Membrane Filter Procedure Applied in the Field

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IN THE PLANNING of public health procedures appropriate for field use or in the event of a natural or wartime disaster, there is a recognized need for quick detection of bacterial pollution of water supplies. For this purpose, application of membrane filter techniques has been proposed, primarily because the 4 to 5 days required to perform coliform enumerations can be reduced to 18 to 20 hours by use of the membrane filter, without apparent loss of validity of results.

Application of the membrane filter in an emergency implies that the tests be completed under field conditions, with minimum availability of standardized laboratory equipment and supplies. It is recognized, also, that in certain situations there may be need for culture incubation by other methods than conventional laboratory incubation. Previous descriptions (*1*, *2*) of EHC Endo medium have specified preparation of the medium on the day of use, thereby necessitating probable preparation under field conditions. In addition, the fragility of glass petri dishes renders them of questionable value in such operations.

The studies which are reported here dealt with (*a*) a specially designed garment to incubate inoculated membrane filters, utilizing body warmth as a source of heat; (*b*) the applicability of more durable containers to field-culture methods; and (*c*) the feasibility of using stored, fully prepared Endo medium to avoid the necessity of mixing the medium in the field.

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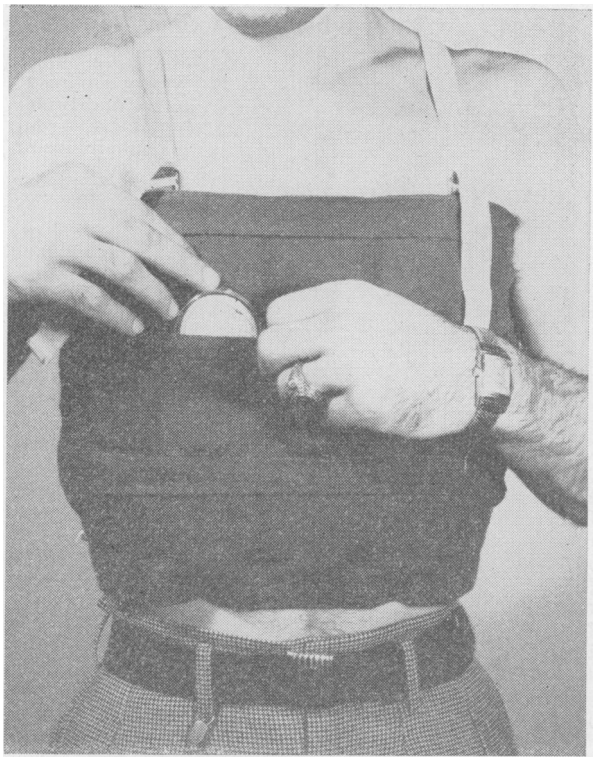

Garment for incubation of inoculated filters by body heat of wearer.

Sample Filtration

Equipment suitable for membrane filtration has been adequately described (*1–4*). All the filtrations in this investigation were made in the laboratory, in order to compare the "field type" of test with the laboratory test as a control procedure. An electric vacuum pump was the only piece of equipment used that might not be available in a disaster area. Samples of water were collected in bulk and were filtered within 2 to 3 hours from the time of collection.

Use of the standard metal or glass-holding apparatus is feasible for field work. The differential pressure across the membrane can be obtained by using a hand-operated bicycle pump with the leathers reversed, a rubber bulb aspirator, or an evacuated container. Field sterilization of the apparatus can be accomplished by immersion for 5 minutes in boiling water, by flaming alcohol, or by the incomplete combustion of methyl alcohol (*1*, *2*, *5*). Resterilization of the filter holder between filtrations of successive samples is not usually re-

quired. Rinsing down the funnel walls with a small amount of sterile water two or three times, after sample filtration but before removal of the membrane, reduces the residual micro-organisms to insignificant levels.

Culture Containers

A satisfactory container should: (*a*) be nonbreakable, of chemically inert material which can be easily cleaned; (*b*) be suitable for sterilization either by heat or by chemical procedures; (*c*) have air- and water-tight seal, to prevent leakage of medium, contamination, or loss of humidity; (*d*) be convenient to use, inexpensive, and readily available.

Probably no single type of container fully meets all of these requirements. Two types of containers have been examined extensively with regard to the above criteria. One was an ordinary 2-oz. ointment tin, 50–60 mm. in diameter, available from most laboratory equipment distributors, large drug stores, or drug supply companies. The other was a disposable plastic petri dish, 50 mm. in diameter. Tests using these containers were run in parallel with the 60-mm. glass petri dishes routinely used in this laboratory for membrane filter studies.

Culture Media

All membranes were incubated for 2 hours on M-enrichment medium (Difco) followed by transfer to EHC Endo medium for final incubation of 16 to 18 hours. The EHC Endo medium used in the medium storage experiments was kept in screw-capped tubes until time for use.

