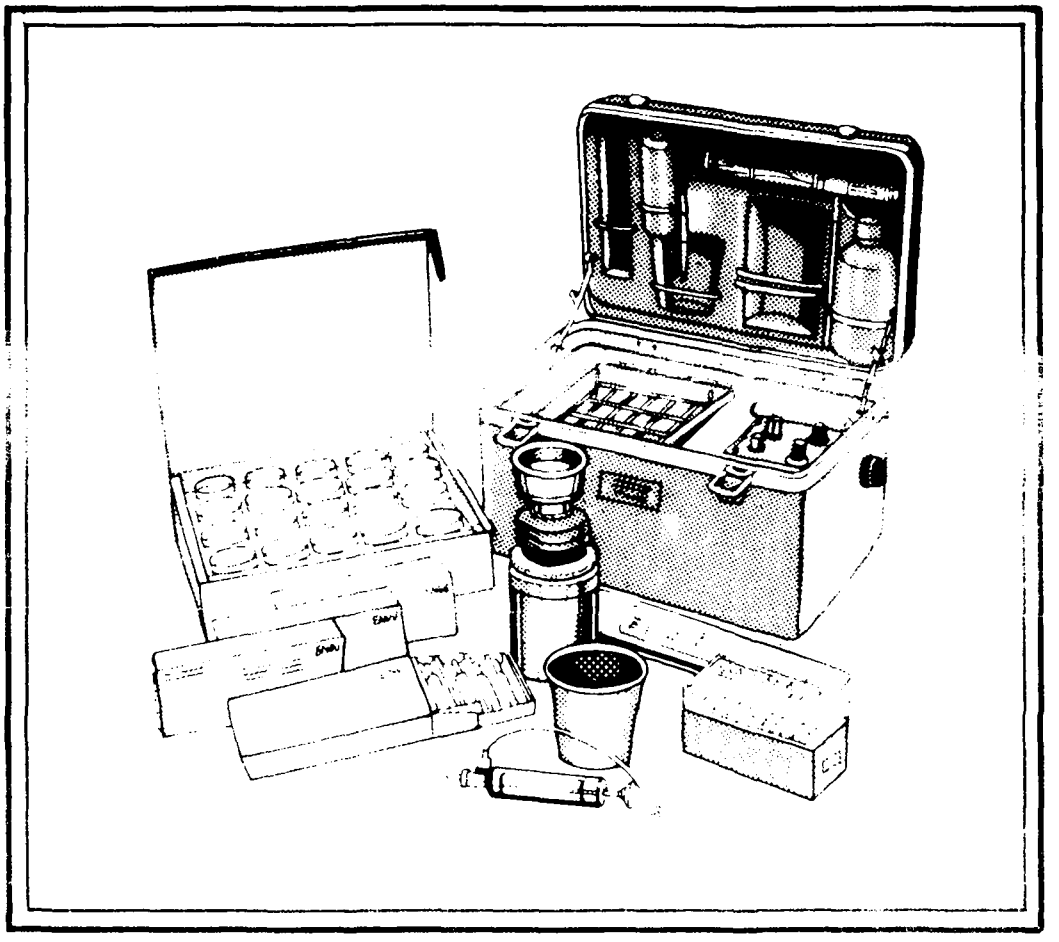
MANUAL FOR BACTERIOLOGICAL ANALYSIS OF NATURAL WATER SUPPLY SOURCES IN DISASTER SITUATIONS

Membrane Filter Method **Portable Equipment**



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PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION

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MEMBRANE FILTER METHOD

PORTABLE EQUIPMENT

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Pan American Health Organization

Environmental Health Program
Emergency Preparedness and Disaster Relief Coordination Program

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1. INTRODUCTION

Among the environmental health measures that must be considered in the wake of a natural disaster is the bacteriological analysis of water supplies. Water contamination is one of the principal public health hazards associated with disasters, since contaminated water can set off an increase in gastroenteritis, diarrhea, and other water-borne diseases. Such contamination may take place at the source, at the treatment plant, or in the distribution system.

Appropriate techniques and portable, sensitive, easy-to-operate equipment are required in order to be able to analyze the bacteriological properties of water supplies in disaster situations.

The use of membrane filters in the bacteriological examination of water (bacterial count, coliform determination, isolation of pathogens) was first considered prior to World War II. Germany and the Soviet Union were the first countries to apply this technique, particularly Germany after many of its laboratories were destroyed during the war.

In 1955 the 10th edition of Standard Methods for Examination of Water, Sewage and Industrial Wastes included the membrane filter technique as a tentatively approved method for the determination of coliforms. In the 11th and 12th editions of the same publication, the method was officially sanctioned for coliform determination. At the present time the membrane filter test is being used as a standard method for evaluating the sanitary properties of water and determining its potability.

This manual is designed to provide a practical guide for bacteriological analysis using the membrane filter technique. It was prepared as an activity of PAHO's Emergency Preparedness and Disaster Relief Coordination Program as part of its component on the training of environmental sanitation professionals for disaster emergencies.

