Comparison of the MF And MPN Techniques In Examining Sea Water

By M. W. Presnell, W. Arcisz, and C. B. Kelly

RESULTS OF A STUDY comparing coliform density values of sea water obtained by the membrane filter (MF) and the most probable number (MPN) procedures establish the former as a reliable technique if due regard is given to turbidities and bacterial densities in determining the volume of sample for filtration. The two techniques gave results 87.1 percent in agreement.

The time- and material-saving features of the MF method as compared with the MPN technique (*1*) make it particularly desirable for examining the frequently large number of samples required for determining sanitary quality of waters in shellfish-producing areas. The Manual of Recommended Practice for the Sanitary Control of the Shellfish Industry (*2*) stipulates that in making bacteriological examinations of shellfish-producing areas the number of samples from each station should be sufficient to give a true picture of the number of coliform organisms present in the water under the various tide and weather conditions occurring during the shellfish-harvesting period. The minimum number of samples required varies, but, where examinations are made to define the line between approved and restricted areas, at least 15 samples per station during the period of survey appear necessary.

Mr. Presnell and Mr. Arcisz are bacteriologists at the Shellfish Sanitation Laboratory of the Environmental Health Center, Public Health Service, at Woods Hole, Mass. Mr. Kelly, chief of the laboratory, is chairman of the American Public Health Association Subcommittee on Laboratory Examination of Shellfish.

The advantages of the membrane filter in the bacteriological examination of fresh water sources have been indicated (*3*, *4*). It has also been suggested that the membrane filter method gives coliform densities comparable to those obtained by the MPN procedure, as applied to fresh water (*5*). Goetz (*6*) reports that in an ocean water survey results obtained by the MF method were more consistent and more accurate than data gathered by standard MPN procedures.

Considering the advantages of the membrane filter method in the bacteriological examination of fresh water, it was deemed advisable to initiate a study to demonstrate the comparative performance of the membrane filter in the examination of sea water. Consequently, the present study, conducted in the Public Health Service Shellfish Sanitation Laboratory at Woods Hole, Mass., was undertaken to determine:

1. The comparison of coliform density values obtained by the membrane filter and the standard MPN procedures.
2. The effect of bacterial densities on the agreement of results by the two methods.
3. The influence of turbidity on the agreement of results by the two procedures.

Materials and Methods

Water samples were collected from three stations selected on the basis of degree of pollution and turbidity. An effort was made to choose stations approximately representative of water qualities defining the three areas of pollution pertaining to shellfish production. Station A represents an approved area (median coliform MPN per 100 ml., 70 or less); station B, a restricted area (median coliform MPN per 100 ml., 70 to 700); and station C, a closed area (median coliform MPN per 100 ml., more than 700).

Samples were collected twice daily from the three stations, at approximately 8:30 a.m. and

1:30 p.m., from November 1952 through February 1953. During this period, tide stages and weather conditions varied widely.

All sample collections were made according to standard procedures (*7*). Sterile, brown-glass bottles with bakelite screw caps, having a capacity of 620 ml., were used for collecting and containing samples.

The elapsed time between the collection and the bacteriological examination of a sample never exceeded 2 hours. Samples were stored at a temperature between 6° C. and 10° C. until examined.

Quantities of a sample used to determine coliform densities by the MPN and the MF methods were from the same bottle. Mixing of the sample was obtained by shaking the sample bottle vigorously 25 times.

Turbidity readings were determined by standard procedures (*7*) and were made on that portion of a sample remaining after appropriate quantities for bacteriological examination had been withdrawn.

The MPN method for determining the bacterial density of a sample consisted of planting 5 replicate quantities in at least 4 decimal dilutions in lactose broth. Gas-producing tubes were confirmed in brilliant green bile broth (*7*).

Sterilization

Filtration techniques, culture media, and incubation conditions employed were those developed and recommended by the Environmental Health Center (*5*). Sterilization of the filtration apparatus, membrane filters, and nutrient pads was accomplished by autoclaving at 121° C. for 15 minutes.

