TO THE INSTRUCTOR

This filmstrip is intended as a teaching tool for basic instruction of the membrane filter technique. Its running time is 11-1/2 minutes. The filmstrip cannot teach the technique by itself, but should be used by an instructor as a part of a planned presentation which includes further explanations, demonstrations, and practice.

The instructor should review the film at least twice before attempting to use it for teaching. This booklet may be used as a classroom reference and lecture guide by making notes opposite the proper points in the filmstrip which will require additional explanation, or demonstration.

It is suggested that the filmstrip be shown to a class at least twice; the first time for familiarization and with subsequent showings interrupted at appropriate points for demonstrations, emphasis, and further explanation.

The record for this filmstrip is a 33-1/3 RPM, microgroove recording, and caution must be taken to use a record player with a proper needle cartridge.

SEE LAST PAGE FOR INFORMATION
ON OBTAINING THE FILMSTRIP

the membrane filter

..... A TEACHING AID TO SUPPLEMENT THE FILMSTRIP

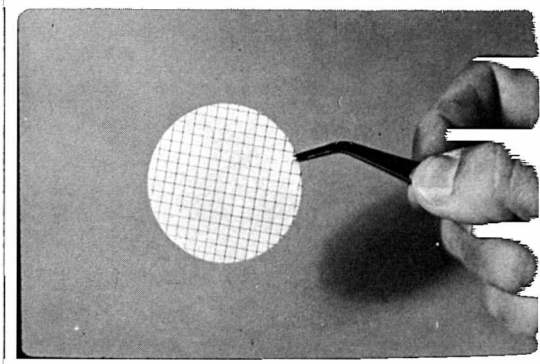
U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Water Supply and Pollution Control and
Communicable Disease Center

Public Health Service Publication No. 749

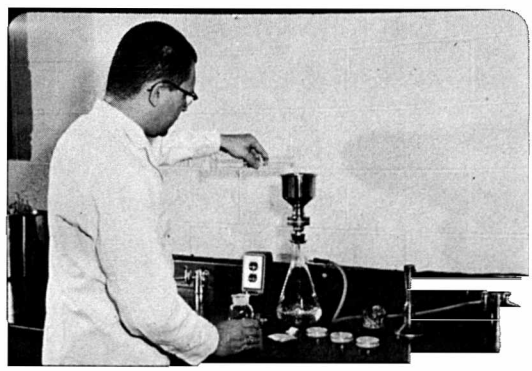
1960

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington 25, D.C. - Price 20 cents.

The membrane filter is a circular disc of a cellulose material approximately two inches in diameter.



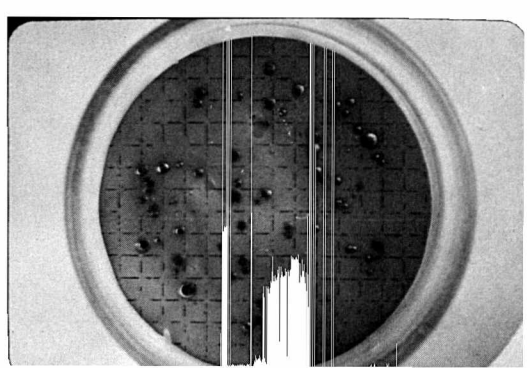
When a liquid or aerosol passes through it, particles larger than the pore size are mechanically retained on the surface of the membrane.

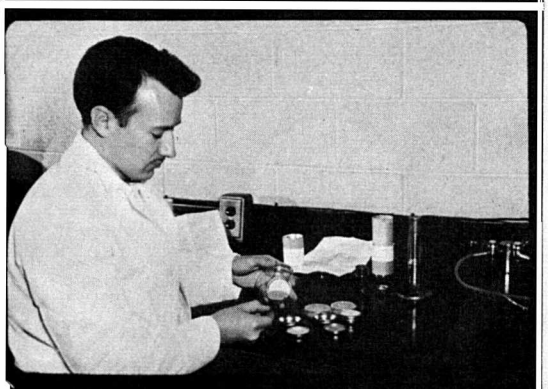


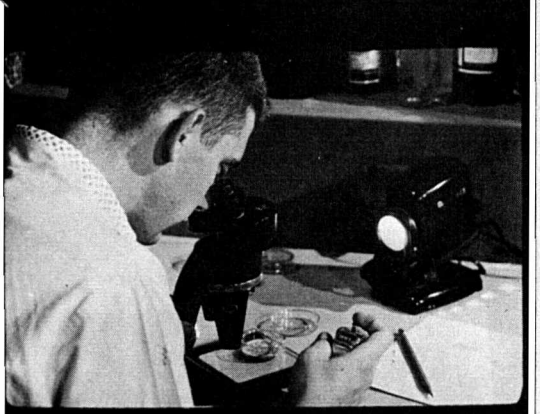
In addition, some particles smaller than the pore size are retained due to electrostatic charges on the particles and filter.

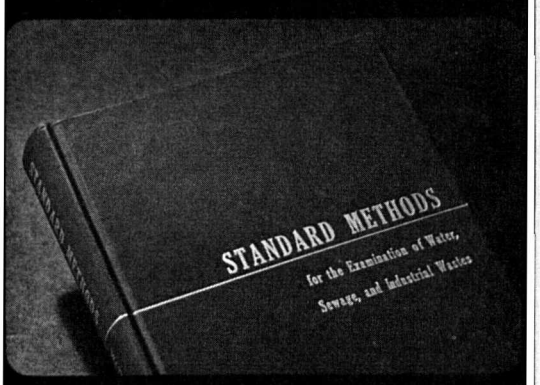
The entrapped organisms are then cultured by media supplied through an absorbent pad.

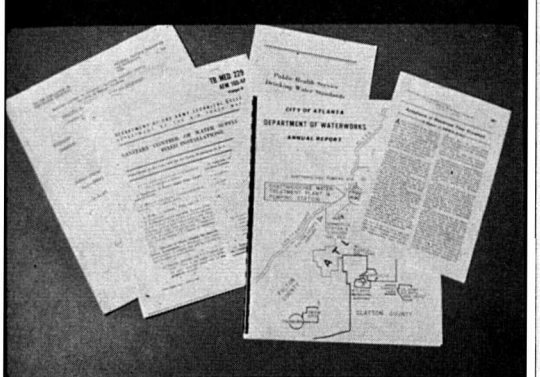
The majority of bacteria, including the common indicators of pollution such as the coliform bacteria, are large enough to be collected by the membrane.