The manual and the set of slides intended to illustrate it are available upon request from:

- Emergency Preparedness and Disaster Relief
  Coordination Program
  Pan American Health Organization
  525 Twenty-third Street, N. W.
  Washington, D.C. 20037, United States of America

or from:

- Eng. Robert Swart
  Pan Caribbean Disaster Preparedness and
  Prevention Project (PCDPPP)
  P. O. Box 1207
  St. John's, Antigua (W.I.)

Portable equipment using a membrane filter is available from at least two firms. A field kit manufactured by Millipore is used as a practical model in this manual. This, however, does not imply that PAHO/WHO approves that equipment or recommends it over others of a similar kind.

2. DEFINITION OF TOTAL COLIFORM GROUP

When the membrane filter (MF) technique is used in coliform determination, the coliforms may be defined, according to the Standard Methods, as nonsporulated gram-negative bacilli, which produce a pink to dark red colony with a golden metallic or yellowish green shine in 18-24 hours when grown in the MF-Endo medium at 37°C±.

3. MEMBRANE FILTER METHOD USING PORTABLE EQUIPMENT

This technique has several strong advantages: it can be used anywhere, it makes it possible to examine a wide variety of water volumes, and it provides a direct reading of the total concentration of coliform bacteria instead of a statistical estimate as is common with the multiple tube technique.

Certain types of samples cannot be filtered because the water is turbid or because of the presence of unusually high populations of noncoliform bacteria or heavy metallic compounds. These difficulties may be encountered in the examination of samples of water from certain wells, reservoirs, small ponds, industrial affluents, and low-quality chlorinated affluents. In turbid samples with a low concentration of coliform bacteria, the use of the multiple tube procedure is recommended.

3.1 Summary of the Method

The filtering procedure consists of using vacuum pressure to pass the water through a 0.45 micron-thick cellulose membrane. The volume that can be filtered also depends on the presence of turbidity. Samples of contaminated water must be diluted before filtering.

In diluting a sample it should be taken into account that in order to determine the total coliform count, the ideal number of colonies on the membrane filter should be from 20-80. With the help of a two-way vacuum valve, the sample is passed through the membrane filter, which is properly placed on the filter stand.

The filter is then placed in a Petri dish containing a medium with agar or a pad impregnated with MF-Endo culture medium.

The inoculated Petri dish is placed in the incubator, reticulated side down, at a temperature of 37°C for 18-24 hours. No more than 30 minutes should elapse between filtration and incubation.

All pink to dark red colonies with a golden or yellowish green metallic sheen are total coliforms.

3.2 Application

Most water samples can be analyzed by the membrane filter method using portable equipment.

3.2.1 Advantages

- A quicker reading is obtained, particularly for coliform group bacteria.

- Larger volume of samples can be examined.

- The readings are more precise than those to be expected with the multiple tube technique.

- The required equipment and supplies take up less space than those for the multiple tube technique.

- The method is ideal for the performance of bacteriological water quality analysis in rural areas lacking electric power or suitably equipped laboratories.

3.2.2 Drawbacks

- Samples with a high number of noncoliform bacteria and a low number of coliform bacteria cannot be examined by this method because of possible suppression of the coliform group bacteria.

- In samples with a low coliform count and a realtively large amount of suspended solids, the bacterial growth may at times produce a continuous film on the surface of the membrane, precluding the possibility of a count.

- Some samples with copper or zinc quantities greater than 1 $\mu g/\ell$ yield irregular results for coliforms.

- The quality of membrane filters may involve such problems as:

  . Presence of toxic wastes from the manufacturing process
  . Irregular porosity
  . Hydrophobic areas
  . The use of ink in drawing the grid lines that is too thick, impeding the growth of the colonies
  . Grid lines drawn in toxid ink
  . Presence of Glycerol.

- Problems with respect to absorbent pads:

  . Presence of sulfite wastes or other substances will inhibit bacterial growth.

- Problems with respect to sterilization of membrane filters:

  . When a filter is sterilized with ethylene oxide, a residue of this gas, which is toxic to bacteria, may be left on the filter.

  . When a filter is sterilized in an autoclave, uneven porosity areas may result unless the time and temperature have been properly controlled.

- In regard to culture medium:

  . These may at times contain nutrients and stains of inadequate quality.

