REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

31 August 1983

DETERMINATION OF TOTAL COLIFORMS IN SEA-WATER BY THE MEMBRANE FILTRATION CULTURE METHOD

REFERENCE METHODS FOR MARINE POLLUTION STUDIES No. 2 Rev. 1

Prepared in co-operation with

WHO

UNEP 1983

Note: This document has been prepared jointly by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) under projects FP/ME/0503-76-05, ME/0503-81-01 and FP/0503-77-03.

Reference Method No. 2 Rev. 1
CORRIGENDUM

In paragraph 8.6 "36 ± 0.1°C" should read "36 ± 1°C"

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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 ten regions and has over 120 coastal States participating in it. [1) 2)]

One of the basic components of the action plans sponsored by UNEP in the framework of Regional Seas Programme is the assessment of the state of marine pollution, 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 are being developed and are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system 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 Standard Organization (ISO) 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 and given once for ever. 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:

Director,
Regional Seas Programme Activity Centre
United Nations Environment Programme
Palais des Nations
GENEVA
Switzerland

1) UNEP: Achievements and planned development of 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.

IE.83-03147

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

The method described is suitable for the determination of total coliforms (3) in coastal bathing waters of temperate and tropical seas. It is designed to be used in sanitary surveillance of bathing beaches.

One advantage of this method is that it avoids false positive readings of colonies from anaerobic bacteria such as Clostrudium perfingens. Many non-coliforms will not grow on the test medium used. The non-coliforms that do grow have to be excluded by using special tests (8.9). A disadvantage of the method is that it also detects coliforms of non-faecal origin and is consequently not a specific test indicating pollution by faecal material.

It uses a membrane filter procedure which allows concentration of the bacteria prior to incubation. It can be employed in alternation with the Multiple-Tube Fermentation (MPN) Test (UNEP/WHO 1983). 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 labor-intensive and, due to the preconcentration of the bacteria in the sample, it is more suitable in situations where low numbers of coliforms are to be estimated. The MPN test should be given preference when the test sample contains high amounts of particulate matter which will hinder the reading of the MFs after incubation.

Since coliforms die when exposed to sea-water, their presence in sea-water indicate only recent contamination by faecal materials. Die-away rates (T-90) depend on salinity, temperature, solar radiation, etc. and must be taken into consideration when interpreting results.

2. REFERENCES

APHA (1981) Standard methods for the examination of water and waste water. American Public Health Association, Washington D.C. (15th edition)

WHO/UNEP (1983) Consultation meeting on methods for monitoring selected pollutants in sewage effluents and coastal recreational water: WHO/UNEP joint project. Rome 24-26 November 1982. WHO regional office for Europe, Copenhagen.

UNEP/WHO (1983) Determination of faecal coliforms in sea-water by the multiple-tube fermentation (MPN) test. Reference Methods for Marine Pollution Studies No. 22 UNEP, Geneva.

UNEP/WHO (in preparation) Guidelines for monitoring the quality of coastal recreational and shellfish-growing waters. Reference Methods for Marine Pollution Studies No. 1, UNEP, Geneva.

3. DEFINITION

Coliform bacteria are aerobic and facultatively anaerobic Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas at 36 ± 1°C. Under the conditions described in this document, the coliform colonies will appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage. Lactose-negative bacteria appear as colourless colonies.

Coliform bacteria detectable by this test include E. coli type I which are of faecal origin and irregular types II and VI which may not be of faecal origin.

4. PRINCIPLES

From sea-water samples taken under sterile conditions, a dilution series is set up according to the number of total coliforms expected in the water sample. Aliquots of this dilution series are filtered through 0.45 u pore size membrane filters. The membrane filters are placed on the surface of M-endo-agar-MF contained in Petri dishes and incubated at 36 ± 1°C for 24 hours. The coliform colonies will appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage.

Residual chlorine, if present, is neutralized by adding thiosulphate to the sampling bottle before sterilization.

Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test using the MacConkey or brilliant green broth.

5. APPARATUS AND GLASSWARE

5.1 Sample bottles of borosilicate glass for surface sea-water, 200-300 ml capacity, wide-mouthed and with ground-glass stoppers.

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 Thermoisolated plastic boxes with cooling pads or similar cooling units (camping equipment) for storage of samples.

5.5 Thermometer, 0 to 50°C, precision ± 1°C, preferably unbreakable plastic type, to be used for checking temperature in plastic boxes (5.4).

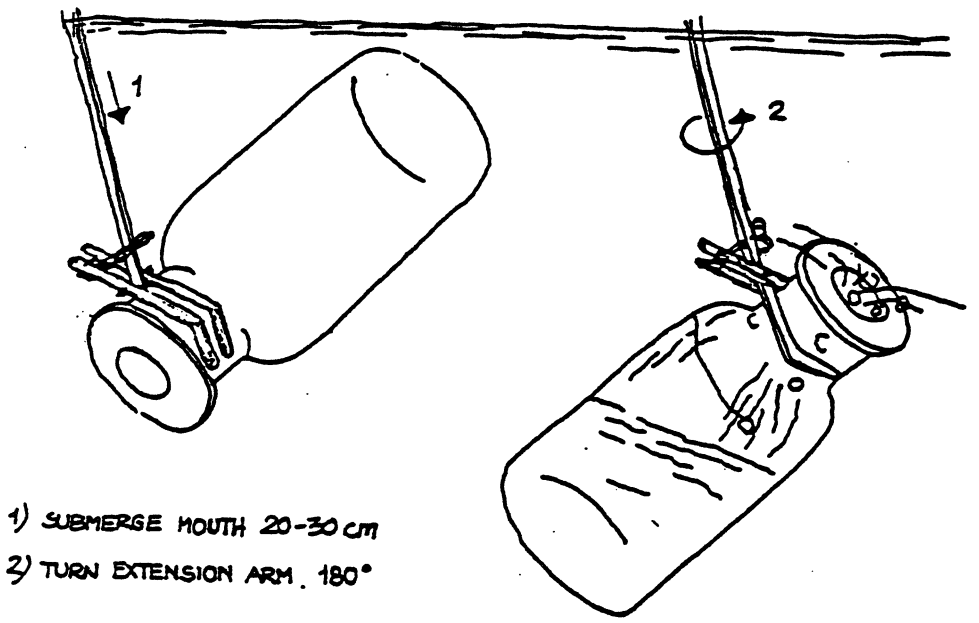

FIGURE 1 : SUBSURFACE SAMPLING WITH EXTENSION ARM

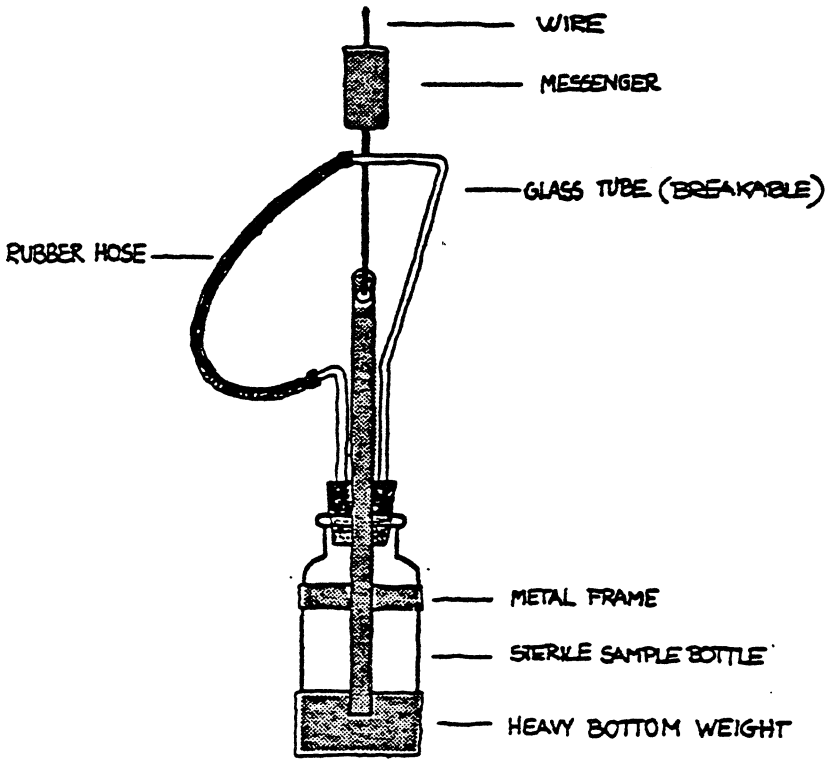

FIGURE 2 : SAMPLER FOR STERILE SUBSURFACE SAMPLING

5.6 Filtration apparatus for 4.7 cm diameter membrane filters (MF) consisting of at least three filter funnels for simultaneous filtration, made of borosilicate glass or other non-toxic sterilizable material, complete with electric or water vacuum pump.

5.7 Air incubator for 36 ± 1°C.

5.8 Stereomicroscope, magnification 10 - 50X; and/or darkfield colony counter, magnification 2 - 3X.

5.9 Autoclave, max 2 atm., electric or gas.

5.10 Drying oven for sterilization at 160°C.

5.11 pH meter, precision ± 0.1 pH units.

5.12 Stainless steel forceps.

5.13 Analytical balance, precision ± 1 mg.

5.14 Refrigerator 4° ± 0.5°C.

5.15 Vibrator (shaker) for mixing liquids in culture tubes.

5.16 Petri dishes of borosilicate glass, diameter 5 cm, complete with stainless steel containers for sterilization, or disposable pre-sterilized plastic Petri dishes.

5.17 Ehrlenmeyer flasks of borosilicate glass for media preparation, capacity 1 and 2 litre.

5.18 Borosilicate glass bacteriological culture tubes.