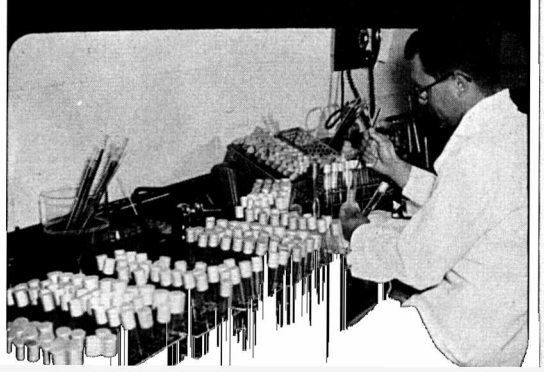












The membrane filter was first used extensively by German technologists in World War II for the examination of water supplies.

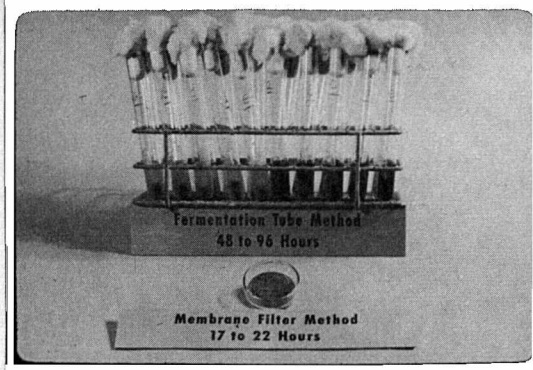
Improvements in this technique have been made by the U. S. Army Chemical Corps Biological Laboratories, the Sanitary Engineering Center of the Public Health Service . . . and others.

As a result of these improvements, the membrane filter technique has been included as a method of analysis in the Standard Methods for the Examination of Water, Sewage and Industrial Wastes.

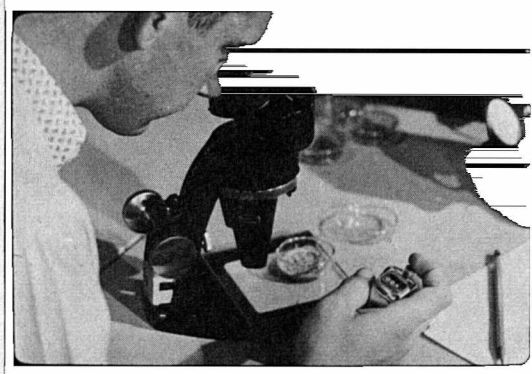
It has been adopted by the Public Health Service . . . the Military Forces . . . and many cities, for the examination of municipal water supplies.

The membrane filter has many advantages over this commonly used fermentation tube technique. One of the most important is the time saved.

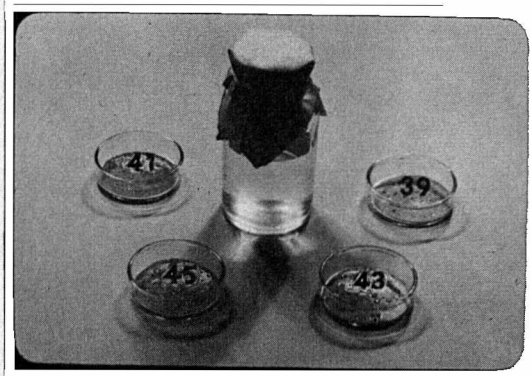
Using the fermentation tube method requires 48 to 96 hours to arrive at a count, as compared with 17 to 22 hours for the membrane filter.



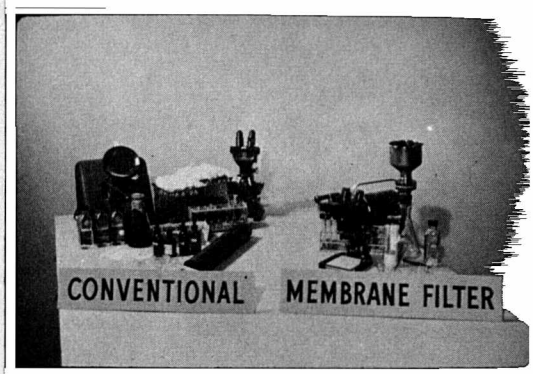
The membrane filter technique produces an actual count and not a statistical approximation as in the fermentation tube technique.



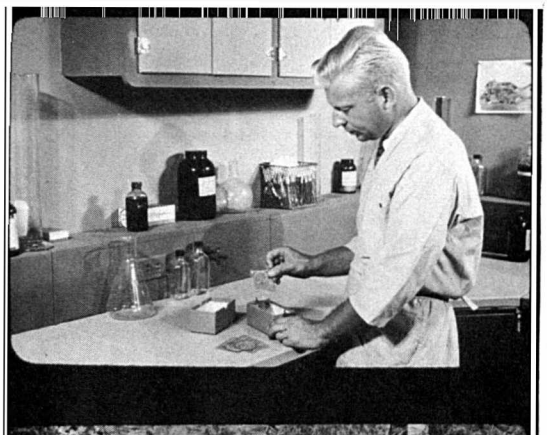
Therefore, with the membrane filter technique, there is a greater degree of reproducibility on examinations of the same sample.

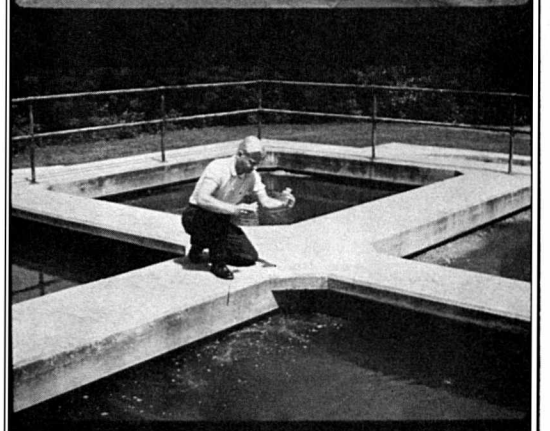


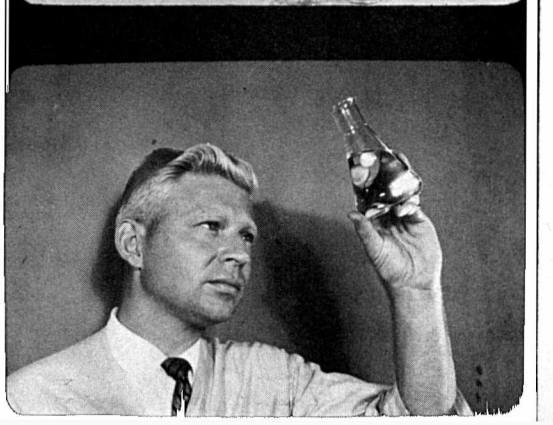
Less space, material, equipment, and labor are required . . .



. . . it's easily adapted for use in field studies . . .







. . . and the test results, in the form of the membrane, are easily preserved for future reference.