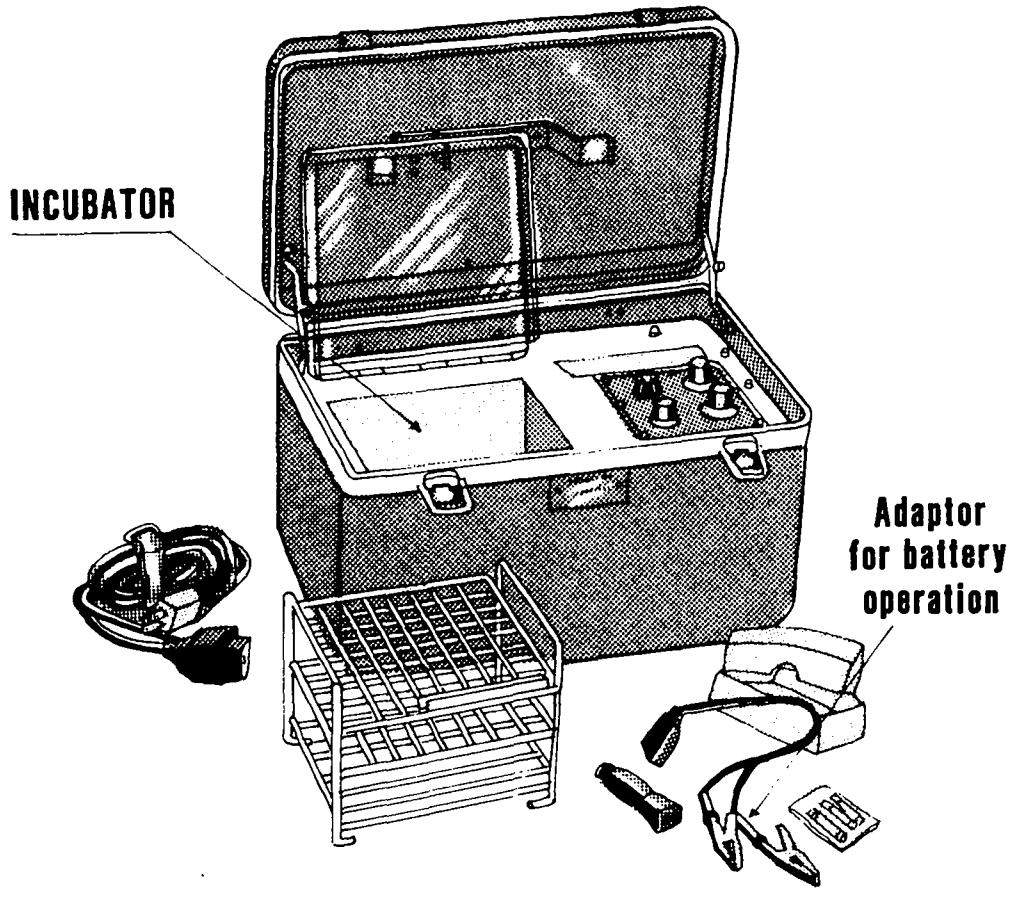
3.3 Equipment, Supplies, and Culture Medium

PORTABLE EQUIPMENT AND ACCESSORIES (Figure 1)

3.3.1 Equipment and Supplies

Portable Incubator

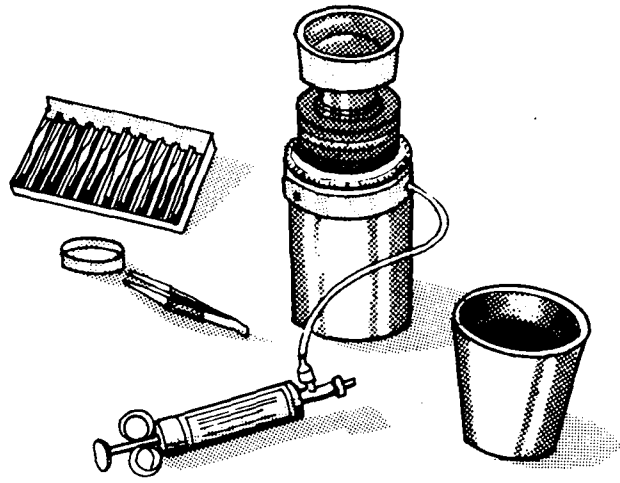
A 27-sample incubator for maintaining the temperature at 37°C works on current or battery.



PORTABLE INCUBATOR (Figure 2)

Stainless Steel Stand and Filter Case

The stand and the filter case should be sterilized in an autoclave at 121°C for 15 minutes or boiled. (See Figure 3)



STAND, FILTER CASE, AND SYRINGE (Figure 3)

Membrane Filters

The filter's bacterial retention capacity should be certified by the manufacturer, and its filtration speed should be satisfactory. (The pore diameters, for bacteriological use, are 0.22 microns for sterilizing liquids and 0.45 microns for retaining bacteria.)

If the filters have not been previously sterilized, they should be placed in an autoclave at 121°C for 10 minutes.

Absorbent Pads for Culture Medium

The pads should be made of filter paper and should be free of inhibiting substances that interfere with the growth of colonies. They should be uniform in thickness to allow the absorption of 1.8-2.2 mℓ of the culture medium. They should be sterilized prior to use in an autoclave at 121°C for 10 minutes.

