REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME OCTOBER 1995

Determination of faecal streptococci in sea water by the multiple test tube (MPN) method

Reference Methods For Marine Pollution Studies No. 23 (Rev.1)

Prepared in co-operation with

WHO

UNEP 1995

This document has been prepared by the World Health Organization (WHO) and issued by the International Atomic Energy Agency, Marine Environment Laboratory (IAEA-MEL) and the United Nations Environment Programme (UNEP) under the project FP/ME/5101-93-03(3033).

For bibliographic purposes this document may be cited as:

UNEP/WHO: Determination of faecal streptococci in sea water by the multiple test tube (MPN) method. Reference Methods for Marine Pollution Studies No. 23, Rev. 1 UNEP, 1995.

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources, and has requested the development of regional action plans. The Regional Seas Programme at present includes 12 regions and has over 140 coastal states participating in it (1), (2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity, and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies is being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The methods are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared by, or in cooperation with, the relevant specialized bodies of the United Nations system as well as other organizations, and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (IS0) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions, the users are invited to convey their comments and suggestions to:

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Coordinating Unit for the Mediterranean Action Plan
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GR-11610 Athens
GREECE

which is responsible for the development and preparation of microbiological and other health-related Reference Methods.

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(1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1, UNEP, 1982.

(2) P. HULM: A strategy for the Seas. The Regional Seas Programme: Past and Future, UNEP 1983.

(3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme for comprehensive support for regional and global marine pollution assessments. UNEP, 1990.

This revised issue of Reference Methods for Marine Pollution Studies No. 23 was prepared by the World Health Organization on the basis of a review of the Method during expert meetings and comments from individual scientists who tested the Method. The assistance of all those who contributed to this revised issue of the Reference Method is gratefully acknowledged.

CONTENTS

1. INTRODUCTION

The original version of this recommended method was prepared by the World Health Organization within the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) and issued by the United Nations Environment Programme as Reference Method for Marine Pollution Studies No. 23 within UNEP's Regional Seas Programme Activity Centre's series.

The method is essentially based on already-existing recognized techniques, and also drawn on the experience of microbiologists in a number of Mediterranean laboratories. In the description, the style used by the International Organization for Standardization (ISO) is followed as closely as possible. While designed primarily with conditions prevailing within the Mediterranean Sea in mind, the method is also, to variable extents, suitable for other similar ecological regions.

The present version of this method incorporates a number of amendments, based on reviews during expert consultation meetings organized by WHO, and on comments received from Mediterranean laboratories using the method within the framework of their national or local marine pollution monitoring programme.

2. SCOPE AND FIELD OF APPLICATION

The method described is suitable for the determination of faecal streptococci in coastal bathing waters of temperature and tropical seas and is designed to be used in sanitary surveillance of bathing beaches.

It is based on the Multiple-Tube Fermentation (MPN) Test and can be employed as an alternative to the Membrane Filtration Culture Method. Whether the Membrane Filtration (MF) Culture Method is preferred to the MPN Test depends on local conditions and personal preferences. In general the MF method is less labour intensive and due to the preconcentration of the bacteria in the sample it is more suitable in situations where low numbers of streptococci are to be estimated. The MPN test should be given preference when the test sample contains amounts of particulate matter which will hinder the reading of the MF's after incubation.

Faecal streptococci normally originate from the intestine of warm-blooded animals and when found in seawater indicate contamination with faecal material. Recent studies indicate that a few variant strains in the faecal streptococci group, may be found in certain plants or plant products. Therefore, wastes from food processing industries may be also a source of organisms yielding positive reaction when tested with this method. Die-away rates (T-90) depend on salinity, temperature, solar radiation, etc. and must be taken into consideration when interpreting the results.

Because of limited survival in the environment, it is not recommended that only faecal streptococci be used when determining water quality. In combination with faecal coliform data (UNEP/WHO 1983a/b), data on faecal streptococci may provide more specific information about pollution sources because certain faecal streptococci are host-specific.

3. DEFINITION

Faecal streptococci are Gram-positive oblong/oval occurring in pairs or in short chains. They will grow in acid dextrose at 35 ± 0.05° C, in brain-heart infusion broth within 2 days at 45° C; in 5 days at 10° C and confirm as catalase negative.

4. PRINCIPLES

Quantitation of faecal streptococci in marine (seawater) samples is accomplished by testing multiple sample portions in an azide based medium for growth at 35° C within 48 hours. Positive findings in the presumptive test phase are confirmed in a second medium (ethyl violet azide broth) for verification of faecal streptococci occurrence.