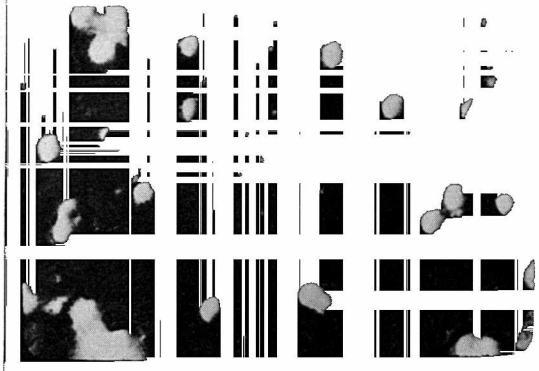
There are some disadvantages, however, to the MF technique. It is not applicable to samples with high bacterial concentrations, which overgrow the surface of the membrane.

Generally unsatisfactory results are obtained in samples containing large numbers of algae, flocs, and other types of suspended solids . . .

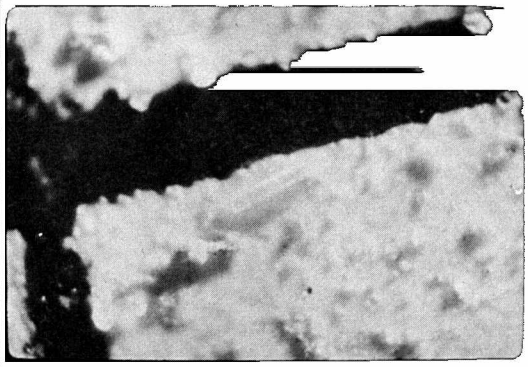
. . . and it requires special instruction of scientific personnel to perform the test and to interpret the results.

The original use of the membrane filter was for the isolation of bacteria in fluids. However, it has been used in other applications . . .

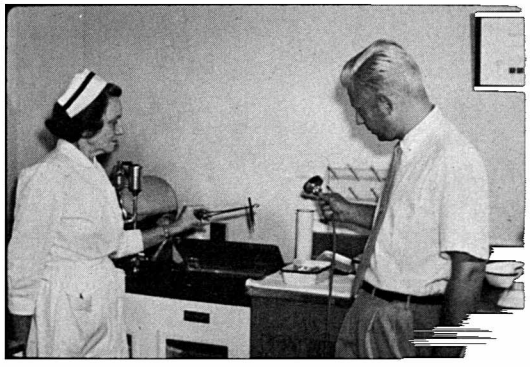
. . . such as the determination of relative size of virus particles . . .



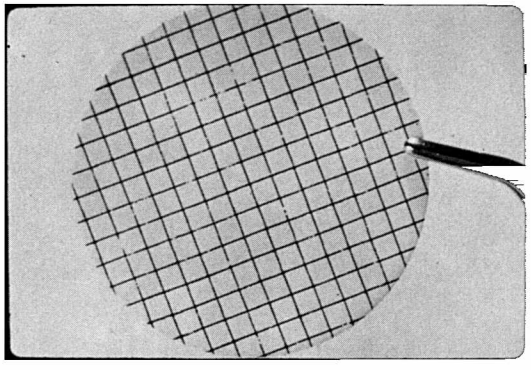
. . . for biological study of algae, and other living organisms . . .



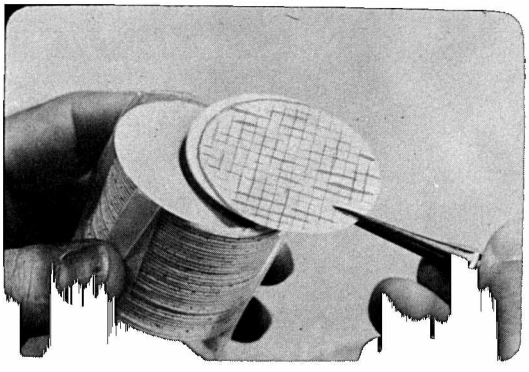
. . . and the collection of particulates from aerosols.

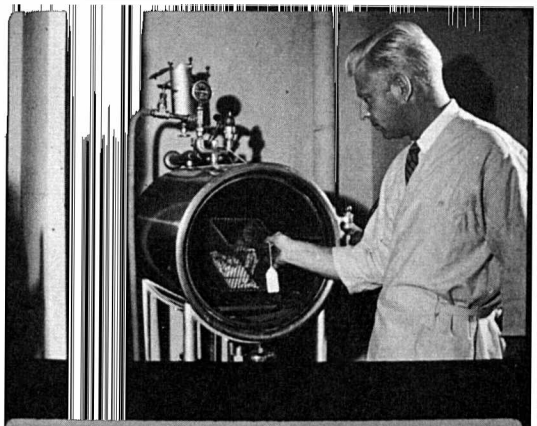


Many filter membranes are marked with grid lines to aid in counting.



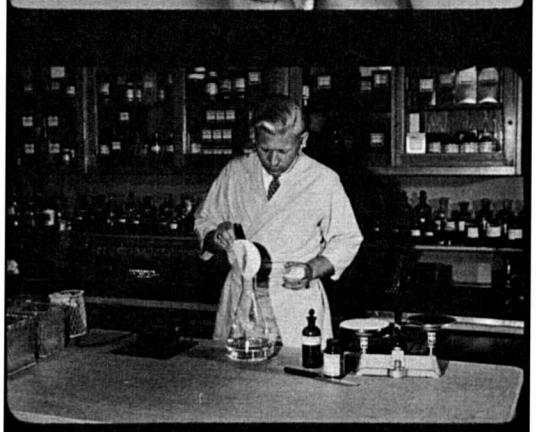
The membranes and absorbent pads are packaged in a variety of ways. One of the most convenient is with the membranes and pads packaged together in sterilized sets.





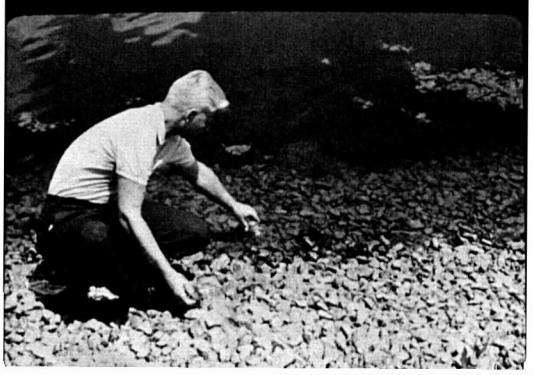
Non-sterile membranes and absorbent pads must be sterilized before use.

The Endo Broth medium, used for water examinations, can be purchased in a ready-to-use liquid broth . . .



. . . or in a dehydrated form . . . and reconstituted in hot water.