Sterilization Procedures

In these investigations, a laboratory autoclave was used to sterilize all materials. For field use, a pressure cooker (such as is used for home-canning purposes) could be substituted.

Equipment, such as funnels, metal containers, and graduates, was sterilized for 15 minutes at 121° C. (15 lbs.). Whenever it was necessary to dry this equipment rapidly, the escape valve of the autoclave was opened to reduce the steam pressure.

(The plastic petri dishes could be sterilized by soaking for 2 hours or longer in 70-percent ethyl alcohol. The alcohol must be allowed to drain from the dishes before using them.)

The membrane filters were separated from the kraft paper dividers (leaving the white blotting paper inserts in place), wrapped loosely in kraft paper (about 10 to each package), and sterilized in the autoclave for 10 minutes at 115° C. (10 lbs.). At the end of the sterilization period, the escape valve was opened to reduce the pressure in the autoclave to atmospheric pressure. (This was essential to protect the membranes from excessive moisture.)

The enrichment medium and EHC Endo base were autoclaved 15 minutes at 121° C. The 20-percent lactose solution was autoclaved 10 minutes at 115° C.

Field Incubation

The incubation assembly (see photograph) consisted of a sleeveless, vestlike garment, adaptable to a torso of almost any size and equipped with adjustable elastic strappings to hold the garment in place and to permit maximum dexterity of the operator. The vest, worn in direct body contact for maximum heat transfer, held 21 incubation containers in pouches located on the front side. Incubation containers were inserted vertically into these pouches so that the bottom (thus, the nutrient pad and membrane) was closest to the body and was separated from the skin by a nylon marquisette fabric which formed the inner lining of the garment. The front side of the pouches was lined with nylon-faced, rubberized cloth to minimize heat losses. A heavy-grade cotton oxford cloth was used as an overlay veneer and extended around the back of the torso, where it was fastened.

Experimental tests consisted of body incubation of inoculated membranes, contained in metal and plastic containers, for the established periods. Parallel tests were made with membranes cultured in glass petri dishes and incubated in a laboratory high-humidity incubator at 35° C.

Results

In table 1, quantitative coliform recovery in the metal and plastic dishes is compared

with recovery in the control test, in which glass dishes were used. Counts are average values of duplicate membranes. All water samples were from farm wells.

Average coliform counts of four replicate samples from a group of rural well waters incubated in the conventional, constant-temperature, high-humidity incubator, and a parallel series incubated at body temperature in the incubator vest, are shown in table 2. Both types of containers, the ointment tin and the plastic petri dish, were used in this evaluation.

Coliform recoveries on stored EHC Endo medium, as compared with the results from freshly prepared EHC Endo medium, are shown in table 3.

These values were based on four replicate filtrations for each of the two media.

Discussion

Comparison of coliform recovery results from the tin and from the plastic containers (table 1) with the recoveries from standard glass petri dishes indicated that any of these containers could be used interchangeably. Laboratory observations demonstrated no consistent variation in either colony size or quality of the characteristic sheen of coliform colonies.

Both the plastic dish and the metal container satisfied most of the criteria outlined for optimum field performance. It was shown that these containers could be incubated in any position without interference from spreading growth caused by condensed moisture falling on the membrane. There appeared to be sufficient adhesive power in a properly saturated nutrient pad to keep the membrane in a fixed position on the bottom of the container. The containers were sufficiently tight to prevent evaporation of the nutrient and to preserve a sufficiently humid atmosphere. (However, several containers were not leakproof, so that medium did seep out. This resulted in staining the incubator vest and occasionally the clothing worn adjacent to the vest.)

Other discomforts to the wearer were associated with continuous wearing of the vest, such as heat buildup and difficulties in sleeping. Under nonemergency conditions, some other means of supplying suitable temperatures, for example, a portable incubator or thermos jar,

Table 1. Average coliform recoveries from experimental tin and plastic containers in relation to standard glass containers

Ointment tin containers				Plastic containers			
Sample No.	Coliforms/100 ml.		Recovery ratios [1]	Sample No.	Coliforms/100 ml.		Recovery ratios [1]
	Standard test	Experimental test			Standard test	Experimental test	
1	2,000	2,300	1.15	11	37	55	1.49
2	600	420	.70	12	900	700	.78
3	180	140	.78	13	220	270	1.23
4	320	330	1.03	14	4	4	1.00
5	260	270	1.04	15	300	320	1.06
6	360	310	.86	16	100	110	1.10
7	140	160	1.14	17	13	14	1.08
8	540	490	.91	18	7,200	8,600	1.19
9	33	50	1.51	19	35	29	.83
10	280	300	1.07	20	120	110	.92
Average			1.02	Average			1.07

[1] "Recovery ratios" are the ratios of the mean number of coliform colonies in the experimental culture containers to the mean number of coliform colonies in the conventional glass Petri dishes (standard test).

