The Use of the Membrane Filter Technique for Testing Water Supplies in the Field

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TWO STUDIES conducted in western national parks during the early summer of 1955 evaluated the feasibility of using the membrane filter field test laboratory to determine the bacteriological quality of the drinking water supplies in the national parks.

The many widely dispersed water supplies in the parks vary from small springs, infiltration systems, wells, or surface supplies to community-type systems. Although the conventional sanitary survey of any water supply system provides data on potential sources of contamination and general adequacy of treatment and distribution, routine examinations for bacteriological safety are needed. The results of such examinations also serve to guide the operation and provide a record which reflects the sanitary quality over an extended period.

Because of the relatively isolated location of many parks and their water supplies, it is often time consuming to collect and mail samples and await reports from State laboratories which may be several hundred miles away. Frequently, in the time between sample collection and examination, a change occurs in the character of the sample, and results may not always reflect the condition of the supply.

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Considering the relatively short season in many park areas, a method of performing a simple, rapid, field test to supplement regular laboratory examinations would be valuable.

The membrane filter technique was introduced into the United States from Europe in 1947. Considerable research has been conducted in the United States, including laboratory studies by the Robert A. Taft Sanitary Engineering Center of the Public Health Service, to develop the technique for the bacteriological examination of drinking water. The membrane filter procedure has been accepted as a tentative method in the 10th edition of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. Portable field test laboratories (MF kits) are available to run tests and obtain results in the field. The time required to obtain results with MF kits is approximately 18 to 20 hours as compared to 2 or 4 days, plus time in transit, with the standard dilution tube test.

Initiation of Studies

Although evidence (*1*) indicated that the MF field kit might be used effectively with national park water supplies, it was considered desirable to run a trial under actual field conditions. The National Park Service requested that the Public Health Service perform special studies relating to the applicability of the membrane filter technique. A project proposal of the work and objectives was outlined. Two duplicate studies of approximately 6 weeks' duration were initiated in June 1955 in national parks. One study was conducted by John D. Eye, professor of sanitary engineering, Virginia Polytechnic Institute, in the Rocky Mountain National Park, Colo. The other study was conducted in the Yosemite and Sequoia-Kings National Parks, Calif., by Ely J. Weathersbee, instructor, Oregon State College. Both investigators, who are sanitary engineers, were called to temporary active duty from the Commissioned Reserve Corps of the Public Health Service.

In addition to studying the applicability of the membrane filter field test laboratory, they conducted sanitary surveys of water supplies sampled for examination by the MF kit. These surveys included inspections of the collection, treatment, and distribution systems of each water supply and a brief study of the drainage areas. In separate reports, the investigators have correlated the results of the bacteriological tests with the sanitary surveys.

Materials and Methods

The MF kit contains the necessary equipment for filtering and incubating the samples and sterilizing the funnel assembly in a rugged, portable carrying case which weighs 30 pounds fully loaded. Membrane filters and dehydrated nutrient media pads were used in the studies. Packs, each containing sufficient membranes and media for six tests, were purchased presterilized in sealed polyethylene bags.

Additional items of equipment were found to be necessary for the tests. These included bottles for collecting water samples and storing sterilized dilution water, hand magnifying lens to assist in counting coliform colonies, improved plastic tape for sealing petri dishes prior to immersing in the thermos bottle incubators, and 95 percent ethyl alcohol for dipping the forceps tips prior to flaming.

The collection of samples and the examinations were conducted in accordance with the procedures outlined in the 10th edition of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. Parallel standard dilution tube tests were run on approximately one-third of all samples collected and results obtained by the two methods were compared. The comparison was made to determine the degree of correlation of the MF kit field results with the standard dilution test results.

Briefly, the MF technique is to filter, under suction, a water sample portion through a small (2-inch diameter) circular, paper-thin disc composed of a cellulose material with pore openings of submicron size (*1*). Any bacteria present in the sample collect on the surface of the filter. The disc is transferred to a petri dish containing an absorbent pad with a small amount of nutrient broth culture media. A small quantity of sterile water is added to the petri dish which is then sealed and incubated in thermos bottles, provided in the MF kit, at approximate body temperature 37° C. After 18 or 20 hours, the dishes are removed from incubation, and those colonies exhibiting a metallic sheen, characteristic of coliform organisms, are counted. Results are recorded as the number of coliform organisms per 100 ml. of sample. Absence of coliforms indicates freedom from contamination.

Usually, in the Rocky Mountain National Park study, the MF kit was taken to the water sampling site where the test was performed. During inclement weather, the field laboratory was established in a ranger dormitory kitchen made available by the park superintendent. A home-type pressure cooker and a small electrically operated incubator were utilized for the standard dilution tube test and also for sample bottle sterilization. In Yosemite, the field laboratory was set up in the sewage treatment plant laboratory where a home-type pressure cooker was available for necessary sterilization of sample bottles, preparation of dilution tubes, and production of sterile water for use in rehydrating nutrient absorbent pads. Both investigators were assisted by park officials in locating water supplies and in obtaining the use of park equipment and facilities.