5.19 Total volume (blow-out) borosilicate glass pipettes of 1, 9, 10 and 20 ml capacity, with stainless steel containers for sterilization.

NOTE: 9 ml capacity pipettes are useful, but not essential.

5.20 Graduated borosilicate glass cylinders of 100, 500 and 1000 ml capacity with glass beakers for cover.

5.21 Small borosilicate glass vials ("Durham vials") to be inserted in culture tubes (5.18).

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

5.23 Heavy wrapping paper.

5.24 Aluminium foil (household quality).

5.25 Membrane filters (MF), pore size 0.45 u, diameter 4.7 cm, or similar, fitting filtration apparatus (5.6).

NOTE: The 0.45 u pore size membrane filter (MF) should be certified by the manufacturer to be free from substances which may hinder the growth and development of bacteria. Maximum recoveries are obtained using membranes composed of mixed esters of cellulose.

5.26 Filter paper.

6. CULTURE MEDIA, CHEMICALS AND STOCK CULTURE

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

6.1 M-endo-agar-MF

Polypeptone	10.0	g
Thiopeptone	5.0	g
Casitone	5.0	g
Yeast extract	1.5	g
Lactose	12.5	g
NaCl	5.0	g
K_2HPO_4	4.375	g
KH_2PO_4	1.375	g
Sodium lauryl sulfate	0.05	g
Sodium desoxycholate	0.10	g
Sodium sulfite	2.10	g
Basic fuchsine	1.05	g
Distilled water (6.6)	1.0	litre
Agar	15.0	g

Preparation: Dissolve the components of the medium in 1 litre of distilled water containing 20 ml ethanol (6.8). Heat to boiling point and promply remove from heat. The final medium should have a pH of 7.2 $\pm$ 0.1. Cool to about 45°C and pour 4-5 ml into each Petri dish. After the agar has

solidified in the Petri dishes invert the Petri dishes and store them in the refrigirator. The dishes with prepared medium can be kept in a refrigerator for up to 30 days if kept in the dark.