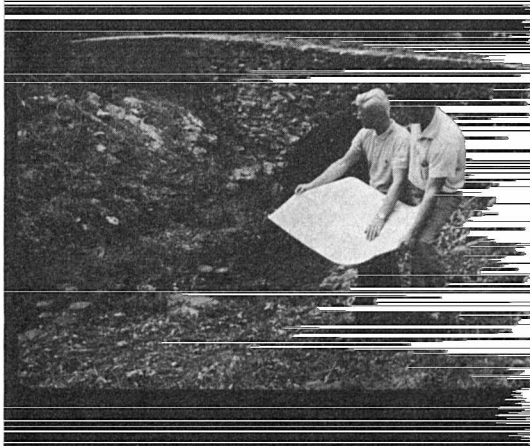
Rehydrated media should be made up in small quantities . . . capped . . . and stored in a dark place.



The objective in examining polluted water is to make a quantitative estimate of the number of coliform bacteria, which represents fecal contamination.

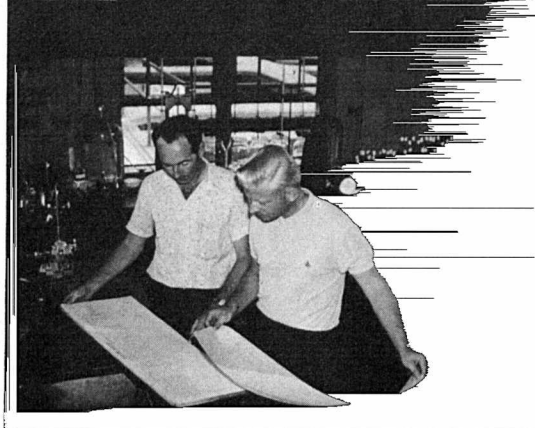
A sample of contaminated water should produce between 20 and 80 coliform colonies, and not more than 250 bacterial colonies of all kinds on a membrane in order for the membrane to be usable.

Before deciding on the volume of the sample to be filtered, collect and study information concerning the source of the water to be sampled . . .

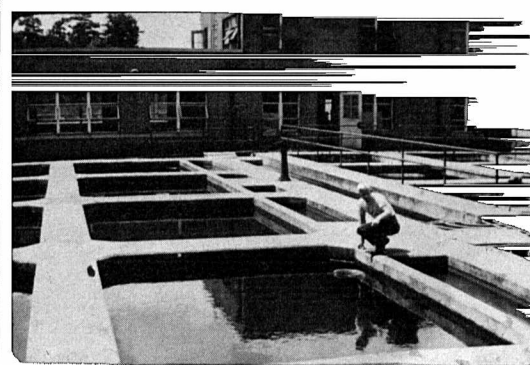


. . . possible pollution from environment . . .

. . . and past history of pollution.



Limiting factors in the quantity of water to be filtered, other than bacterial content, are suspended material, algae, chemical flocs, or miscellaneous debris.



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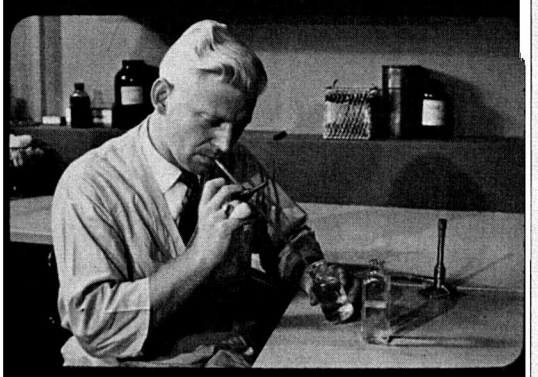
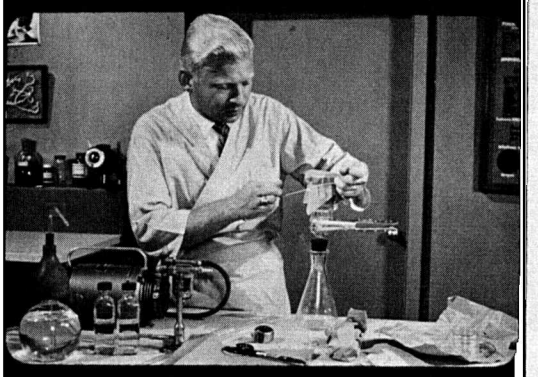
Treated water	100-500 ml.
Well Water	10-50 ml.
Springs	0.1-10 ml.
Heavily polluted water	0.01-1 ml.





Some recommended sample volumes of various classes of water are shown in this chart.

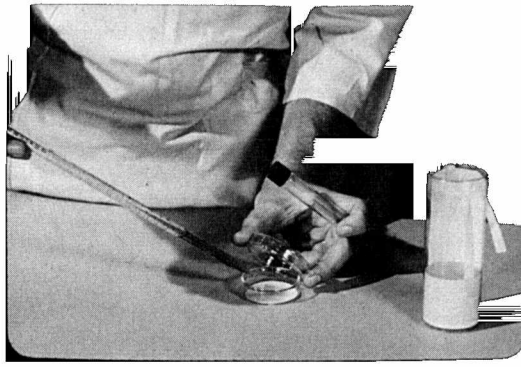
When less than 20 ml. of sample is filtered, the sample should be diluted with sterile dilution water to give a volume of fluid equal to at least 20 ml.

The basic equipment needed for the MF technique is simple, and can be found in most bacteriology laboratories.

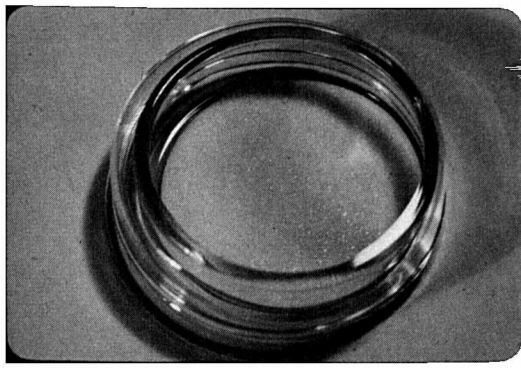
All material and equipment must be sterilized according to standard laboratory practice.

Before filtration, place a sterile absorbent pad into a sterile Petri dish.

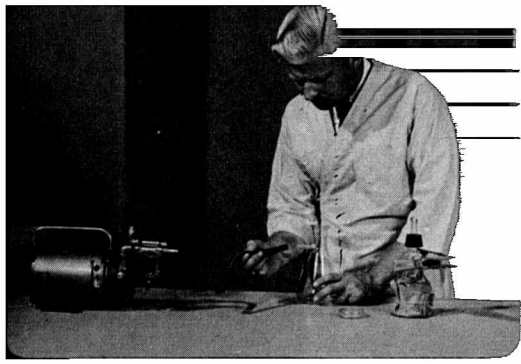
Add approximately 2 ml. of the nutrient, Endo Broth, to the absorbent pad.