Five sample portions for each multiple test tube dilution (10 ml, 1 ml, 0.1 ml, etc.) are inoculated into individual tubes of azide dextrose broth. After 24 and 48 hours incubation, all culture tubes are examined for the presence of turbidity.

The frequency of positive reactions in the different dilutions of confirmed cultures is used for the calculation of the most probable number (MPN) of faecal streptococci in the water sample.

5. APPARATUS AND GLASSWARE

5.1 Sample bottles of borosilicate glass or autoclavable plastic bottles with screw-cap tops, 200-250 ml capacity, wide-mouthed and with ground-glass stoppers are used to collect marine (seawater) samples.

5.2 Sample rod of non-corrosive material with a clamp to hold the sampling bottle (figure 1).

5.3 Subsurface sampler of the type shown in figure 2, or similar, complete with plastic rope and weight.

5.4 Thermo-insulated plastic container with prefrozen packs of chemical gel (camping equipment) for storage of samples.

5.5 Thermometer, 0 to 50° C, precision ± 1° C, to be used for checking temperature in sample collection containers (5.4). Thermometer, 0° to 50° C, precision ± 0.5° C, to be used for checking temperature during incubation.

5.6 Air or water incubator adjusted to 35 ± 0.5° C with suitable test tube racks for incubating multiple tube cultures (5.15).

5.7 Autoclave, maximum 2 atm., electric or gas.

5.8 Drying oven for sterilization of glass sample bottles and pipettes at 160° C for 2 hours.

5.9 pH meter, precision ± 0.1 pH units.

5.10 Balance for media preparation, precision ± 1 mg.

5.11 Refrigerator with temperature range of 2-8° C.

5.12 Vibrator (shaker) for mixing sample aliquots in serial dilution (optional).

5.13 Ehrlenmeyer flasks of borosilicate glass for media preparation, capacity 1 and 2 litres.

5.14 Borosilicate glass bacteriological culture tubes with sufficient capacity to receive 20 ml of medium.

5.15 Total volume (blow-out) borosilicate glass pipettes of 1, 9 and 10 ml capacity with stainless steel containers for sterilization.

Note: 9 ml capacity pipettes are optional.

5.16 Graduated borosilicate glass cylinders of 100, 500 and 1000 ml capacity with glass beakers, aluminium foil or wrapping paper for cover.

5.17 Bacteriological loops made from 22-24 Chromel gauge, nichrome or platinum-iridium. Diameter of the loop: 3 mm.

5.18 Heavy wrapping paper or aluminium foil wrap.

5.19 Test tube racks, rust resistant, with capacity for 15 or 25 test tubes (preferably).

6. CULTURE MEDIA, REAGENTS AND STOCK CULTURE

Note: The composition of the media is based on one litre solutions or similar units. Before preparation of media and solutions, the actual needs have to be established and adequate amounts must be chosen accordingly.

6.1 Preparation of Materials, Media, Reagents

All glassware and apparatus (5) should be washed with non-toxic detergent (6.8), followed by a thorough (complete) rinse with hot tap water and then rinsed at least three times with distilled water (6.6).

6.2 Sterilization of Glassware and Equipment

6.2.1 Surface sample bottles (5.1). Clean all sample bottles as described under (6.1) and dry. Plastic sample bottles can only be sterilized in the autoclave (121° C for 15 minutes). Be sure screw-cap tops are loose fitting during sterilization to avoid bottle collapse due to steam pressure in autoclaving. Sterilize glass sample bottles preferably in a drying oven (5.8) for two hours at 160° C. Before sterilization, place a small piece of filter paper in the neck of the bottle to prevent the ground glass stopper