NOTE: Do not autoclave the medium.

NOTE: The agar surface should not become too dry because if the agar is dry the MF will not adhere well to the agar surface (8.5) and hinder the diffusion of nutrients to the colonies on the MF.

6.2 MacConkey broth

6.2.1 Medium

Sodium taurocholate	5.0 g
Lactose	10.0 g
NaCl	5.0 g
Peptone	20.0 g
Distilled water (6.6)	1.0 litre

Preparation: Dissolve components by shaking. Adjust pH to 7.1 $\pm$ 0.1 with diluted HCl and then add the bromo-cresol purple solution (6.2.2). Add inverted vials (5.21) to clean culture tubes (5.18, 8.1) and dispense sufficient medium into the culture tubes so that the inverted vials are at least partially covered after the entrapped air in these vials has been driven out during autoclaving and close the tubes with cotton plugs. Autoclave (5.9) the closed culture tubes at 121°C for 15 minutes.

6.2.2 Bromo-cresol purple solution

Preparation: Dissolve 1 g of bromo-cresol purple in 99 ml of 95% ethanol (6.8).

6.3 Brilliant green bile broth

Oxgall, dehydrated	20.0 g
Lactose	10.0 g
Peptone	10.0 g
Brilliant green	13.3 mg
Distilled water (6.6)	1.0 litre

Preparation: Dissolve components by shaking. Add inverted vials (5.21) to clean culture tubes (5.18, 8.1) and dispense sufficient medium into the culture tubes so that the inverted vials are at least partially covered after the entrapped air in these vials has been driven out during autoclaving and close the tubes with cotton plugs. Sterilize by autoclaving (5.9) at 121°C, preferably for 12 minutes, but not exceeding 15 minutes. After sterilization, cool the broth as quickly as possible. Final pH should be 7.2 $\pm$ 0.2. Test the samples of the finished product for performance using control stock cultures (6.9).

6.4 Phosphate buffer (pH = 7.2)

K_2HPO_4	3.0 g
KH_2PO_4	1.0 g
Distilled water (6.6)	1.0 litre

6.4.1 P-buffer for filtration

Preparation: Dissolve components and autoclave at 121°C for 15 minutes.

6.4.2 P-buffer for dilutions

Preparation: Dissolve components and dispense 9 ml in test tubes used for dilutions in the dilution series (8.4) and autoclave (5.9) at 121°C for 15 minutes.

6.5 Thiosulphate solution

Preparation: prepare a 10 per cent sodium thiosulphate solution in distilled water (6.6) and sterilize by filtration (e.g. through a sterile MF (8.2.4, 8.2.5)).

6.6 Distilled water

Use only water distilled in all-glass or all-quartz distillation apparatus. De-ionized water is also acceptable if produced in apparatus not releasing toxic substances.

NOTE: Commercially available distilled water is often produced in copper and zinc apparatus and is highly toxic for coliforms. Before using such water its toxicity should be checked with a stock culture of E. coli (6.9).

6.7 Detergents for cleaning glassware and apparatus

Use only detergents recommended by the supplier for bacteriological use. If such a detergent is not available, check normal household detergents with a biotest using a stock culture of E. coli (6.9).

NOTE: Never use toxic chromic-sulphuric acid mixture for cleaning glassware.

6.8 95 per cent ethanol per analysis.

6.9 Stock culture of E. coli.

7. SAMPLING

For a sampling plan, follow Reference Method No. 1 (UNEP/WHO, in preparation).

7.1 Sampling of surface water

Attach clean sterilized sample bottle (8.2.1) to the clean sampling rod (5.2). Immediately before submerging the sample bottle, remove the ground glass stopper from the bottle without touching the stopper cone. 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 with the mouth of the bottle downwards, in order to avoid contamination by 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.