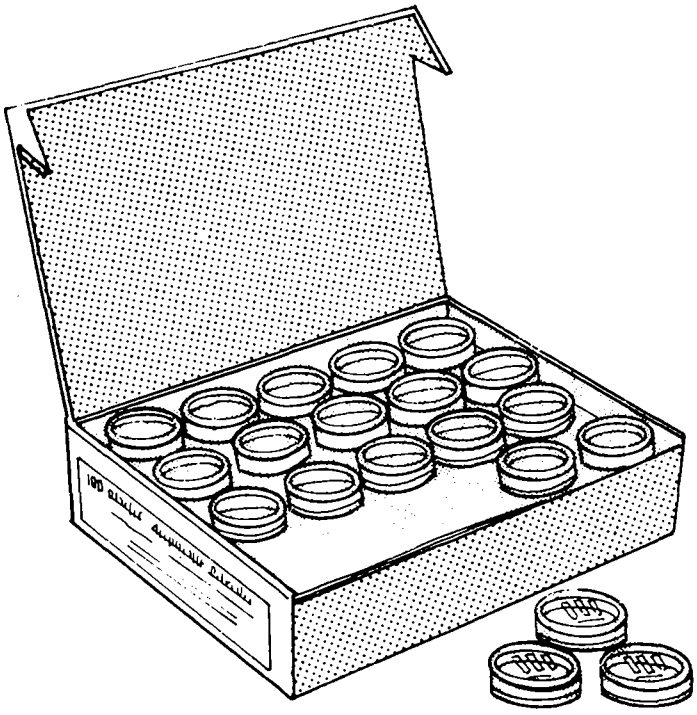
As an alternative, these pads may be replaced by culture broth to which 1.5% agar has been added. This preparation should be placed in the Petri dishes with care to prevent bubbles from forming; the surface should remain smooth and damp.

Forceps

Forceps with round ends and a smooth straight point should preferably be used. Forceps with rough surfaces may damage the membranes. When not in use they should be kept in alcohol, and they should be flamed before use in an analysis.

Petri dishes

Petri dishes 50-60 mm in diameter and 12 mm high are used in the membrane filter technique. Plastic dishes that can be sealed hermetically are preferred.



PETRI DISHES (Figure 4)

Precipitation Cups

Cups containing 95% ethyl alcohol for sterilizing the forceps.

Sampling Containers

Made of stainless steel.

Sampling Bottles

100 mℓ capacity, sterile, made of neutral glass or autoclavable plastic, nontoxic.

Alcohol Burner

Pipettes

Sterile glass or plastic.

Supplies for Preparing Culture Medium

Glass or stainless steel container.

3.3.2 Culture Medium

MF-Endo Broth

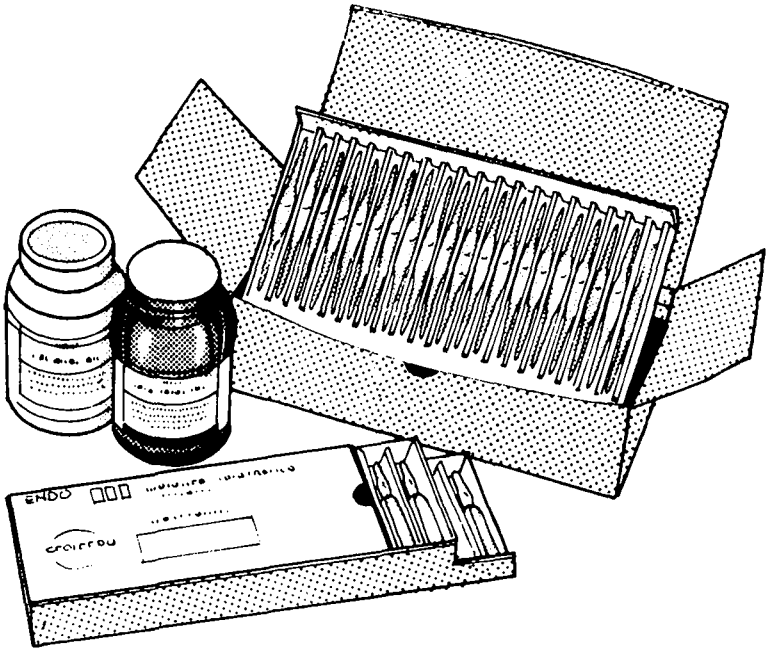
Formula

Polypeptone	10.00 g
Triopectone	5.00 g
Trypticase	5.00 g
Yeast extract	1.50 g
Lactose	12.50 g
Sodium chloride	5.00 g
Potassium dihydrogen phosphate	1.375 g
Dipotassium hydrogen phosphate	4.375 g
Sodium lauryl sulphate	0.05 g
Sodium deoxycholate	0.10 g
Sodium sulfite	2.10 g
Gasic fuchsin	1.05 g
Distilled water	1 ℓ
Ethyl alcohol, 95%	20 mℓ

Preparation

Dissolve 4.8 grams of the medium in 100 mℓ of distilled water containing 2 mℓ of 95% ethyl alcohol. Mix and heat the medium to the boiling point, withdrawing it frequently from the flame. Cool to 45°C. Do not sterilize.