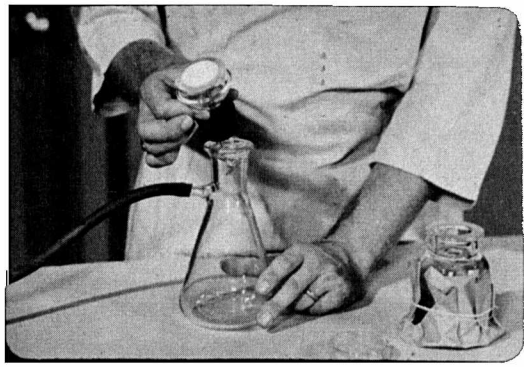
Complete saturation of the pad is important. Insufficient nutrient may cause disappointing results.



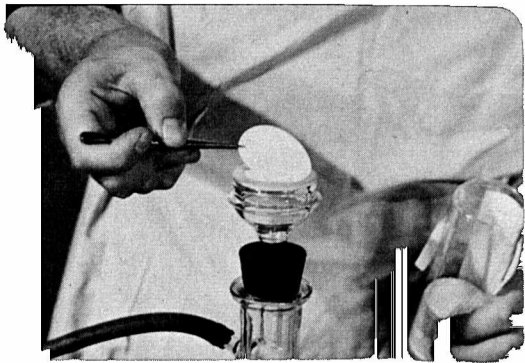
For filtration . . . begin by attaching a vacuum line to the side tube of a filtering flask . . .

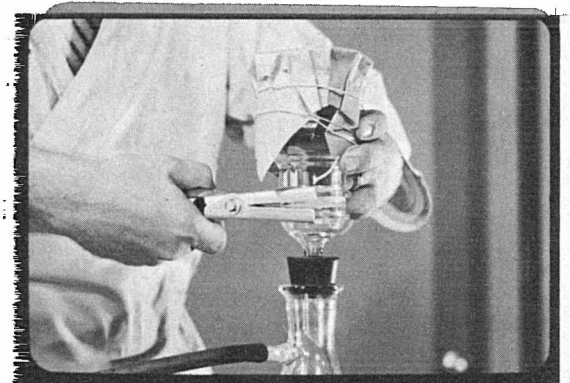


. . . and insert the filter receptacle into the flask.

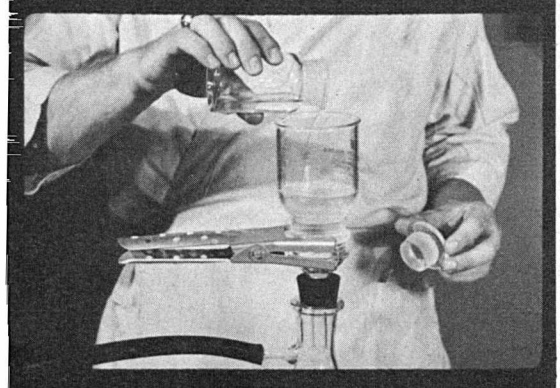


Place a sterile membrane, grid side up, onto the filter receptacle.



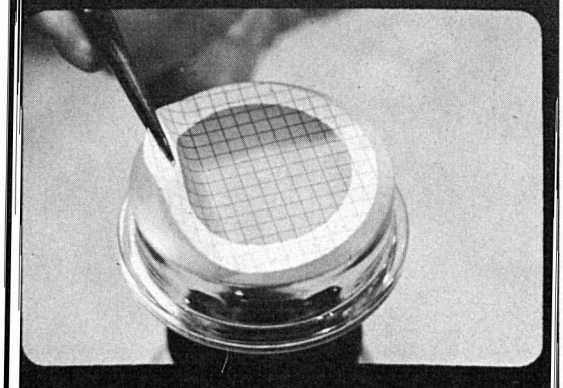


Clamp the funnel in place. The assembly must be closed tightly to prevent leakage or by-passing of the filter membrane.

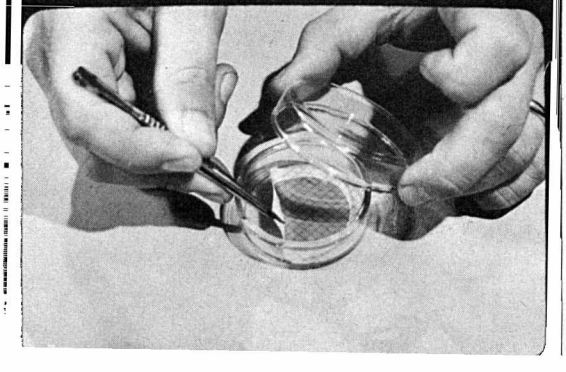


Apply the vacuum, and filter the sample. As the water passes through the membrane, all particulate material, including living bacteria, are retained on its surface.

After the water sample is filtered, the inside of the funnel is rinsed with sterile water to wash down any residual drops of the sample.

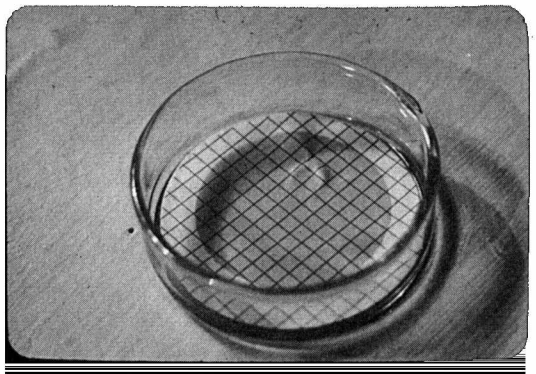


Remove the membrane from the receptacle . . .

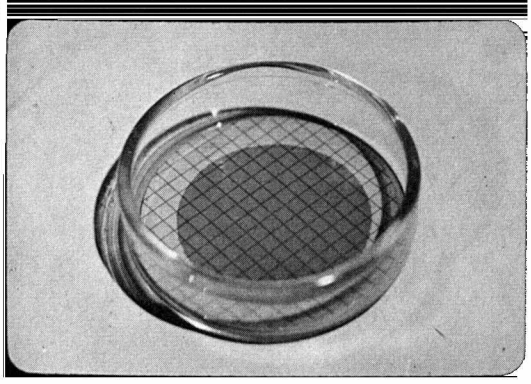


. . . and carefully "roll" it, grid side up, onto the absorbent pad containing the Endo Broth.