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 and store the samples in the clean thermoisolated box (5.4) with cooling pads at about 4°C. Keep samples in the dark avoiding exposure to more than +10°C. Separate bottles from each other with clean wrapping paper (5.23) to avoid breakage. Check the temperature with a thermometer (5.5) every three hours. Report irregularities in the test report. Label sample bottles indicating the sampling station, time of sampling and other factors relevant to the interpretation of the results.

7.2 Sampling of subsurface water

Lower the sterilized subsurface sampler (8.2.2) 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 thermoisolated box (5.4). Proceed as for sampling of surface water (7.1).

NOTE: It is known that the die-away rate of coliforms 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 filtered and incubated the same day. If this is not possible, the samples should be stored at 4°C and analysed not later than 24 hours after sampling.

The water sample represents the test solution.

8. TEST PROCEDURE

8.1 Washing of glassware and equipment

All glassware and apparatus (5) should be washed with non-toxic detergent (6.7), first rinsed thoroughly with hot tap water and then rinsed at least three times with distilled water (6.6).

8.2 Sterilization of glassware and equipment

8.2.1 Surface sample bottles (5.1). Clean the sample bottle as described under 8.1. Dry and sterilize it in a drying oven (5.10) for three hours at

160°C. Before sterilization, place a small piece of filter paper (5.26) in the neck of the bottle to prevent the ground glass stopper from sticking after cooling. After cooling to ambient temperature in the drying oven, remove this filter paper with sterilized forceps (8.2.6) and fit the ground glass stopper securely into the neck of the bottle. Put the bottles into detergent-cleaned thermoisolated boxes (5.4). Separate the bottles from each other with clean wrapping paper (5.23) to avoid breakage.

NOTE: If residual chlorine is suspected in the water sample, add, aseptically, 0.1 ml of a 10 per cent thiosulphate solution (6.5) for each 100 ml sample to the contents of the sample bottle before sterilization. This amount is sufficient to neutralize about 15 mg of residual chlorine per litre.

8.2.2 Subsurface sampler (5.3). Clean the subsurface sampler as described under (8.1), rinse with tap and distilled water (6.6). Enclose each sampler in heavy wrapping paper (5.23) or aluminium foil (5.24) and sterilize in an autoclave (5.9) for 15 minutes at 121°C.

8.2.3 Petri dishes (5.16) and pipettes (5.19). Clean dishes and pipettes, complete with a cotton plug in the mouthpiece, are put into suitable stainless steel containers and sterilized in a drying oven (5.10) for three hours at 160°C.

NOTE: Disposable pre-sterilized plastic Petri dishes may be more economical to use than reusable glass Petri dishes.

8.2.4 Filter funnels of filtration apparatus (5.6). Loosen the filter-holding assembly slightly and wrap the whole filter-funnel in heavy wrapping paper (5.23) or aluminium foil (5.24). Sterilize in an autoclave (5.9) for 15 minutes at 121°C, or in a drying oven (5.10) for 3 hours at 160°C.

8.2.5 Membrane Filters (MF) (5.25). Remove the paper separator (if present) and place 10 to 12 clean MFs into Petri dishes (5.16). Autoclave (5.9) for 15 minutes at 121°C. At the end of the sterilization let the steam escape rapidly in order to minimize the accumulation of condensate on the MFs.

NOTE: Sterilized MFs are commercially available.

8.2.6 Forceps (5.12). Sterilize forceps by dipping them into 95 per cent ethanol (6.8) and flaming them.

8.3 Selection of sample size and dilution series

The MFs should ideally have from 20 to 80 colonies after incubation. If previous experience for planning the dilution series for clean sea-water is not available, filter the following volumes of the original sample: 100 ml, 10 ml, 1 ml and 0.1 ml (figure 3). For contaminated waters the dilutions have to be greater.