The MF kits, reputedly among the best available at the time of the studies, were ordered shortly before the initiation of the field work. Despite mechanical difficulties experienced with the MF kits early in the studies, the equipment performed in a satisfactory manner after adjustments. Most of the difficulties were related to ill-fitting petri dish holders, leakage of funnel apparatus, and wetting of membrane cultures in petri dishes in the thermos bottles because of leakage of parafilm tape supplied with the kit.

One major difficulty, which persisted throughout the course of the studies, concerned the method of incubation of the membrane filter cultures. Thermos bottles supplied with the kits are used for incubation. Under low temperature conditions prevailing during the studies, a significant drop in temperature, as much as 20° F. 4 hours after starting the incubation of cultures, occurred in these thermos bottles.

Although the exact effects of such variation from required incubation temperatures were not evaluated, evidence indicated that the results were adversely affected. More recent improvements in MF kits also provide built-in electrically heated incubators that are adaptable to battery or to standard power sources.

As many of the individual tests were performed where the samples were obtained, open air conditions interfered with the use of the kit. Turbidities were abnormally high in the surface supplies during the early period of the studies because of the spring runoff. Because of the turbidity, difficulty in filtering enough water to obtain significant growth was reported from both study sites. Also, on some samples sediment deposited on the membrane so spread bacterial growth that coliform organisms could not be identified or counted.

The use of dehydrated nutrient media pads was considered convenient and satisfactory. However, possible inhibition of coliform growth related to the use of this nutrient method was suspected in the Rocky Mountain Park study.

Results of Parallel Tests

The determination of the agreement of the MF test with the standard dilution tube test was included in the studies. Temporary laboratory facilities and equipment to run the standard methods test were assembled in the parks. In addition, some samples were sent to State health department laboratories for examination.

Standard dilution tube tests were run on approximately one-third of all samples collected for examination by the membrane filter technique. With the standard dilution tube method, an estimate of the most probable number (MPN) of coliform organisms is calculated, and test results are reported in coliforms per 100 ml. of sample. In the MF test, a direct count of coliforms on the membrane filter are made, and results are also reported per 100 ml. of sample.

In comparing the results of the two tests on the same sample of water, the 95 percent confidence limit was applied to the standard dilution tube test results in recognition of the bias in the standard method.

In the present studies, the coliform counts obtained by the MF test were generally lower than those obtained by the standard dilution tube test. In some cases, the lower counts were caused by excessive turbidity that limited the amount of sample that could be filtered as mentioned previously.

The results of only 17 of the 123 total parallel tests were in disagreement by the two methods (table). The number of results in disagreement was higher in the Rocky Mountain National Park study than at Yosemite and Sequoia-Kings National Parks. Considering the difficulties encountered, the agreement of the two methods is deemed satisfactory and indicates that the membrane filter test results are reliable.

Comparison of results of parallel membrane filter and standard dilution tube tests applying the 95 percent confidence limit to the MPN obtained by the standard dilution tube test

Park study area	Number of samples	Number of samples agreeing	Number of samples disagreeing	Percent agreement
Rocky Mountain	69	56	13	81
Yosemite and Sequoia	54	50	4	93
Both studies	123	106	17	86

Conclusions

1. The 86 percent agreement in the results obtained by the membrane filter technique and

the standard dilution tube test on the same samples is considered satisfactory. Elimination of mechanical difficulties experienced would undoubtedly further increase the agreement.

2. Results of the membrane filter tests were known in less than 1 day and were directly applicable to the evaluation of individual water supplies which had been surveyed during the course of the studies. When the samples were mailed to central laboratories, results of the standard dilution tube method could not be obtained in less than 7 to 10 days.

3. The samples collected from the surface water supplies during the early spring runoff generally contained high turbidities which were difficult to analyze by the membrane filter method.

4. Satisfactory results were obtained when the portable testing kit was set up in a central shelter within the parks where samples were brought for analysis. Exposure to open air conditions affects the operation of the kit if the analysis is made at the sampling site.

5. The operation of the kit is simple and rapid; however, specialized training and familiarity with the equipment is necessary for obtaining results which can be properly evaluated.

6. The membrane filter method can be used for bacteriological examination of isolated water supplies as found in national parks. However, the studies clearly indicated the need for improvements in the portable field laboratories (MF kits), particularly in the method of incubation.

REFERENCE

(*1*) Clark, H. F., Geldreich, E. E., Jeter, H. L., and Kabler, P. W.: The membrane filter in sanitary biology. Pub. Health Rep. 66: 951–977, July 20, 1951.

Public Health Training Program

Under a new Public Health Service training program, 266 public health workers are now enrolled in graduate training in more than 40 schools. Authorized by Congress on July 23, 1956, the program went into effect in the fall semester with awards of almost $1 million in training grants to schools and individuals.

Upon completion of their studies, most of the trainees will be employed in State and local health departments, thus helping to relieve the acute personnel shortage that has prevented many areas from making full use of modern knowledge about the prevention and control of disease.

Under the awards, 130 nurses are being trained for public health nursing positions through grants totaling $377,618 to 32 schools of nursing. Grants totaling $260,137 to 10 schools of public health are training 47 students who are specializing in various other types of public health activities.

In addition, training grants were awarded directly to 89 persons: 7 physicians, 6 dentists, 5 dental hygienists, 39 sanitary engineers and other sanitation specialists, 3 veterinarians, 5 nutritionists, 18 health educators, and 6 persons from other professions concerned with public health.