It was found that satisfactory sterilization of membrane filters by autoclaving was obtained by the following method:

Approximately 10 filters as shipped, that is, between nutrient pads and paper disks, were placed in covered petri dishes and autoclaved. Care was taken to limit the height of the stack of filters per petri dish to a point sufficiently low to prevent the top of the dish from contacting the stack of filters. This precaution was necessary since it was found that the weight of the petri dish cover exerted sufficient pressure at sterilization temperatures to cause adherence of the membrane filter to its protective coverings. Filters and pads were stacked vertically in petri dishes. Edges of filters were not allowed to project from between their protective covers. Complete coverage of each filter during autoclaving prevented the edges of the filters from curling and becoming brittle.

Table 1. Coliform densities obtained by MF and MPN methods, station A [1]

Sample No.	Turbidity (p.p.m.)	MF count per 100 ml.[2]	MPN per 100 ml.
1	5	2	4.5
2	5	170	920
3	5	5	2
4	5	2	2
5	5	2	2
6	20	17	33
7	20	3	7.8
8	20	1	13
9	10	4	4
10	10	5	2
11	10	59	79
12	10	4	6.8
13	10	71	79
14	10	2	17
15	5	3	2
16	5	18	46
17	5	11	2
18	10	5	13
19	10	8	7.8
20	5	5	13
21	5	5	4.5
22	10	5	17
23	10	2	2
24	5	3	17
25	5	20	22
26	5	2	1.8
27	10	1	1.8
28	10	9	2
29	5	7	4.5
30	20	2	2
31	5	12	23
32	10	2	4.5
33	10	6	7.8
34	10	4	4.5
35	10	32	46
36	10	11	17
37	5	1	6.8
38	5	1	1.8
39	10	3	33
40	10	6	7.8
41	10	29	17
42	10	4	2
43	10	22	13
44	5	110	33
45	5	35	33
46	5	3	4.5

[1] 20 ml. filtered per membrane filter. [2] Total count of 5 membrane filters.

Sample Volumes

To increase the accuracy of colony counts and to minimize the suppressive effects of excessive

Table 2. Coliform densities obtained by MF and MPN methods, station B

Sample No.	Turbidity (p.p.m.)	Calculated MF count per 100 ml.	MPN per 100 ml.
1	5	50	49
2	5	180	490
3	10	290	700
4	10	270	170
5	10	150	220
6	10	480	790
7	10	110	490
8	10	40	220
9	30	120	230
10	30	80	170
11	30	890	2, 400
12	20	470	700
13	30	840	3, 500
14	50	910	2, 400
15	20	360	790
16	50	1, 200	5, 400
17	10	280	1, 100
18	20	340	170
19	5	730	1. 700
20	20	160	490
21	20	2, 800	7. 900
22	20	2, 100	6, 400
23	20	2, 000	4, 900
24	20	12, 000	11, 000
25	5	310	490
26	20	120	230
27	20	500	490
28	30	650	640
29	30	2, 000	2, 300
30	30	600	1, 100
31	50	3, 100	4, 900
32	30	1, 100	790
33	50	4, 000	4, 900
34	10	560	790
35	10	250	460
36	20	2, 500	1. 400
37	20	1, 000	3, 500
38	10	650	4, 300
39	20	2, 500	4, 900
40	20	1, 100	2, 300
41	20	2, 700	11, 000
42	10	2, 000	1, 300
43	20	440	950
44	10	1, 500	1, 300
45	5	500	330

bacterial density on coliform recovery rates, an attempt was made to filter sample volumes yielding not more than 350 total colonies per membrane filter. In the present study, the choice of sample volumes filtered per membrane filter from the three sampling stations warrants discussion.

On the basis of previous coliform densities obtained by the MPN method, filtration of 100 ml. of sample from station A was indicated. Though it was possible to pass as much as 600 ml. of water (average turbidity 8 p. p. m.) from station A through a filter without its being clogged, volumes in excess of 50 ml. produced a matlike overgrowth. By using 5 membranes and filtering five 20-ml. portions of sample instead of one 100-ml. portion, no overgrowth was noted, and the total colony count of the 5 membranes was never in excess of 350 colonies.