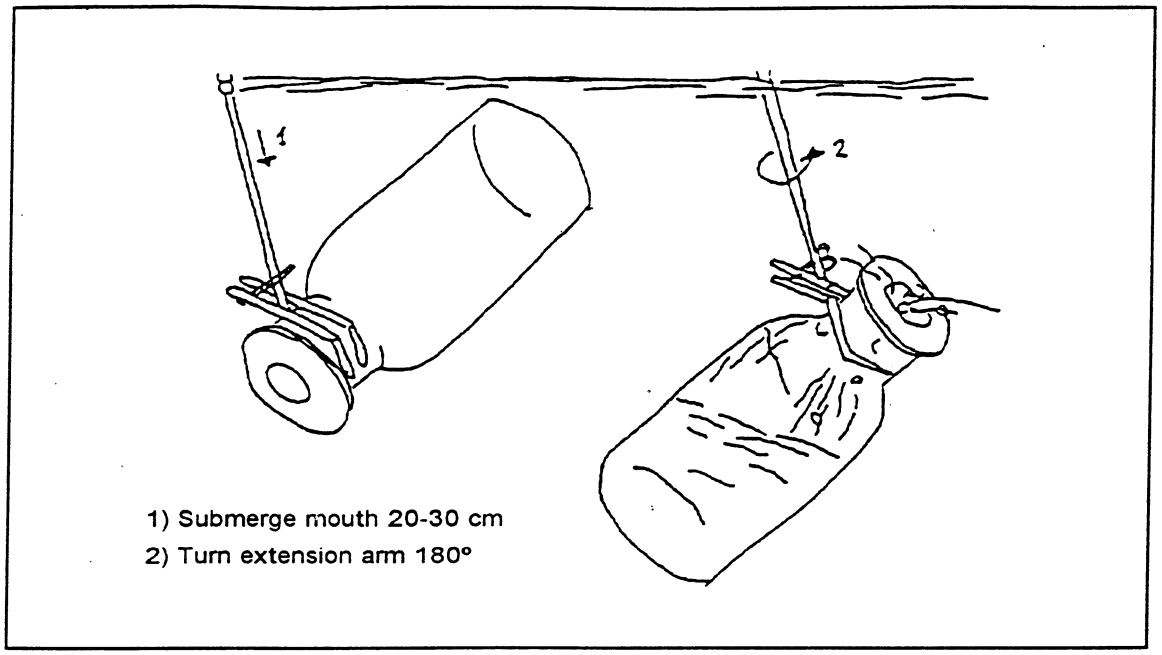

Figure 1. Subsurface sampling with extension arm.

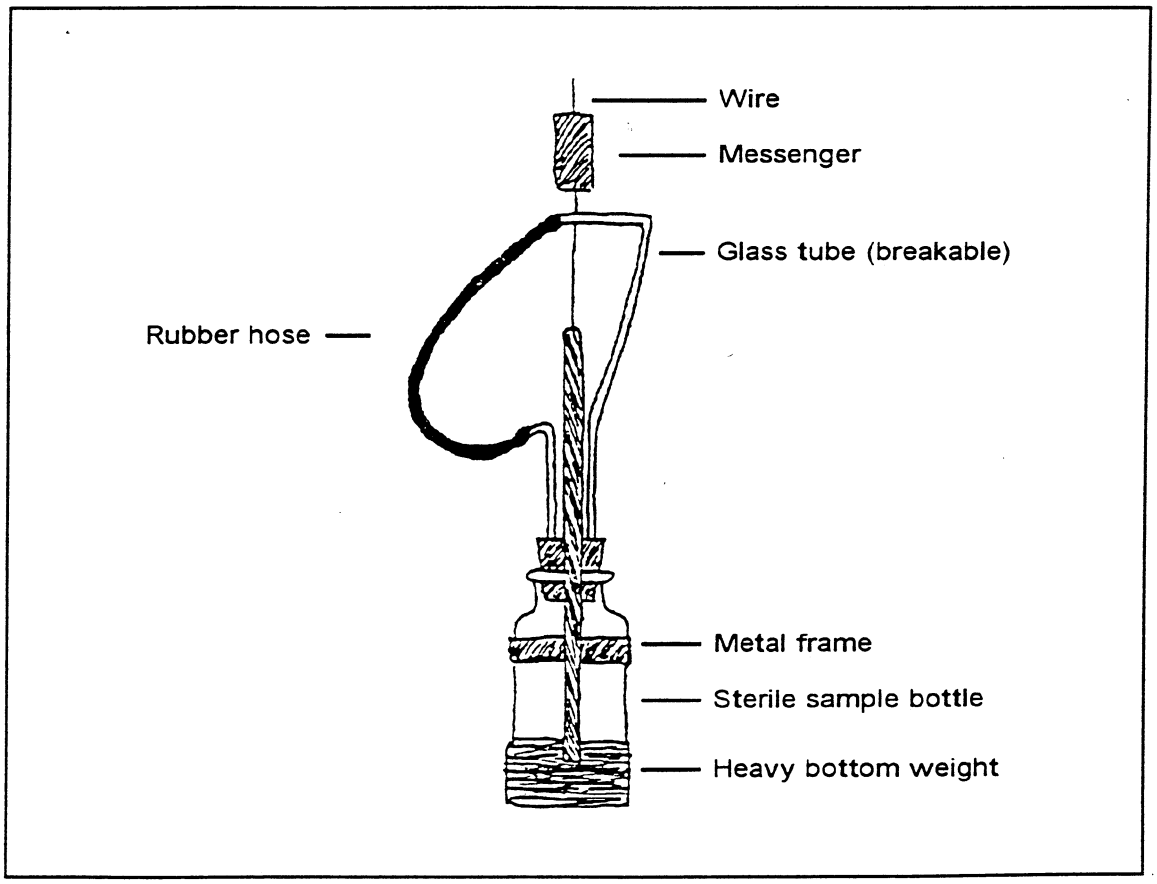

Figure 2. Sampler for sterile subsurface sampling.