Use 1.8-2.0 mℓ of medium for each absorbent pad. It is recommended that the medium be prepared on the same day it is to be analyzed. It should be kept refrigerated; even so, it can be stored for no more than a maximum of 4 days.



CULTURE MEDIUM (Figure 5)

3.4 Sampling

Sterile 125 mℓ bottles should be used for taking bacteriological samples. Samples for bacteriological water analysis in disaster situations should be collected at the source (well or spring), at the municipal supply facilities and private systems, and at water mains and storage tanks. (For further information on the taking of samples, see Vigilancia de la Calidad del Agua Potable, World Health Organization. Geneva, 1977.)

In lakes, reservoirs, and rivers, the abundance of bacteria varies and depends on the depth and hour at which the sample is taken.

To collect the sample, dip the bottle some 15-20 cm below the surface of the water. This should be done quickly to avoid collecting any floating material. The mouth of the bottle should be pointed against the flow of the current to prevent hand contact with the water sample.

To collect samples from a well, plump water from the well for 5-10 minutes and take a sample in a sterile bottle.

To collect a sample from a faucet, select one with no leaks and let the water run 2-5 minutes.

Since the bacterial population is strongly influenced by temperature, it is important to keep the samples refrigerated at 4°C even after they reach the laboratory, or to begin the analysis immediately with the help of portable equipment.

3.5 Procedure

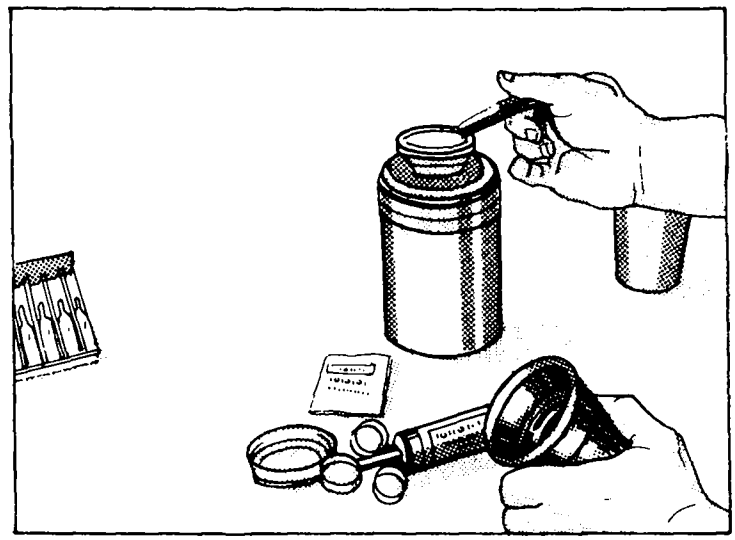
The filtering equipment should be sterilized before beginning each series of filtrations. If it is necessary to sterilize the equipment several times during the day, it is advisable to expose the funnel and the stand to ultraviolet light for 2 minutes. The equipment may also be sterilized in boiling water for 5 minutes.

An alcohol burner is attached to the stand. This is used to sterilize the filtering cup.

Table 1: RANGE OF SAMPLE VOLUMES FOR TOTAL COLIFORM USING THE MEMBRANE FILTER METHOD

Type of Sample	Volume Required (mℓ)				
	100	50	10	1	0.1
Lakes, reservoirs	x	x	x		
Springs, wells	x	x	x		
Surface sources for water treatment plants		x	x	x	

- Place the filter case aseptically on the stand and set the filter in place with the help of sterile forceps. (See Figure 6)



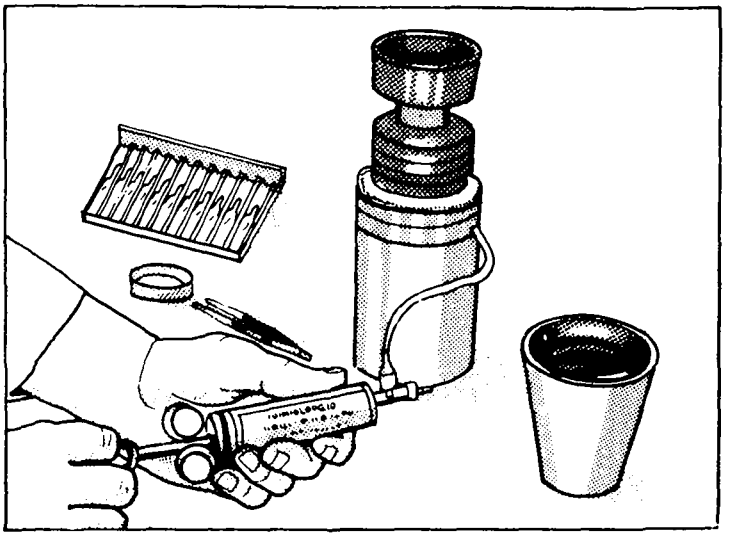
FILTER ON STAND (Figure 6)

- Carefully set the upper part or funnel on the base of the filter case and secure it firmly by turning the blue neck clockwise.