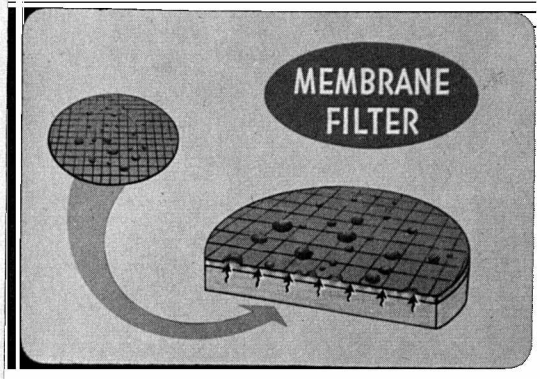
If air pockets are trapped under the membrane, light spots will appear on its surface. Remove the membrane and repeat the "rolling" process.



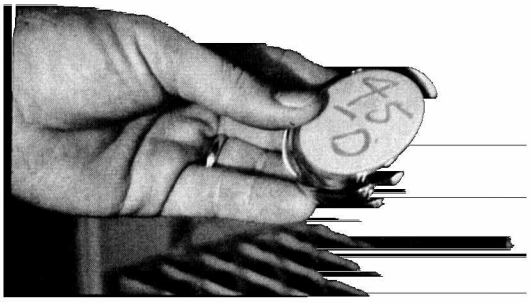
The surface of the membrane should acquire a uniform pink color in a few minutes.



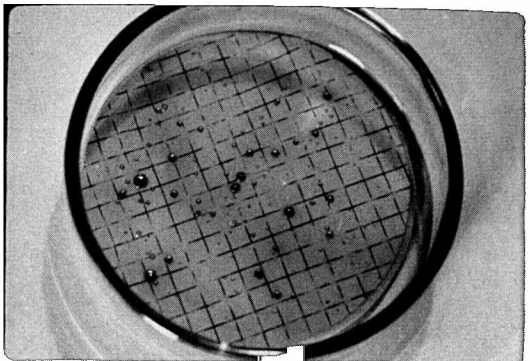
The absorbent pad feeds the Endo Broth by diffusion through the pores in the membrane to the growing bacteria on the surface.

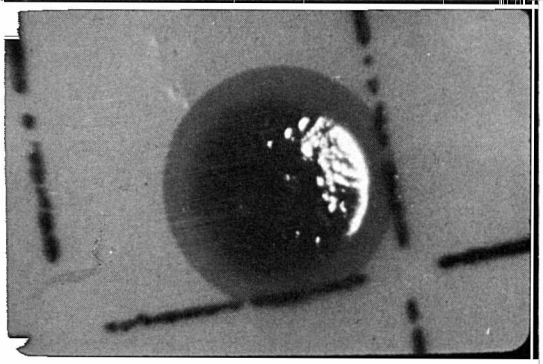


Cover the dish and place it, inverted, into a humidity controlled incubator at 35° C. for 17 to 22 hours.

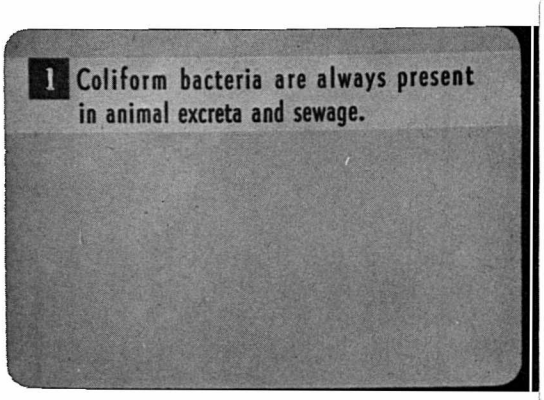


Any living bacteria capable of growing in the incubation period on the medium selected, will develop visible colonies.

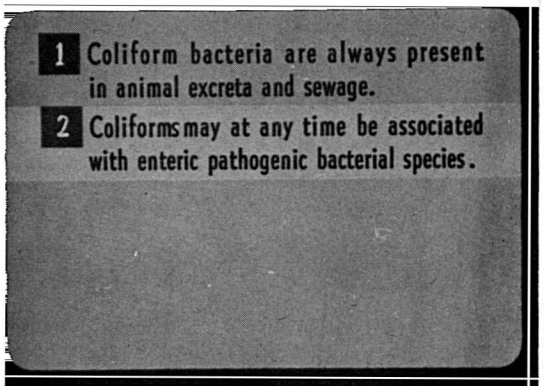




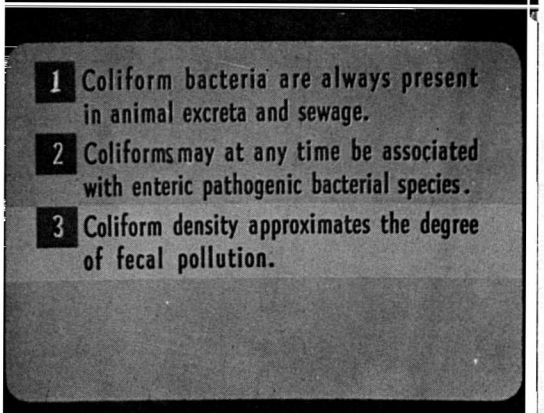
The coliform bacteria have a characteristic dark color with a golden metallic sheen over the surface, which differentiates them from others.



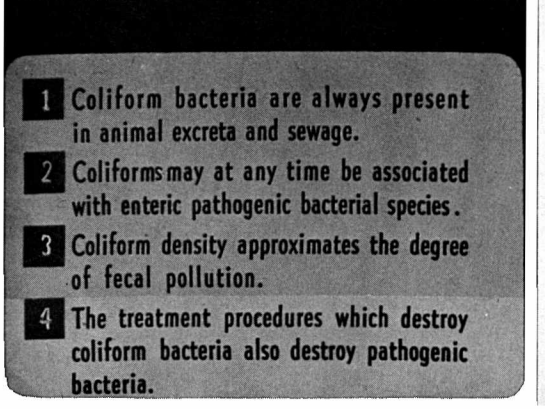
The sanitary quality of water is indicated by the presence of coliform bacteria because . . . one . . . coliform bacteria are always present in animal excreta and sewage . . .



. . . two . . . coliforms may, at <u>any</u> time, be associated with enteric pathogenic bacterial species . . .