from sticking after cooling. The paper insert should be left in place until time of sample collection. At that time, discard the paper insert by flipping it out of bottle. Do not insert it into the sample or remove with fingers because of chance contamination. Put bottles into detergent-cleaned thermo-insulated boxes (5.4). Separate the bottles from each other with clean wrapping paper (5.18) to avoid breakage.

Note: If residual chlorine is suspected in the water sample, add 0.1 ml of a 10 per cent thiosulphate solution (6.7) for each 100 ml of sample to the contents of the sample bottle before sterilization. This amount is sufficient to neutralize about 15 mg of residual chlorine per litre.

6.2.2 Subsurface sampler (5.3). Clean the subsurface sampler as described under 6.1, rinse with tap and distilled water (6.6). Enclose each sampler in heavy wrapping paper (5.18) and sterilize in an autoclave (5.7) for 15 minutes at 120° C.

6.2.3 Pipettes (5.15). Clean pipettes as described under 6.1 then insert a cotton plug in the mouthpiece and place into suitable stainless steel containers prior to sterilization in a drying oven (5.8) for two hours at 160° C.

6.3 A-1 Broth (Direct Test)

	strength	
	single	double
Beef extract	4.5 g	9.0 g
Tryptone or polypeptone	15.0 g	30.0 g
Glucose	7.5 g	15.0 g
Sodium chloride	7.5 g	15.0 g
Sodium azide NaN_3	0.2 g	0.4 g
Distilled water	1.0 litre	1.0 litre

Preparation: Dissolve ingredients in distilled water (6.6). The pH should be 7.2 ± 0.2 after sterilization.

Place 5 clean culture tubes in each of 3 rows of a test tube rack (more in case the expected MPN of faecal streptococci is high). Add 10 ml of double strength azide broth (6.3) to the first row of these culture tubes. Into the second and successive rows transfer 10 ml of single strength azide dextrose broth. Close all prepared tubes with metal caps or cotton plugs. Autoclave (5.7) the prepared media tubes at 121° C for 15 minutes. Check tubes of sterile medium for a final pH of 7.2 ± 0.2 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures (6.8). *S. faecalis* should produce turbidity within 24 hours incubation at 35° C. *E. coli* growth (turbidity) should not occur.

Note: Double strength broth is only necessary in the first row where 10 ml of test tube solution is added to the culture tubes.

6.4 Ethyl Violet Azide Broth

Tryptone or biosate	20.0 g
Glucose	5.0 g
Sodium chloride NaCl	5.0 g
K_2HPO_4	2.7 g
KH_2PO_4	2.7 g
NaN_3 (sodium azide)	0.4 g
Ethyl violet	0.00083 g
Distilled water	1.0 litre

Preparation: Dissolve ingredients in distilled water (6.6). The pH should be 7.0 ± 0.2 after sterilization.

Place 5 clean culture tubes in each of 3 rows of a test tube rack (more in case the expected MPN of faecal streptococci is high). Dispense 10 ml of single strength ethyl violet azide broth (6.4) into the culture tubes. Into the second and successive rows transfer 10 ml of single strength azide dextrose broth. Close all prepared tubes with metal caps or cotton plugs. Autoclave (5.7) the prepared media tubes at 121° C for 15 minutes. Check tubes of sterile medium for a final pH of 7.2 ± 0.2 after autoclaving. Test a sample of the sterilized medium for performance using control stock cultures (6.8). *S. faecalis* should produce turbidity within 24 hours incubation at 35° C. *E. coli* growth turbidity) should not occur.

6.5 Phosphate Buffered Water

Preparation: To prepare stock phosphate buffer solution, dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4), in 500 ml distilled water, adjust to pH 7.2 ± 0.5 with 1N sodium hydroxide (NaOH), and dilute to 1 litre with distilled water.

Add 1.25 ml stock phosphate buffer solution and 5.0 magnesium chloride solution (38 g anhydrous $MgCl_2$ l^{-1} distilled water) to 1 litre distilled water. Dispense in amounts that will provide 99 ± 2.0 ml or 9 ± 0.2 ml after autoclaving for 15 minutes. Final pH should be 7.2 ± 0.1 peptone water (alternative dilution water): repeat material from 6.6 in total coliform write up.

