
Maryland	3	7			2	1	
District of Columbia		2					
Virginia	1	2			1	19	6
West Virginia		2					1
North Carolina	1	6			1	45	
South Carolina		1				4	5
Georgia	1	6		1	4	41	6
Florida		4				21	
East South Central		**21**			**2**	**74**	**37**
Kentucky		4				10	20
Tennessee		10			1	39	4
Alabama		4			1	14	7
Mississippi		3				11	6
West South Central		**30**		**5**	**14**	**346**	**31**
Arkansas		1		4	3	30	1
Louisiana		3		1	4	2	
Oklahoma					2	17	3
Texas		26			5	297	27
Mountain	**6**	**17**		**1**	**1**	**119**	**1**
Montana	1	1				10	
Idaho		5			1	22	
Wyoming	2					2	
Colorado		3				32	
New Mexico						3	
Arizona		5				42	1
Utah	3	3		1		8	
Nevada							
Pacific		**88**			**5**	**63**	
Washington		7				19	
Oregon		5				3	
California		76			5	41	
Alaska		1					
Hawaii							

[1] Including cases reported as streptococcal sore throat. [2] Including cases reported as salmonellosis.

FOREIGN REPORTS

CANADA

Reported Cases of Certain Diseases—Week Ended June 16, 1951

Disease	Total	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia
Brucellosis	3					2	1				
Chickenpox	1,095	6		41	8	113	579	51	31	117	149
Diphtheria	1	1									
Dysentery, bacillary	3					3					
Encephalitis, infectious	1						1				
German measles	491			66		80	201		5	50	89
Influenza	34			28	3		1	2			
Measles	1,274	3		80	32	249	336	56	53	344	121
Meningitis, meningococcal	7					1	3	3			
Mumps	663	8		15		215	267	27	51	36	44
Poliomyelitis	6					2	4				
Scarlet fever	297	1		2		69	35	48	18	53	71
Tuberculosis (all forms)	274	13		3	44	114	26	31	3	8	32
Typhoid and paratyphoid fever	25					21	1				3
Venereal diseases:											
Gonorrhea	291	7		6	3	58	55	16	29	29	88
Syphilis	80	2		9	3	38	7	2	8	2	9
Primary	8			1			1		3	2	1
Secondary	2						1		1		
Other	70	2		8	3	38	5	2	4		8
Other forms	1										1
Whooping cough	113				1	24	42	4	1	23	18

FINLAND

Reported Cases of Certain Diseases—May 1951

Disease	Cases	Disease	Cases
Diphtheria	56	Scarlet fever	2,219
Dysentery	5	Typhoid fever	2
Meningitis, meningococcal	6	Venereal diseases:	
Paratyphoid fever	53	Gonorrhea	476
Poliomyelitis	8	Syphilis	15

WORLD DISTRIBUTION OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

The following tables are not complete or final for the list of countries included or for the figures given. Since many of the figures are from weekly reports, the accumulated totals are for approximate dates.

CHOLERA

(Cases)

Place	January–April 1951	May 1951	June 1951—week ended—				
			2	9	16	23	30
ASIA							
Burma	1,113	320	62	26	25	9	
Akyab	7						
Bassein	228	120	5	4	5		
Mergui		106	14	11	6	2	
Moulmein	168	47	25	7	8	7	
Rangoon	22	10					

Place	January–April 1951	May 1951	June 1952—week ended— 2	9	16	23	30
ASIA—continued							
India	35,952	6,348	2,086	2,061	1,856	[1] 850	
Bombay	3						
Calcutta	2,088	1,157	256	233	222	182	
Cuddalore	7						
Lucknow		3	3	8	1		
Madras	144	62	39	21	17	9	
Nagpur	68		1	8	1		
Negapatam	87						
Trichinopoly	100	5	2		3		
Tuticorin	34						
India (French):							
Karikal	36						
Pondicherry	143	5					
Indochina:							
Cambodia	[2] 39	[3] 4		[3] 3	[3] 6		
Viet Nam	19	4	2	1	1		
Cantho		2					
Haiphong	3						
Soc Trang	2				1		
Pakistan	9,267	2,615	[1] 119	[1] 37	[1] 15		
Chittagong	26	16	4		2		
Dacca	46	5					
Thailand	1						

[1] Preliminary. [2] Includes imported cases. [3] Suspected.