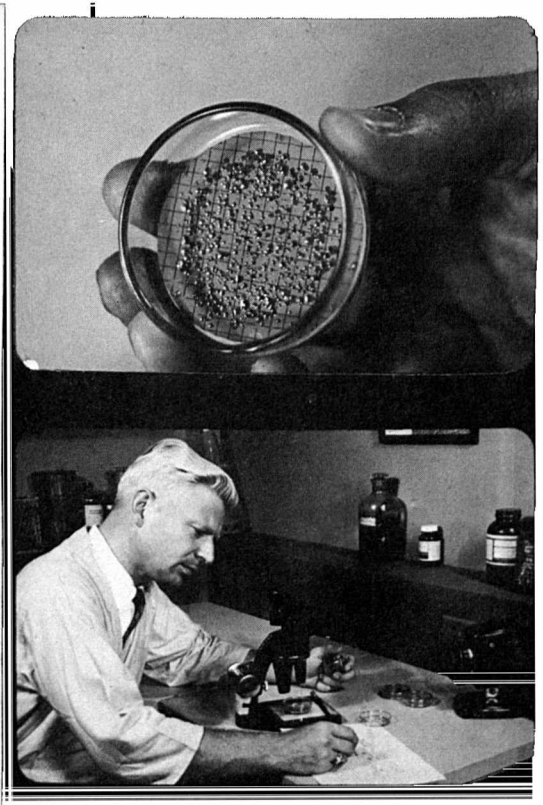


. . . three . . . coliform density approximates the degree of fecal pollution . . .



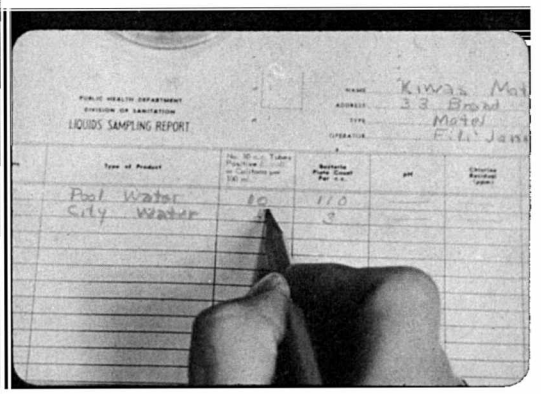
. . . and four . . . the treatment procedures which destroy coliform bacteria also destroy pathogenic bacteria.

After incubation each membrane is inspected for excessive crowding of colonies and growth running together. These are discarded.

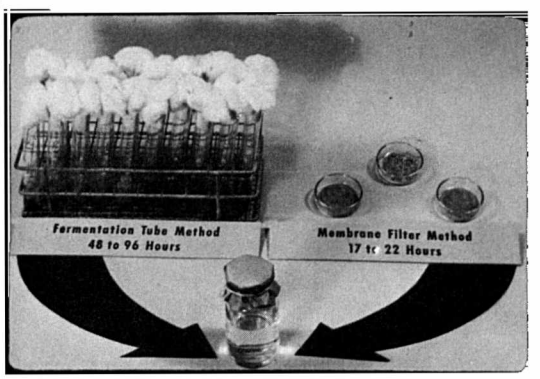


The remaining membranes are counted for coliform colonies. A magnification of 10 to 15 diameters is used to aid in the examination of the surface sheen.

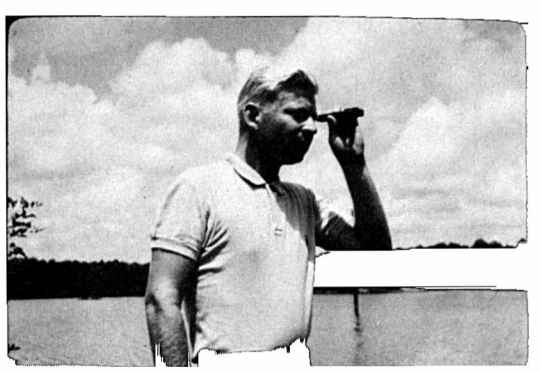
All data are reported on the basis of coliforms per 100 ml. of the sample. The average density of potable water should not exceed one coliform per hundred ml. per month for all samples.

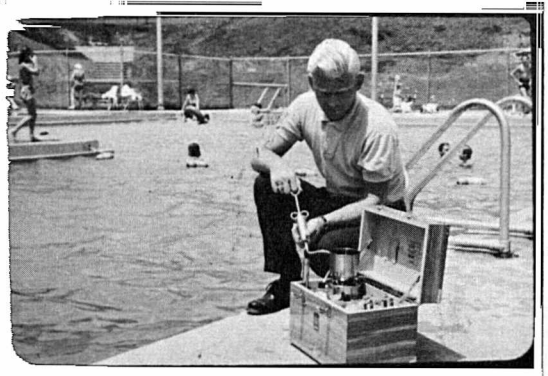


The fermentation tube method and membrane filter method will not produce the same coliform count. However, correct interpretation of the results of each procedure leads to the same sanitary classification of any water sample.

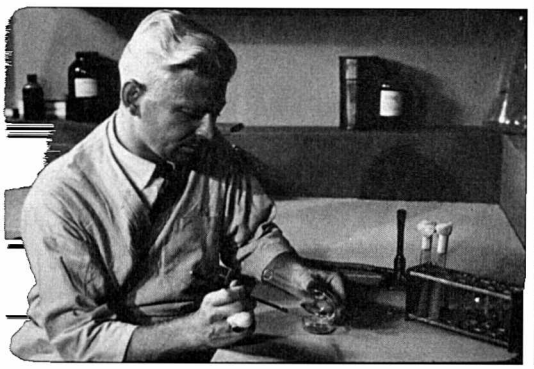


All bacterial tests on waters should be correlated with sanitary surveys appropriate to the type and source of the supply.

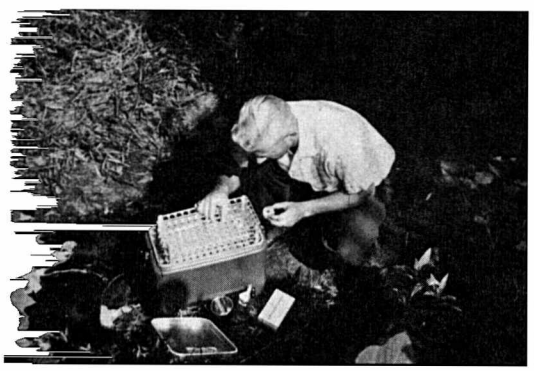




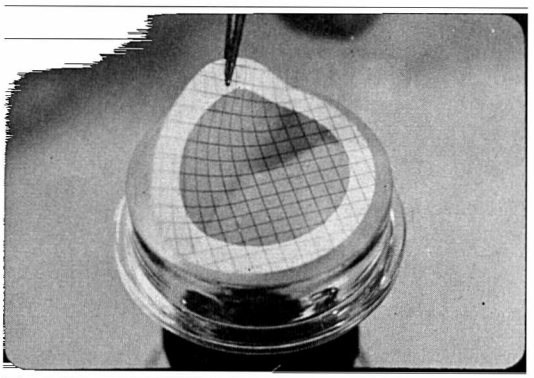
The MF procedure has a variety of other uses in the investigation of sanitation problems. It permits the determination of total plate counts from swimming pool waters, which are useful in determining operational efficiency . . .