Do not suspend bacteria in any dilution water for more than 30 minutes at room temperature because death or multiplication may occur.

6.6 Distilled Water

Use only water distilled in all-glass or all-quartz distillation apparatus or reverse osmosis (RO) water. Deionized water is also acceptable if produced in apparatus not releasing toxic substances.

6.7 Thiosulphate Solution

Prepare a 10 per cent sodium thiosulphate ($Na_2S_2O_3$) stock solution. To a 120 ml sample bottle, add 0.1 ml of the 10% solution of $Na_2S_2O_3$ before sample bottle sterilization.

6.8 Detergents for Cleaning Glassware and Instruments

Do not use any detergents other than those recommended by official of bacteriological materials. If such detergents are not available, carry out an assay with a household detergent, using a strain of *E. coli* as a bio-test.

Note: You should never use a solution with a sulphochromic acid base for washing glassware. The solution is toxic to bacteria.

6.9 Strains for Quality Control

Strains of *Streptococcus faecalis* or *Streptococcus faecium.*

7. SAMPLING

Details of a sampling plan are provided in Part I of these guidelines.

7.1 Sampling of Surface Water

Label sample bottle at collection site indicating the sampling station, time of sampling and other factors relevant to the interpretation of the results. Attach clean sterilized sample bottle (6.2.1) to the clean sampling rod (5.2). Immediately before submerging the sample bottle, remove the ground glass stopper from the bottle without touching the inner surface of the bottle and stopper or screw cap. Immerse the bottle from the bow of the boat or from the windward side while the boat is moving forward slowly. Push the bottle with the sampling rod 25 cm under the water surface film, then turn the sample bottle upwards and take the sample (figure 1). The sterilized sample bottle may also be filled directly by hand. Position bottle with neck pointing slightly upward and opening directed away from the hand. Plunge the bottle below the water surface with a sweeping motion against the current.

Retrieve the bottle and discard some water, if necessary, so that some air space remains in the closed bottle. This space is needed for homogenizing the water sample at the receiving laboratory. Replace the glass stopper or screw cap and store the samples in the clean, thermo-insulated sample container (5.4) with prefrozen packs of chemical gel at about 4° C, avoiding exposure to more than + 10° C. Keep samples in the dark. Separate bottles from each other with clean wrapping paper (5.18) to avoid breakage.

7.2 Sampling of Subsurface Water

Label sample bottle at collection site indicating the sampling station, time of sampling and other factors relevant to the interpretation of the results. Lower the sterilized subsurface sampler (6.2.2) after attaching it to a clean plastic rope, without letting the weight disturb the bottom sediments (figure 2). Release the messenger and after one minute retrieve the sampler and store it in a thermo-insulated sample collection container (5.4). Proceed as for sampling of surface water (7.1).

Note: It is known that the die-away rate of faecal streptococci at ambient temperature in the presence of light is very high. Therefore, all efforts should be made so as not to collect more samples than can be analyzed and incubated the same day. If this is not possible, the samples should be stored at 4° C and analyzed not later than 24 hours after sampling.

8. TEST PROCEDURE

8.1 Selection of Sample Size and Dilution Series

Select a dilution series for each sample that will ensure positive tubes in the lowest dilution row and negative tubes in the higher dilution rows. If previous experience for planning the dilution series for clean seawater is not available, transfer 10 ml of the seawater to the first row containing 5 tubes of double strength azide dextrose broth, 1 ml to the second row of 5 tubes and 0.1 ml to the third row of 5 tubes. All rows after the first row (if 10 ml sample portions are used) should contain only single strength azide dextrose broth.

In polluted seawater the dilution should be extended to 5 dilution rows (the first receiving 10 ml from the seawater sample into double strength azide dextrose broth) or 5 dilution rows of single strength azide dextrose broth into which the seawater sample is transferred after the appropriate serial (10 fold) dilutions (figure 3).

8.2 Incubation in Azide Dextrose Broth at 35 + 0.5° C for 48 hours (Presumptive Test)

Each sample and all dilutions must be vigorously shaken to insure uniform dispersion of bacteria in each sample portion tested.

Using sterile pipettes (6.2.3) transfer 10 ml portions of the water sample (7) into each of 5 sterilized culture tubes containing double strength azide dextrose broth (6.3). Next transfer with a sterile pipette (6.2.3) 1 ml sample portions (7) into 5 sterilized culture tubes containing single strength azide dextrose broth (6.3).