- Connect the syringe to the filtering equipment with a plastic tube.

- To safeguard against contamination, some 50 mℓ of sterile water should be filtered through the equipment prior to beginning filtering the samples.

- Pour the samples into the funnel or filtration receptacle, pumping the syringe to start the filtration. (See Figure 7)

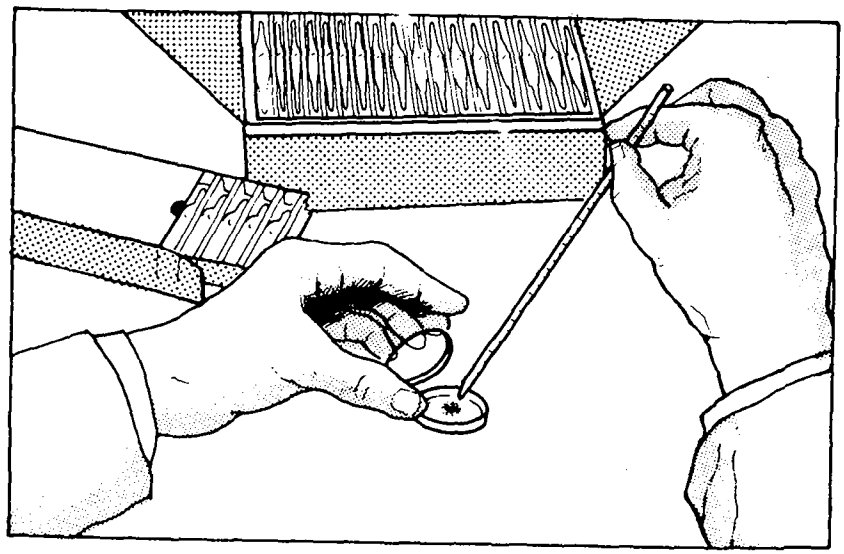


PUMPING THE SAMPLE THROUGH THE FILTER (Figure 7)

- Filter the sample through the 0.45 micron-thick cellulose membrane.

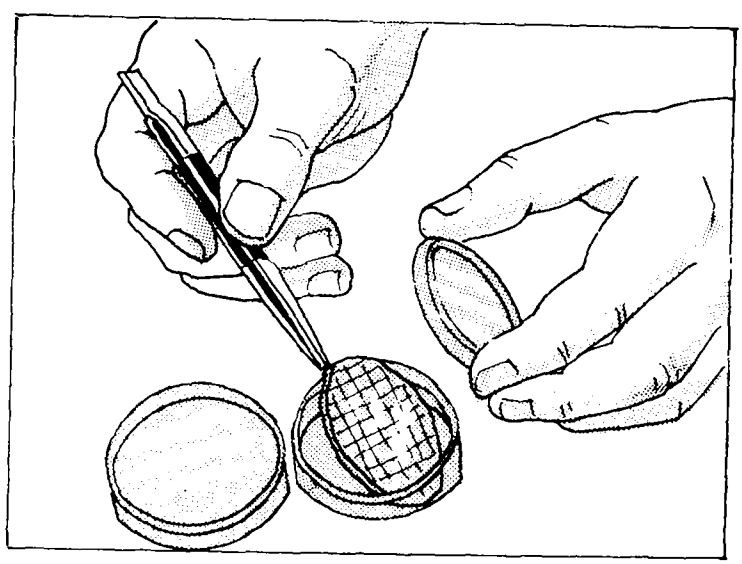
- After the samples have been filtered, wash the funnel 3 times with 20-30 mℓ of sterile water. Detach the funnel.

- Add 1.8 to 2.0 mℓ of culture medium to the absorbent pad using a sterile pipette. (See Figure 8)



ADDING THE CULTURE MEDIUM (Figure 8)

- With sterile forceps remove the membrane from the filtering unit and place it very carefully on the Petri dish with MF-Endo agar or pad with MF-Endo broth. (See Figure 9)



FILTER ON PAD (Figure 9)

- It is important to keep air bubbles from forming in the membrane. Any bubbles that are formed can be eliminated by pressing down gently along the edges of the membrane with sterile forceps.

- Incubate the samples with the grid face down for 18-24 hours at 37°C.

- For analysis in rural areas, the equipment should be connected first to be battery of the vehicle so that the incubator can be kept at a constant temperature. This equipment operates on 6 and 12 volts.

- No more than 30 minutes should elapse between filtration and the start of incubation.

- All pink or red colonies with a metallic sheen are total coliforms.