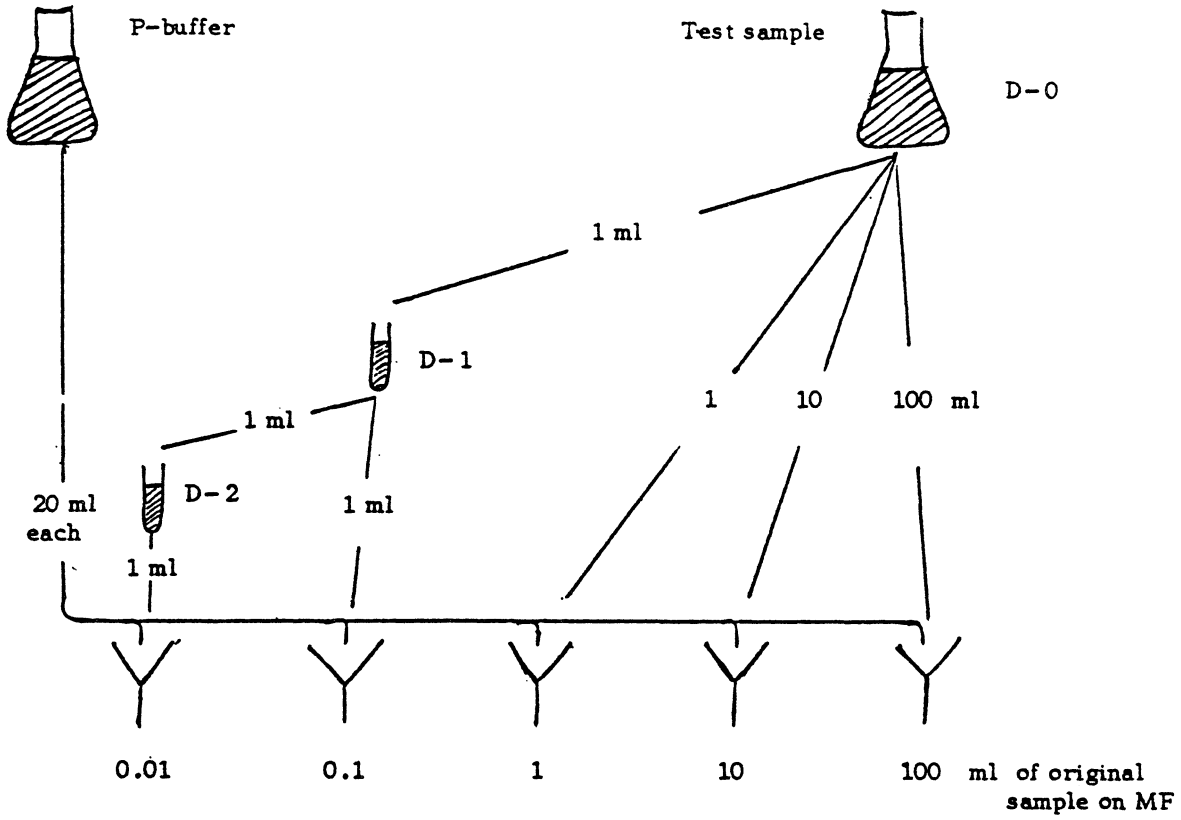

FIGURE 3 : SCHEME OF PREPARATION OF DILUTION SERIES AND OF THE FILTRATION PROCEDURE

8.4 Preparation of the dilution series

Before taking aliquots from the original sample or from the dilutions these must be vigorously shaken in order to guarantee that representative aliquots are taken.

Prepare the dilution series by taking with a sterilized pipette (8.2.3), after vigorously shaking the sample (7), 1 ml from the original sample (figure 3, dilution: D-0) and transfer this 1 ml into a culture tube containing 9 ml of P-buffer (6.4.2) to make the first dilution (D-1). Agitate the tube on a mixer (5.15) or shake it vigorously by hand. Continue the preparation of the dilution series by taking 1 ml from the first dilution (D-1) and mixing it in a new culture tube containing 9 ml of phosphate buffer (6.4.2) in order to obtain the second dilution (D-2), etc.