For preparing further dilutions shake sample again then transfer with a sterile pipette (6.2.3) 1 ml of water sample (7) into a test tube containing 9 ml of phosphate buffer or peptone water (6.5) (dilution D-1). Mix vigorously by hand or optionally with vibrator (5.12). Using a sterile pipette, transfer aseptically (6.2.3), 1 ml of D-1 into each of the 5 culture tubes containing 10 ml single strength azide dextrose broth (6.3).

If necessary, for further dilutions transfer 1 ml of the D-1 dilution into a test tube containing 9 ml of phosphate buffer or peptone water (6.5) to obtain dilution D-2, 1 ml of dilution D-2 into 9 ml of phosphate buffer (6.5) to obtain dilution D-2, 1 ml of dilution D-2 into 9 ml of phosphate buffer (6.5) to obtain dilution D-3, etc. Shake each succeeding dilute aliquot prior to transferring dilute sample portions to culture tubes containing single strength azide dextrose broth.

Incubate the series of azide dextrose broth culture tubes in an incubator (5.6) at 35 ± 0.5° C for 48 hours.

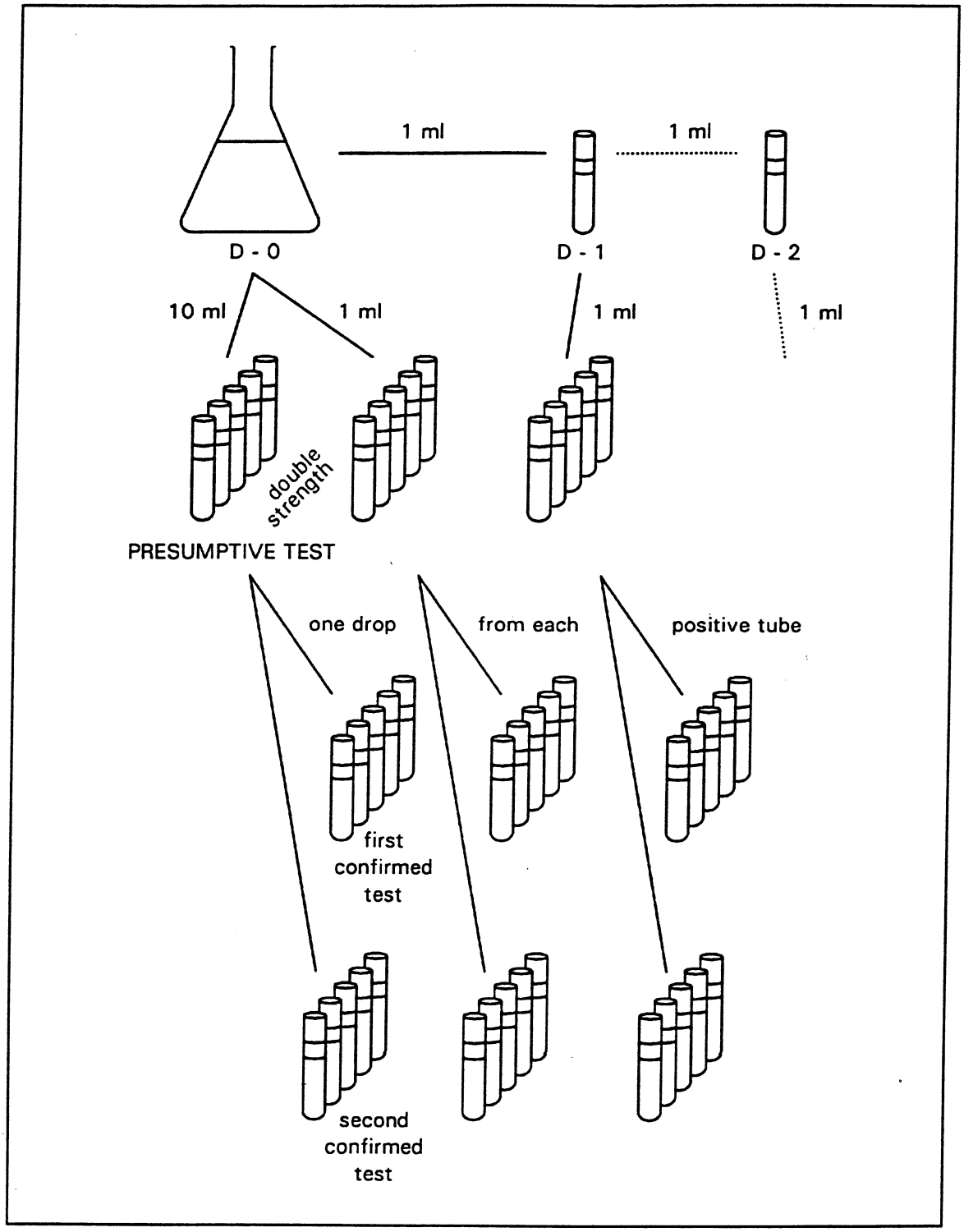

Figure 3. Scheme of preparation of dilution series.