3.6 Results

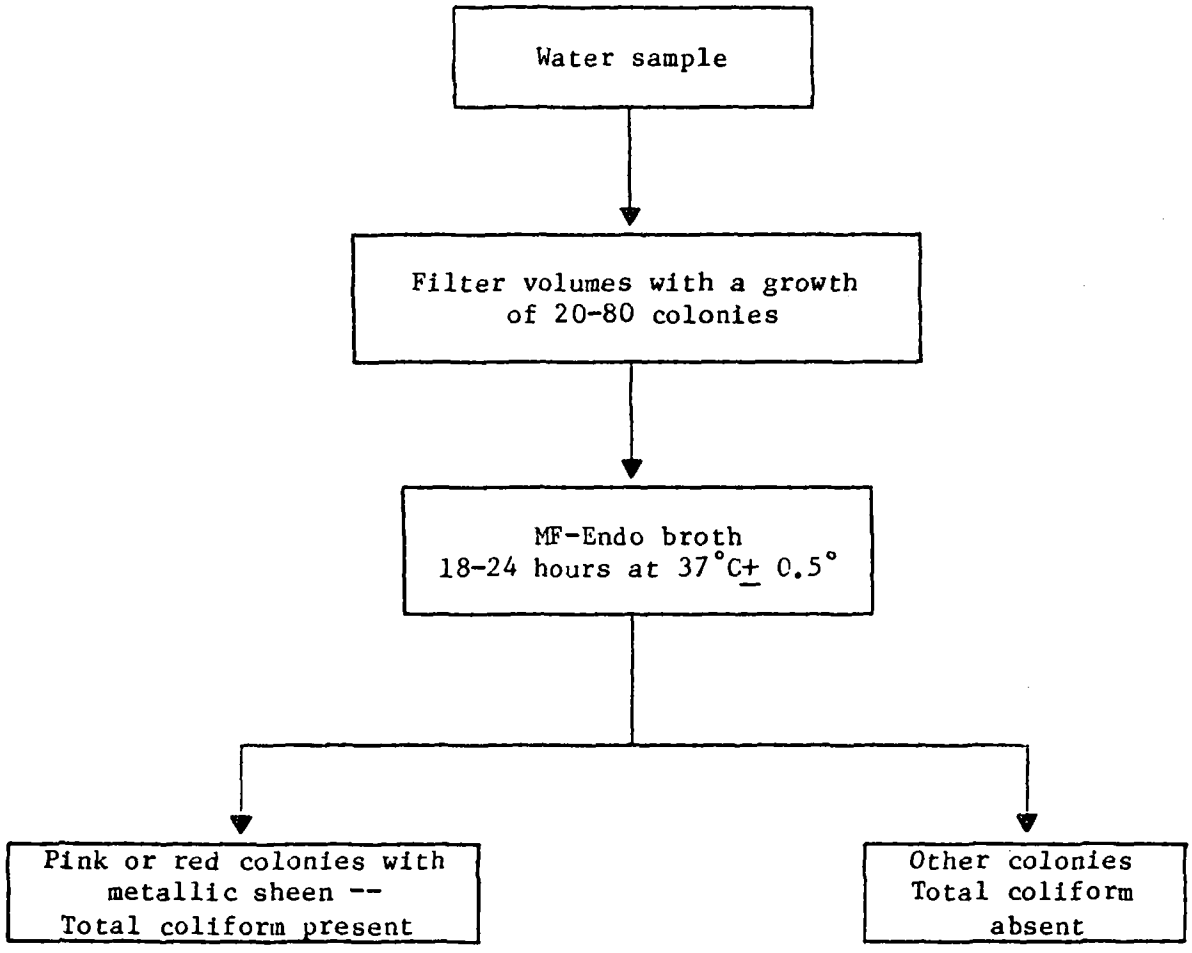
- Select those membranes that have 20-80 pink or red colonies with a metallic sheen.

- Count the colonies using a stereoscopic microscope with 10-15x magnification and fluorescent light.

- The following formula is used for calculating the total coliform:

$$\text{No. of total coliform counted/100 m}\ell = \frac{\text{No. of coliform colonies counted}}{\text{Volume of original sample filtered}} \times 100\ \text{m}\ell$$

DIAGRAM FOR THE DETECTION OF TOTAL COLIFORM
MEMBRANE FILTER TECHNIQUE (Figure 10)



3.7 Water Monitoring Recommendations

The monitoring of bacteriological water quality should begin immediately after a disaster occurs. The following are among the measures to be considered for maintaining the sanitary quality of the water:

- Daily monitoring of residual chlorine in the water in the public supply system.

- Increasing the pressure of the water to counter contamination.

- Protecting the supply system by cleaning and disinfecting water mains, storage tanks and wells.

3.8 Logging the Data

The following items should be considered in logging the data:

- Sample number
- Date and hour it was taken
- Date and hour it was analyzed
- Sampling location
- Volume filtered
- Number of total coliform colonies counted.

The "volume filtered" data make it possible to calculate the number of total coliform colonies counted per 100 mℓ. Table 2 can be used to register a number of samples.