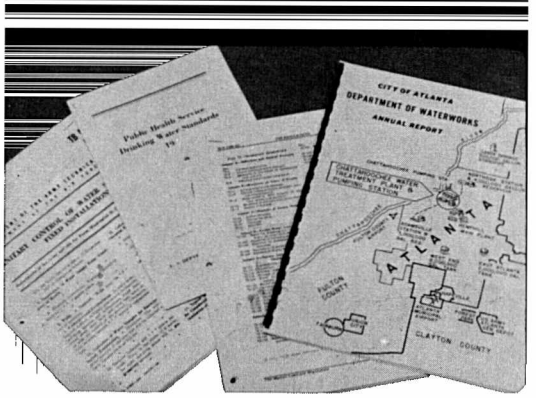
. . . and has promise in the use of other indicators of pollution such as the fecal streptococci group.



The MF procedure is more adaptable to field use than other bacterial procedures for estimating pollution organisms.



The development of the membrane filter has at last furnished a rapid and accurate technique for the quantitative estimation of the bacterial content in water, air, and food.



It has attained wide acceptability by many organizations.

The MF method provides a technique for the direct counting of coliform bacteria . . .

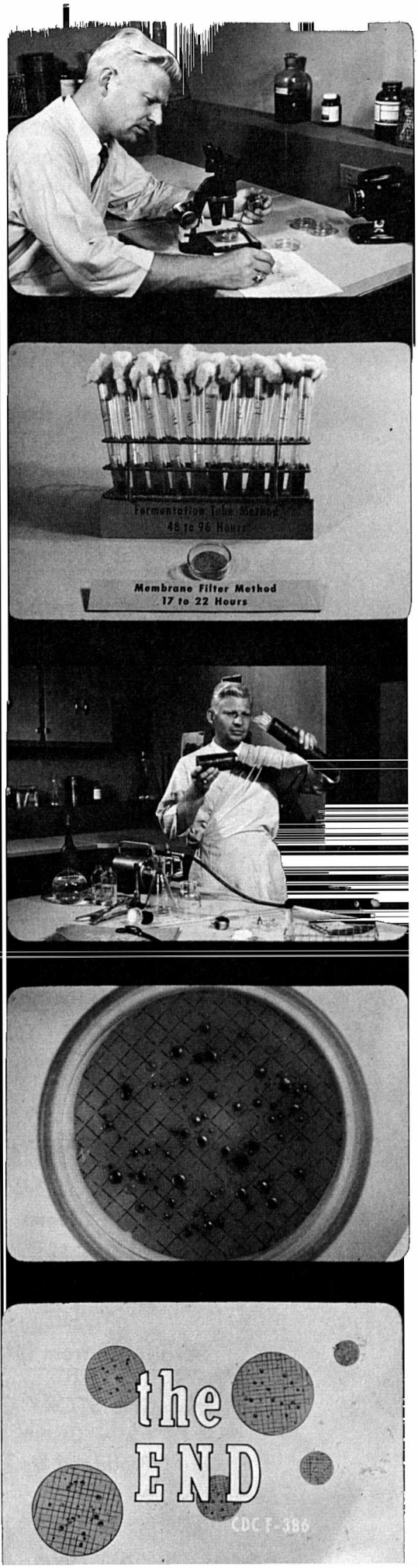
. . . in an elapsed time of 17 - 22 hours as compared with other procedures requiring 48 hours or longer . . .

. . . and it requires a minimum of space, equipment, and labor.

The membrane filter technique for the bacteriological analysis of water is a method that is more certain and precise in results than other methods now in use.

THE END

F-386



TO OBTAIN FILMSTRIP: If not available from your State Health Department, the filmstrip may be borrowed from the Film Library of the Communicable Disease Center. Address requests to:

Chief, Communicable Disease Center
Public Health Service
U. S. Department of Health, Education, and Welfare
Post Office Box 185
Chamblee, Georgia

Requests should specify exact showing date and alternate, and should reach CDC at least two weeks in advance. All requests will be confirmed. Filmstrip will be loaned for short periods, not to exceed one week. When needed for a longer period request a special loan, listing the number of showings planned and audience expected. Borrower is expected to pay return postage and insurance.

ADDITIONAL FILMSTRIPS IN THE WATER SUPPLY FIELD

Available from CDC Film Library

FILTRATION PLANTS (F-132a) 1953
FS - 35mm, color, sound, 59 frames, 8 minutes

FUNCTIONING OF GAS FEED CHLORINATORS, Part I:
VISIBLE VACUUM CHLORINATOR (F-146a) 1954
FS - 35mm, color, sound, 57 frames, 12 minutes

FUNCTIONING OF GAS FEED CHLORINATORS, Part 2:
VOLUME METERING CHLORINATOR (F-146b) 1954
FS - 35mm, color, sound, 42 frames, 10 minutes

AN INTRODUCTION TO BACKSIPHONAGE AND
CROSS CONNECTIONS (F-171) 1957
FS - 35mm, color, sound, 75 frames, 11 minutes

A LARGE WATER TREATMENT PLANT (F-165) 1954
FS - 35mm, color, sound, 84 frames, 12 minutes

OPERATION OF HYPOCHLORINATORS (F-225) 1957
FS - 35mm, color, sound, 57 frames, 11 minutes

SAMPLING AND TESTING DRINKING WATER (5-140) 1950
FS - 35mm, color, sound, 74 frames, 8 minutes

Available from PHS Regional Offices (see opposite page).

THE STORY OF WATER SUPPLY - 1958
FS - 35mm, color, sound, 82 frames, 15 minutes
Produced by The American Water Works Association

For additional information on the membrane filter techniques and on healthful aspects of water supply and water quality control, write to:

Department of Health, Education, and Welfare
Attn: Regional Medical Director, PHS
For: Water Supply Consultant

located at the following addresses:

Region I & II	Room 1200, 42 Broadway New York 4, New York
Region III	700 East Jefferson Street Charlottesville, Virginia
Region IV	Room 164 50 Seventh Street, N. E. Atlanta 23, Georgia
Region V	Room 712 New Post Office Building 433 West VanBuren Street Chicago 7, Illinois
Region VI	2305 Federal Office Building 911 Walnut Street Kansas City 6, Missouri
Region VII	Ninth Floor 1114 Commerce Street Dallas 2, Texas
Region VIII	Room 551 621 Seventeenth Street Denver 2, Colorado
Region IX	447 Federal Office Building Civic Center San Francisco 2, California

or

Division of Water Supply and Pollution Control
Public Health Service
U. S. Department of Health, Education, and Welfare
330 Independence Avenue, S. W.
Washington 25, D. C.

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