PLAGUE

(Cases)

AFRICA							
Belgian Congo	12	5		1			
Stanleyville Province	12	5					
British East Africa:							
Tanganyika	[1] 42						
Madagascar	127	4			1		
Union of South Africa	13						
Orange Free State	13						
ASIA							
Burma	235	11	1		1		
Rangoon	[2] 1						
Tavoy	2						
India	5,673	281	[3] 41	[3] 13	[3] 10	[3] 26	
Allahabad	[2] 109	[2] 17	[2] 1	[2] 1			
Bombay	1						
Calcutta	16	4					
Cawnpore	8						
Lucknow	15						
Nagpur	11						
Indochina:							
Cambodia	7	19	10	1			
Pnom Penh	7		2	1			
Viet Nam	45	28	2	4			
Baria				2			
Cap St. Jaques			1				
Phanthiet	24	18	1	2			
Phu Kok Island	9	10					
Indonesia:							
Java	5						
Bandoeng	1						
Djakarta	[2] 1						
Jogjakarta	2						
Semarang	[2] 1						
Madura	12						
Timbang	12						
Thailand	7						
SOUTH AMERICA							
Brazil	4						
Ceara State	4						
Ecuador	14						
Chimborazo Province	8						
Loja Province	6						

[1] Includes suspected cases. [2] Imported. [3] Preliminary figure.

SMALLPOX

(Cases)

Place	January–April 1951	May 1951	June 1951—week ended—				
			2	9	16	23	30
AFRICA							
Algeria	44	2					
Bechuanaland	127						
Belgian Congo	933	213	67	30	30		
British East Africa:							
Kenya	2						
Nyasaland	36	4			7		
Tanganyika	145	102					
Uganda	10	5					
Cameroon (British)	5						
Cameroon (French)	83	54			[1] 23	[2] 15	
Egypt	1						
Ethiopia	8	2					
French Equatorial Africa	88	1					
French West Africa	1,975	237			116	161	
Dahomey	340	19			[1] 7	[2] 1	
Guinea	9					[2] 1	
Ivory Coast	182	27			[1] 2	[2] 4	
Mauritania	6	7			[1] 2	[2] 1	
Niger Territory	738	36			[1] 4	[2] 54	
Senegal	3					[2] 1	
Sudan	497	130			[1] 82	[2] 87	
Upper Volta	200	18			[1] 19	[2] 12	
Gambia	1						
Gold Coast	333	4					
Morocco (French)	6						
Mozambique	88	9	3				
Nigeria	4,809				2		
Rhodesia:							
Northern		1		1			
Southern	253						
Sierra Leone	23						
Sudan (Anglo-Egyptian)	18			4			
Togo (French)	30	5					
Tunisia	3	2					
Union of South Africa	349	1					
ASIA							
Afghanistan	233	66					
Arabia	3						
Aden	[3] 2						
Oman	1						
Burma	560	22	4	4	7		
Ceylon	11	33	2				4
China	4						
India	161,094	24,051	[4] 5,589	[4] 4,299	[4] 3,789	[4] 1,836	
India (French)	2,085	199	50	20	29		
India (Portuguese)	115	25					
Indochina:							
Cambodia	75	3			2	17	
Viet Nam	434	528	73	85	69	58	
Indonesia:							
Borneo	1,083	19					
Java	131	14					
Iran	228	11	5	5			
Iraq	121	13		16	1	8	1
Japan	52		1				
Korea	474						
Pakistan	27,377	5,458	498	[4] 22	[4] 171	[4] 5	
Straits Settlements	1						
Syria			1				
Thailand	33						
Turkey	[5] 120						
EUROPE							
Great Britain:							
England: **Brighton**	15						
Portugal	9						
Netherlands		52	1				1
SOUTH AMERICA							
Brazil	3						
British Guiana	11						
Colombia	25						
Equador	107	2					
Paraguay	20						
Venezuela	41						

[1] June 1-10, 1951. [2] June 11-20, 1951. [3] Imported. [4] Preliminary figure. [5] Corrected figure.