It is postulated that certain types of extraneous matter on the membrane surface serve as a "blotter" to hold fluid which encourages diffuse growth of organisms, thus preventing colony formation. It was noted that overgrowth by noncoliform organisms rarely occurred on filters having moderate to light depositions of extraneous matter. Granular extraneous matter when unaccompanied by fine suspended siltlike material did not interfere with colony formation or serve as a bridge for coalescence.

Experiments employing various turbidity concentrations and various particle sizes and shapes would have to be performed before any definite relationship between turbidities and colony growth and formation could be established.

Bacterial densities in waters from stations B and C showed such wide morning-to-afternoon and day-to-day fluctuations that it was impossible to obtain desired results by employing only one volume of sample for filtration. Satisfactory membrane counts were obtained by filtering 20-ml., 10-ml., and 1-ml. portions from station B and 1-ml. and 0.1-ml. portions from station C. For increased accuracy, duplicate membranes were examined for each sample volume.

Though average turbidities of waters from stations B and C were relatively high, 20 and 14 p. p. m., respectively, no clogged and few overgrown filters resulted even from the maximum volume of water filtered, that is, 20 ml.

Results

Data obtained from stations A, B, and C are presented in tables 1, 2, and 3.

Table 1 is self-explanatory. In tables 2 and 3, the method of calculating membrane counts per 100 ml. needs description. Valid membrane counts from all volumes of a sample filtered were included in calculating coliform counts per 100 ml. Counts were deemed valid

if filters were free from evidence of overcrowding, overgrowth, and excessive noncoliform organisms. Calculations were made as follows:

$$\frac{\text{Total number coliform organisms} \times 100}{\text{Total volume sample filtered}} = \text{Number organisms per 100 ml.}$$

For example, in sample 9 at station B, membrane counts from all sample volumes filtered (duplicate 20-ml., 10-ml., and 1-ml. quantities) were valid. Totaling coliform counts and volumes of water filtered and applying the above equation gives:

$$\frac{75 \text{ organisms} \times 100}{62 \text{ ml. of sample}} = 120 \text{ organisms per 100 ml. of sample}$$

Table 3. Coliform densities obtained by MF and MPN methods, station C

Sample No.	Turbidity (p.p.m.)	Calculated MF count per 100 ml.	MPN per 100 ml.
1	5	2, 500	7, 900
2	5	9, 500	7, 900
3	5	860	1, 300
4	5	500	460
5	5	9, 900	11, 000
6	5	1, 200	3, 300
7	30	5, 300	4, 900
8	30	12, 000	35, 000
9	20	4, 800	3, 300
10	20	7, 600	17, 000
11	10	16, 000	11, 000
12	10	4, 700	3, 300
13	20	54, 000	24, 000
14	20	5, 000	4, 900
15	20	2, 000	4, 900
16	20	19, 000	35, 000
17	20	7, 700	7, 900
18	20	3, 500	11, 000
19	20	5, 300	4, 900
20	20	76, 000	54, 000
21	20	14, 000	13, 000
22	20	140, 000	110, 000
23	5	84, 000	110, 000
24	5	150	78
25	10	2, 900	7, 900
26	20	1, 800	2, 300
27	10	3, 800	11, 000
28	20	23, 000	23, 000
29	20	140, 000	130, 000
30	20	7, 000	13, 000
31	20	4. 200	4, 600
32	20	7, 400	13, 000
33	10	3, 300	3. 300
34	20	6, 800	3, 300
35	10	16, 000	33, 000
36	20	2, 800	1, 300
37	20	6, 000	11, 000
38	10	11, 000	4, 900
39	10	4, 800	7, 900
40	20	23, 000	46, 000
41	10	5, 100	3, 300
42	10	5, 600	4, 900
43	10	3, 800	3, 200
44	20	1, 000	2, 200
45	10	240. 000	130. 000
46	5	14, 000	23, 000
47	10	13, 000	13, 000
48	5	91, 000	220, 000