Table 2. Average coliform recoveries from tin and plastic containers using conventional and body incubation

Sample	Ointment tin containers (coliforms/100 ml.)		Recovery ratios	Sample	Plastic containers (coliforms/100 ml.)		Recovery ratios
	Standard incubation	Body incubation			Standard incubation	Body incubation	
1	220	220	1.00	11	660	530	0.80
2	2,300	2,800	1.22	12	880	1,000	1.14
3	420	540	1.28	13	270	270	1.00
4	140	240	1.71	14	380	530	1.39
5	330	300	.91	15	320	300	.94
6	260	300	1.15	16	110	130	1.18
7	300	270	.90	17	14	18	1.29
8	170	170	1.00	18	8,600	9,200	1.07
9	480	640	1.33	19	1,100	1,500	1.36
10	50	50	1.00	20	29	32	1.10
Average			1.15	Average			1.13

may better serve the purpose of incubation in field examinations of water supplies.

Steam or hot-air sterilization of the metal culture containers resulted in development of scale and rust after repeated use. Both types of culture containers were considered to be disposable, and could be taken into the field in a sterile condition, used once, and discarded. The low cost of these containers made the practice economically feasible.

According to the data in table 2, it appeared that when the body served as the source of heat, coliform recoveries were equal to those obtained from conventional incubation. Colonies incubated by body heat were noticeably smaller, necessitating occasional extension of incubation time to insure complete development and optimum differentiation of coliform colonies. The smaller size often was advantageous, because it helped to minimize interference with coliform differentiation due to overcrowding and confluence of colonies.

Frequent random temperature observations in the pouches of the incubation vest, made over a 6-week period in which the vest was used, indicated maintenance of a relatively constant and uniform temperature range. The limits were 33.5° C. and 35.5° C.

From the data in table 3, it appeared that limited-duration storage of the EHC Endo medium had no discernible effect on quantitative coliform recovery. Similarly, colony size and sheen characteristics appeared not to be adversely affected with storage up to 3 or 4 weeks. Thus, fully prepared Endo medium, previously mixed in the laboratory, can be taken out into the field for medium-transfer purposes.

These results suggest application of field incubation procedures to small-scale surveys in remote areas where accurate results must be obtained quickly by starting or completing incubation at the sampling site. The body incubation modification is particularly applicable

Table 3. Average coliform recoveries on stored and freshly prepared medium

Number of days stored	Recovery ratios of stored Endo media at—	
	Room temperature	4° C.
19	0.96	0.93
25	1.09	.96
28	1.15	1.02
32	1.09	1.00

to civil defense activities and disaster relief, where laboratory facilities may not be conveniently available.

Conclusions

1. Both tin and plastic containers possess the merits of simplicity, mechanical durability, and membrane filter adaptability.

2. Equally satisfactory results are obtainable by use of the metal or the plastic container as a culture dish for membrane filters.

3. The incubator vest is an acceptable device for utilizing body heat for the incubation of membrane filters under field or emergency conditions.

4. The EHC-modified Endo medium may be stored for 3 or 4 weeks without destroying the usefulness of the medium.

REFERENCES

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(*3*) Goetz, A., and Tsuneishi, N.,: Application of molecular filter membranes to the bacteriological analysis of water. J. Am. Water Works Assoc. 43: 943–984 (1951).

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Public Health Service Staff Announcements

Dr. Harold M. Janney has been appointed medical director of the Bureau of Prisons, Department of Justice. Since 1950 Dr. Janney has been medical director of the United States Penitentiary, Atlanta, Ga. He has had progressively responsible appointments in the Federal prison system since his initial Public Health Service assignment as medical staff officer at the Federal Reformatory, Chillicothe, Ohio, in 1936.

Dr. Joseph O. Dean, formerly associate chief of the Bureau of State Services, Public Health Service, on September 15 was named as an assistant to the medical director of the Bureau of Indian Affairs, Department of the Interior. Prior to his assignment as associate chief in 1949, he served as district director and regional medical director of Region VII, with headquarters in Kansas City, Mo.

Dr. Dean was appointed to the Regular Corps of the Public Health Service in 1929, and received his master of public health degree in 1937 from the Johns Hopkins School of Hygiene and Public Health. During his career, he served at quarantine stations in New York and New Orleans. From 1937 to 1941 he made studies of public health administration in rural areas and also of the activities of various county health departments. From 1941 to 1944 he was stationed in San Juan, Puerto Rico, as medical consultant.

During World War II, Dr. Dean was concerned with activities relating to emergency health and sanitation, serving first as assistant chief of the Division of States Relations and later as chief of the Office of Surplus Property. From October 1946 to October 1947, he was assistant chief of the Division of Commissioned Officers.