8.5 Filtration procedure

Begin filtration with the highest dilution (e.g D-2) in order to avoid contamination from samples containing bacteria in higher concentrations. Use a sterilized filtration funnel (8.2.4) for each dilution series. Place the sterilized MF (8.2.5) with flamed sterilized forceps (8.2.6) over the porous

plate of the filtration apparatus (8.2.4). Carefully place the matching funnel unit over the receptacle and lock it in place. Add into the funnel about 20 ml of buffer solution (6.4.1). With a sterilized pipette (8.2.3) add 1 ml of the D-2 dilution into the buffer solution in the funnel. Filter with a partial vacuum. Wash the funnel walls with approximately 20 ml of buffer solution (6.4.1). Filter with a partial vacuum. Wash the funnel walls two more times with 20 ml of buffer solution (6.4.1) and filter again. Unlock and remove the funnel, immediately remove the MF with flamed sterilized forceps (8.2.6) and place the MF on the agar surface of the medium contained in the Petri dish (6.1.1) with a rolling motion to avoid the entrapment of air. Before filtering the next dilution (D-1) in the same manner, pass 20 ml of buffer solution (6.4.1) through the assembled filtration unit.

8.6 Incubation

The Petri dishes containing the MFs on agar (8.5) are sealed and incubated (5.7) immediately for 24 hours at 36 $\pm$ 0.1°C. As a sterility check, incubate also one blank (without MF), i.e. a Petri dish containing agar (6.1) only.

8.7 Interpretation

Count with a stereomicroscope or similar (5.8) only colonies which appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage. If the number of dubious colonies is greater than 10 per cent of the total number of colonies, test dubious colonies either by MacConkey broth test (8.9.1) or brilliant green bile broth test (8.9.2).

8.8 Estimation of precision

Check the precision of the technique at periodic intervals (at least once every season) by preparing three independent series of dilutions (8.4) using the same sample, i.e. repeating the four last consecutive dilution steps described under 8.3 and 8.4 (figure 3). The water sample used should be collected during the routine monitoring programme at a coastal station typical of the area. The dilution series should be selected in such a way so that one dilution step yields three MF counts which satisfy the 20 to 200 colonies requirements expressed in section 9.1.

Filter each individual dilution following procedure 8.5. Incubate according to procedure 8.6. Report MF counts following the procedure described in sections 9.1 and 9.2 taking into consideration interpretation method of section 8.7. Results should be reported in the test report (table 2, item 9).

Calculate the total coliform concentrations of the original sample for each of the replicate results, according to section 9.3 and report the results in the test report (table 2, item 10).

For each dilution step having the three MF counts between 20 and 200 total coliform colonies, calculate: the mean concentration, the concentration range, the standard deviation of the concentrations, and the coefficient of variation of the concentrations, and record them in the test report (table 2, item 11).

If the sample does not yield at least 20 colonies per membrane filter in one dilution, prepare a test solution from a stock culture (6.9) and repeat the estimation of precision.

NOTE: Coefficient of variation (%) = $\frac{\text{standard deviation}}{\text{mean}} \times 100$

8.9 Confirmatory test

8.9.1 MacConkey broth test: With a flamed bacteriological loop (5.22) transfer the suspected colony from the MF into a culture tube containing MacConkey broth (6.2.1) and incubate at 36 ± 1°C for 48 hours. Coliforms will develop gas which is trapped in the inverted Durham vials, and acid which turns the violet-like colour of the original broth into a yellowish colour.

8.9.2 Brilliant green bile broth test: With a flamed bacteriological loop (5.22) transfer the suspected colony from the MF into a culture tube containing brilliant green bile broth (6.3) and incubate at 36 ± 1°C for 48 hours. Coliforms will develop gas which is trapped in the inverted Durham vials.

NOTE: The MacConkey broth test is equivalent to the brilliant green bile broth test. Either can be used for confirmation.