Table 2: DETERMINATION OF TOTAL-COLIFORM/MEMBRANE FILTER METHOD

PAGE No. ___ OF _____

PROJECT_____ ANALYST _____

No. of total coliform counted/100 mℓ = $\frac{\text{No. of coliform colonies counted}}{\text{Volume of original sample filtered}}$ x 100 mℓ

Sample Number	Date and Hour Taken	Date and Hour Analyzed	Sampling Location	Volume Filtered	Total-Coliform Counted	Number of Total-Coliform/ 100 mℓ

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TEXT FOR SLIDES

PROCEDURE FOR DETERMINATION OF TOTAL COLIFORM

Membrane Filter Method Using Portable Equipment

The environmental health measures to be considered in the wake of a natural disaster include, among others, bacteriological analysis of the water supplies.

The classical method to investigate coliform bacteria is with the use of multiple fermentation tubes. A newer procedure based on the use of membrane filters is being employed as a standard method for evaluating the sanitary quality of water and determining its potability.

In disaster situations and in the absence of adequate laboratory facilities, portable membrane filter equipment is used in performing the bacteriological analysis of water.

00 **PAHO Logo**

01 **Title frame**

02 **Portable membrane filter kit**

The portable membrane filter kit for bacteriological analysis of water consists of an incubator with a capacity for 27 samples that can be operated on either current or battery, a stainless steel stand and filter case, a syringe for producing a vacuum, and a stainless steel sampling container.

03 **Other basic components of the kit**

In addition to this equipment, other basic components are Petri dishes, pipettes, culture medium, membrane filters, forceps, and absorbent pads.

04 Necessary items to prepare culture medium

A scale, sterile distilled water, 95% ethyl alcohol, a test tube, a beaker, and sterile spatulas are needed for preparing the culture medium. The culture medium for total coliform is MF-Endo Broth.

05 Weighing amount of culture medium

Weigh the necessary amount of the medium (dehydrated MF-Endo broth).

06 Adding sterile distilled water to the medium

Add sterile distilled water containing 95% ethyl alcohol. It is advisable to prepare only the amount of medium that is to be used, since it can only be stored for a maximum of 96 hours.

07 Dissolving the medium

Dissolve the culture medium by heating it gradually, bringing it close to the fire, and then withdrawing it, until the boiling point is reached.

08 Assembling the filtering unit

Begin to assemble the filtering unit by inserting the forceps in the slot.

09 Feeding alcohol into the burner

Using the lid of the bottle of ethyl alcohol, feed alcohol into the burner attached to the base of the filtering unit.

10 Sterilizing the filtering unit

Use the burner to sterilize the filtering unit before performing the analysis. Both the filter case and the sampling container have to be properly sterilized. Sterilization time is 15 minutes.

11 Placing the filtering unit over the sampling containter

After the system is sterilized, continue assembling the filtering unit. Set the base of the unit over the sampling container.

12 Setting the filter on filter case

Place the membrane filter on the filter case using sterile forceps.

13 Securing filter case

Then carefully set the upper part or funnel on the base of the filter case, secure firmly, and turn the blue neck clockwise.

14 Placing absorbent pad in the Petri dish

Place the absorbent pad in the Petri dish with the pad distributor.

15 Adding the medium to pad

Add 1.8-2.0 mℓ of the culture medium to the pad.

16 Pouring distilled water through the filter prior to filtering sample

As a safeguard against contamination, about 50 mℓ of sterile water should be pumped through the filter before starting the analysis.

17 Filtering sample

Homogenize the sample and then filter it.

18 Pumping sample with syringe

Begin pumping the sample with the syringe through the 0.45 micron-thick cellulose membrane.

19 **Placing membrane in Petri dish**

Detach the funnel and with sterile forceps remove the membrane from the filtering unit and place it very carefully on the pad with M-Endo medium.

20 **Placing sample in the incubator**

Incubate the sample with the grid face down for 18-24 hours at 37°C.

21 **View of equipment in the laboratory**

Here, the equipment is installed in the laboratory. The samples are in the incubator that forms part of the portable kit.

22 **Pluging incubator into extension cord**

When analyses are to be performed in a rural area, begin by connecting the equipment to the battery of the mobile unit.

23 **Connecting clamps to battery**

For doing this, the system includes a set of clamps that can be connected to the poles of the battery.

24 **Equipment inside the movile unit**

Having connected the equipment to the mobile unit, continue with the incubation of the samples.

25 **Incubation in process**

26 Typical coliform colonies

After 18-24 hours observe the typical total coliform colonies. These are pink or red with a metallic sheen. To calculate the results, select those membranes that have 20-80 pink colonies with a metallic sheen.

27 Formula to calculate total coliform count

The findings may be expressed as follows:

$$\text{No. of total coliform counted/100 m}\ell = \frac{\text{No. of coliform colonies counted}}{\text{Volume of original samples filtered}} \times 100\ \text{m}\ell$$

28 Credits