TYPHUS FEVER *

(Cases)

Place	January–April 1951	May 1951	June 1951—week ended—				
			2	9	16	23	30
AFRICA							
Algeria	39	11					
Belgian Congo	1						
British East Africa:							
Kenya	9						
Somaliland	1						
Uganda	2	2					
Zanzibar		1					
Egypt	60	8	1				
Eritrea	9	4					
Ethiopia	287	48					
Gold Coast	2						
Libya:							
Cyrenaica	1	2		1	1		
Tripolitania	7	3					
Morocco (French)	2						
Morocco (Spanish)	12						
Nigeria	1						
Tunisia	11	4		1			
Union of South Africa	24						
ASIA							
Afghanistan	289	79					
Ceylon	2	1					
India	46	15	5	4	2	4	
India (Portuguese)	30	2					
Indochina: Viet Nam	25	6					
Iran	195	20	8		12	5	
Iraq	27	10	3	1	2	3	
Israel	2						
Japan	10						
Korea	72						
Pakistan	11	2					
Syria	1						
Transjordan	39	3	1			1	
Turkey	76	17	1	2	10	5	2
EUROPE							
Germany (French Zone)			1				
Great Britain:							
Island of Malta	1						
Portugal	28						
Sicily	5						
Spain		1					
Yugoslavia	224						
NORTH AMERICA							
Costa Rica	[1] 5						
Guatemala	11						
El Salvador	[1] 4						
Jamaica	[1] 6	[1] 2					
Mexico	[2] 40						
Puerto Rico	1						
SOUTH AMERICA							
Chile	62	11	4		3	4	
Colombia	32						
Ecuador	[2] 312						
Paraguay	11						
Venezuela	[1] 9		1				

*Reports from some areas are probably murine type, while others include both murine and louse-borne types.

[1] Murine.

[2] Includes murine type.

YELLOW FEVER

(C—cases; D—deaths)

Place		January–April 1951	May 1951	June 1951—week ended—				
				2	9	16	23	30
AFRICA								
Gold Coast	C	[1] 21	[1] 6				[2] 1	
Accra	C	[1] 4						
Adeiso	C	[1] 6						
Sierra Leone	C	[2] 2						
Koinadugu District	C	[2] 2						
Freetown	C	[2] 2						
NORTH AMERICA								
Panama	C	1						
Bocas Del Torro Province	C	1						
SOUTH AMERICA								
Brazil	D	[3] 400						
Goiaz State	D	[3] 400						
Anapoli	D	[4] 1						
Goiania	D	[4] 2						
Goiaz	D	[4] 5						
Inhumas	D	[4] 1						
Jaraqua	D	[4] 6						
Mineiros	D	[4] 2						
Niquelandia	D	[4] 3						
Pirenopolis	D	[4] 1						
Porangatu	D	[4] 1						
Rio Verde	D	[4] 2						
Uruacu	D	[4] 2						
Matto Grosso State	D	3						
Colombia	D	13						
Boyaca Department	D	1						
Otanche	D	1						
Caqueta Commissary	D	2						
Montanita	D	1						
Meta Territory	D	1						
North Santander Department	D	3						
La Vega	D	3						
Santander Department	D	6						
Campohermoso	D	1						
Guamales	D	1						
Maradales	D	1						
Tambo Redondo	D	1						
Venegas	D	1						
Ecuador	C	61						
Esmeraldas Province	D	1						
Atacames	D	1						
Quininde	D	1						
Santo Domingo de Los Colorados	C	58						
San Meguel	D	1						
Peru	D	4						
Huanuco Department	D	1						
Junin Department	D	1						
Loreto Department	D	1						
San Martin Department	D	1						

[1] Includes suspected cases. [2] Suspected. [3] The number of deaths Dec. 1–Feb. 20, 1951, was estimated to be 400 and the number of cases was estimated to be 2,000. [4] Confirmed deaths.

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