All azide dextrose broth tubes showing turbidity after 24 and 48 hours must be subjected to the confirmed test with ethyl violet azide broth (8.3). After 24 hours of incubation, enumerate the positive azide dextrose tubes (growth and resultant turbidity of the broth) in Table 3, Paragraph 8, Column 1a of the Analytical report (10). Between 24 and 48 hours, new tubes of azide dextrose broth may become positive. Therefore, note down the total number of positive dextrose azide tubes in Column 1b.

8.3 Incubation in Ethyl Violet Azide Broth at 35° C for 24-48 hours (Confirmed Test)

Three row azide dextrose broth presumptive test: after incubation for 24 hours in azide dextrose broth at 35° C (8.2), a second test tube series is transferred with flame sterilized transfer loop (5.17) three loop-fulls from each azide dextrose broth culture tube that provided a positive reading into culture tubes situated in identical positions on the tube rack containing ethyl violet azide broth (6.4).

Five row azide dextrose broth presumptive test: after incubation for 24 hours in azide dextrose broth at 35° C (8.2) select the highest dilution in which the row of 5 tubes gives positive readings in all 5 tubes (no negative readings should exist in lower dilutions) and all positive tubes in succeeding higher dilutions, then transfer with sterilized transfer loop (5.17) three loop-fulls from each azide dextrose culture tube that provided a positive reading into test tubes situated in identical positions on the tube rack containing ethyl violet azide broth (6.4).

Incubate all confirmation cultures (ethyl violet azide broth) at 35 ± 0.5° C in an incubator (5.6) for 24 hours. The presence of faecal streptococci is indicated by the formation of a purple bottom at the base of the tube, or occasionally by a dense turbidity. After 24 hours of incubation, record all positive tubes in table 3, paragraph 8 under column 2a of the Analytical report (10) and discard them.

At the same time inoculate all negative ethyl violet azide tubes again by transferring with flame sterilized transfer loop (5.17) three loop-fulls from each original azide dextrose culture tube that provided a positive reading after incubation for 24 hours and any 48 hours positive culture (turbidity) in azide dextrose broth at 35° C (8.2).

Under completion of a 48 hour incubation for all ethyl violet azide tubes, record positive and negative results on the sample test report (table 3).

9. EXPRESSION OF RESULTS

9.1 Calculation of Faecal Streptococci per 100 ml of Seawater Sample

Based on the data in the test report for positive results in various dilutions, calculate the MPN (Most Probable Numbers) value using table 2.

If sample portions of 10 ml, 1 ml and 0.1 ml per test tube have been used, take the number of recorded positive readings from the 24-48 hour incubation in ethyl violet azide broth (8.3) reported in table 3, item 8, under 24 + 48 (2c) and find the corresponding most probable number (MPN) from table 2. Enter this value in the test report (table 3, item 9).

When more than three dilutions are employed, the results of only three of these are used in computing the MPN. For MPN calculation purposes, select the highest dilution that has all tubes positive and the next two following dilutions that will have some negative tubes.

Occasionally, positive plates may be encountered beyond the three serial dilutions. In those cases, and for purposes of calculating the MPN, include such additional results in the third row. These skip combinations of positive plates should occur with a frequency lower than 1% of the MPN tests performed. Higher frequencies would suggest laboratory error in pipetting sample dilutions. Select the dilution in which the row of five tubes gives positive readings in all 5 tubes (no negative readings should exist in lower dilutions) and the two next succeeding higher dilutions. Find the MPN (Table 2) corresponding to the number of positive tubes in these three dilutions and adjust the value by multiples of 10 depending on the starting dilution below 10 ml.

Table 1 illustrates an example of the numbers of positive test obtained with a series of 5 consecutive dilutions: 5-5-3-2-1. For MPN calculations the highest dilution selected would be that using 1 ml of sample. The final combinations of positive plates reported would be 5-3-3, instead of 5-3-2. The MPN value corresponding to the 5-3-3 combination would be 170 faecal streptococci/100 ml (Table 2), and not the MPN value of 140 faecal streptococci/100 ml associated to the 5-3-2 combination. The MPN density thus obtained should be adjusted for dilution by multiplying for as many powers of 10 as dilutions were performed with the first plate series considered below 10 ml. For example, a MPN value of 5-3-3 would be expressed as 170 faecal streptococci/100 ml when the starting dilution is 10 ml, but it would be counted as 1700 faecal streptococci/100 ml when the starting dilutions is 1 ml, and 17000 faecal streptococci/100 ml when the largest sample volume used was 0.1 ml.