Table 4. Comparison of coliform densities obtained by MF and MPN methods using 95-percent confidence limit of recorded MPN as a base line

Station	Average turbidity (p.p.m.)	Number of samples	Number of samples agreeing	Number of samples disagreeing	Percent agreement
All stations	14	139	121	18	87. 1
A	8	46	36	10	78. 3
B	20	45	37	8	82. 3
C	14	48	48	0	100

Table 4 gives the percentage of agreement between bacterial densities obtained from samples by the MF and MPN methods. The 95-percent confidence range of the recorded MPN (table 1) was used as a baseline for comparison. For a 5-tube, 3-dilution test, the 95-percent confidence range is the difference between the lower and upper limits; namely, 0.3 to 2.9 times the calculated MPN (*8*). The 95-percent confidence limits were obtained by multiplying the recorded MPN's by the factors 0.3 and 2.9. Those MF counts falling within these limits were considered in agreement.

The percentage agreement of results by the two methods was higher for waters of high coliform density than for waters of low and moderate coliform content.

Summary and Conclusions

Coliform densities were obtained by simultaneous examination of sea water samples by the MF and MPN methods. Comparison of the data revealed that the two techniques gave results 87.1 percent in agreement.

Results obtained by the MF and MPN methods from waters having large coliform counts gave greater percentage agreement than results obtained from waters having a low coliform count.

Water turbidity can greatly influence the coliform recovery rate and should be considered

in conjunction with the bacterial density in determining the volume of sample to be filtered.

The use of small replicate volumes of water is indicated where a single large volume from sources having low bacterial numbers produces overgrowth.

On the basis of results from the present study, it is concluded that the membrane filter method is a reliable technique for determining the coliform densities of sea water if due regard is given water turbidities and bacterial densities in determining the volume of sample for filtration.

REFERENCES

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PHS Advisory Council Appointments

Dr. Wallace M. Yater, director of the Yater Clinic in Washington, D. C., was appointed during November 1953 to the National Advisory Health Council, which advises the Surgeon General on grants in research and public health work. Dr. Yater, a past president of the District of Columbia Medical Society, has been active in clinical and pathological research. He was director of the department of medicine of Georgetown University's medical school and also served as head of the university hospital's department of medicine from 1930 to 1945. He served as civilian consultant to the Medical Department of the Army from 1942 until 1947.

Frank A. Robbins, Jr., for many years a ranking officer of the Harrisburg Hospital in Pennsylvania and a member of the American Public Welfare Association, was appointed during November to the National Advisory Arthritis and Metabolic Disease Council, which advises and makes recommendations on activities of the National Institute of Arthritis and Metabolic Diseases and on grants for research in these fields. Mr. Robbins served as secretary of the Department of Public Assistance of Pennsylvania from 1947 to 1951. A retired steel company executive, he is a member of the American Iron and Steel Institute and of the Engineer's Society of Pennsylvania.

Dr. E. Cowles Andrus, president-elect of the American Heart Association, was appointed to the National Advisory Heart Council. He is one of five newly appointed members of this 15-man body. The council makes recommendations on programs of the National Heart Institute and serves as recommending authority for research and teaching grants on diseases of the heart and circulation. Also named to this council were:

Dr. Owen H. Wangensteen, chief of the department of surgery and professor of surgery at the University Hospital, who has been chairman of the department of surgery at the University of Minnesota since 1930; **Daniel Sherby,** director of the Continental Bank of Cleveland, who was a member of the National Advisory Policy Committee for Health and Welfare, and has served with the Federal Government in other consultative capacities; **Helen L. Curry,** president of the Kansas Council of Women, an organization acting as a clearinghouse for health and other civic activities of 29 statewide women's groups; **Louis E. Leverone,** Chicago, president of the Nationwide Food Service, Inc., and active in the field of business aviation and vocational training, boys clubs, and a number of civic groups.