Table 1. Example for computing the density of faecal streptococci in a water sample analyzed by the MPN method.

Sample portion ml	Positive tubes
10	5
1	5 (X)
0.1	3 (X)
0.01	2 (X)
0.001	1

Dilution Factor: = 10/1 = 10
MPN (5-3-3) = 170 x 10 = 1700 faecal streptococci/100 ml

95% confidence limits:
lower: 80 x 10 = 800 faecal streptococci/100 ml
higher: 410 x 10 = 4100 faecal streptococci/100 ml

9.2 Precision of Results

Select the 95 per cent confidence limits for the MPN selected in (9.1) from table 2 and enter them in the test report (table 3, item 9).

Table 2. MPN index and 95% confidence limits for various combinations of positive and negative results when five 10-ml portions, five 1-ml portions and five 0.1-ml portions are used.

No. of Tubes Giving Positive Reactions out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower	Upper
0	0	0	<2	–	–
0	0	1	2	1.0	10
0	1	0	2	1.0	10
0	2	0	4	1.0	13
1	0	0	2	1.0	11
1	0	1	4	1.0	15
1	1	0	4	1.0	15
1	1	1	6	2.0	18
1	2	0	6	2.0	18
2	0	0	4	1.0	17
2	0	1	7	2.0	20
2	1	0	7	2.0	21
2	1	1	9	3.0	24
2	2	0	9	3.0	25
2	3	0	12	5.0	29
3	0	0	8	3.0	24
3	0	1	11	4.0	29
3	1	0	11	4.0	29
3	1	1	14	6.0	35
3	2	0	14	6.0	35
3	2	1	17	7.0	40
3	3	0	17	5.0	46
4	0	0	13	5.0	38
4	0	1	17	7.0	45
4	1	0	17	7.0	46
4	1	1	21	9.0	55
4	1	2	26	12	63
4	2	0	22	9.0	56
4	2	1	26	12	65
4	3	0	27	12	67
4	3	1	33	15	77
4	4	0	34	16	80
5	0	0	23	9.0	86
5	0	1	30	10	110
5	0	2	40	20	140
5	1	0	30	10	120
5	1	1	50	20	150
5	1	2	60	30	180
5	2	0	50	20	170
5	2	1	70	30	210
5	2	2	90	40	250
5	3	0	80	30	250
5	3	1	110	40	300
5	3	2	140	60	360
5	3	3	170	80	410
5	4	0	130	50	390
5	4	1	170	70	480
5	4	2	220	100	580
5	4	3	280	120	690
5	4	4	350	160	820
5	5	0	240	100	940
5	5	1	300	100	1300
5	5	2	500	200	2000
5	5	3	900	300	2900
5	5	4	1600	600	5300
5	5	5	≥1600	--	--

10 TEST REPORT

Table 3. Faecal streptococci in seawater samples.

1. Sampling area
country: _____
area: _____

2. Sampling point (station)

code number: _____
longitude: _____
latitude: _____

3. Time of sampling hour: ___.___ day: _____ month: ____ year: _____

4. Sampling and environment conditions
Sampling depth: _____
Temperature at sampling depth: _____
Salinity at sampling depth: _____
Container number: _____
Duration of storage: _____
(other factors which may influence the results should be reported under 10)

5. Time of inoculation hour: ___.___ day: ___/___/___
6. Start of incubation hour: ___.___ day: ___/___/___
7. End of incubation hour: ___.___ day: ___/___/___

8. Number of positive reactions

Volume in ml transferred	Azide dextrose		Ethyl violet azide		
	24h (1a)	48h (1b)	24h (2a)	48h (2b)	24h + 48h (2c)
10					
1					
0.1					
0.01					
0.001					
0.0001					

9. Test results:

Ethyl violet azide broth (24 + 48 h)
_____ F. strep./100 ml

95% confidence limits:
____;____ F. strep./100 ml

10. Anomalies observed in the test procedure:

_____
_____
_____

11. Full address of the institution which carried out the analysis:

_____
_____
_____
_____

12. Name(s) and signature(s) of the person(s) who carried out the analysis:

_____
_____
_____

Date: _____

11. REFERENCES

APHA (1985) Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C. (16th edition).

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Issued by

Oceans and Coastal Areas Programme Activity Centre
United Nations Environment Programme

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