9. EXPRESSION OF RESULTS

9.1 Report the number of coliform colonies on individual MFs after the incubation has been completed and adjust this count after the confirmatory tests, if necessary, have been made. Use only MFs with a total number of colonies (i.e. coliforms plus non-coliforms) between 20 and 200. Retain only two significant digits of the counted number of coliform colonies per filter.

Indicate the results obtained for each filter separately in the test report (table 1, item 9).

9.2 Express the results in terms of total coliforms per 100 ml of sample, using the following equation:

$$\text{total coliforms per 100 ml sample} = \frac{\text{adjusted number of coliform colonies}}{\text{ml of sample filtered}} \times 100$$

Indicate the results obtained for each dilution separately in the test report (table 1, item 10). Report also the results obtained on MFs with less than 20 coliform colonies per filter. If there are no coliform colonies on the filter report the results as "<1 coliform per 100 ml."

9.3 Compute the number of total coliforms per 100 ml sample and report it as the final test result (table 1, item 11). If there are MFs containing between 20 and 200 characteristic colonies in two consecutive dilutions calculate the mean of these dilutions and report it as final test result.

9.4 Record in the test report (table 1, item 12) anomalies observed in test procedure (confluent growth of colonies, deviation from temperature prescribed for sample storage and incubation, etc.).

10. TEST REPORT

Fill in the test report (tables 1 and 2) giving full details in every column.

Table 1 : Test Report on Total Coliforms in Sea-Water Sample

1. Sampling area

   1.1 country: _____ 1.2 area: _____

2. Sampling point (station)

   2.1 code number: _____ 2.2 longitude: _____

   2.3 latitude: _____

3. Time of sampling

   3.1 hour: _____ 3.2 day: _____ 3.3 month: _____

   3.4 year: _____

4. Sampling and environmental conditions

   4.1 depth of sampling: _____ 4.2 container number: _____

   4.3 temperature at sampling depth: _____

   4.4 salinity at sampling depth: _____

   4.5 duration of storage: _____

   4.6 other factors which may influence the results should be reported under 12.

5. Time of filtration

   5.1 hour: _____ 5.2 day: _____

6. Start of incubation

   6.1 hour: _____ 6.2 day: _____

7. End of incubation

   7.1 hour: _____ 7.2 day: _____

8. Confirmatory tests carried out (mark test used)

8.1 MacConkey: _____ 8.2 Brilliant green: _____

9. Number of colonies per individual filter

dilution	ml of original sample filtered	total coliform colonies
D-0	100	_____
D-0	10	_____
D-0	1	_____
D-1	0.1	_____
D-2	0.01	_____
D-3	0.001	_____
D-4	0.0001	_____

10. Number of total coliforms/100 ml sample

dilutions	n/100 ml
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

11. Test result: _____ total coliforms/100 ml samples

12. Anomalies observed in the test procedure:

_____

_____

_____

_____

_____

13. Full address of the institution which carried out the test:

_____

_____

_____

_____

_____

14. Name(s) and signature(s) of the person(s) who carried out the test:

_____

_____

Date: _____

Table 2 : Test Report on Precision Estimation

1. Sampling area

   1.1 country: _____ 1.2 area: _____

2. Sampling point (station)

   2.1 code number: _____ 2.2 longitude: _____

   2.3 latitude: _____

3. Time of sampling

   3.1 hour: _____ 3.2 day: _____ 3.3 month: _____

   3.4 year: _____

4. Sampling and environmental conditions

   4.1 depth of sampling: _____ 4.2 container number: _____

   4.3 temperature: _____ 4.4 salinity: _____

   4.5 duration of storage: _____

   4.6 other factors which may influence the test result should be reported under 12.

5. Time of filtration

   5.1 hour: _____ 5.2 day: _____

6. Start of incubation

   6.1 hour: _____ 6.2 day: _____

7. End of incubation

   7.1 hour: _____ 7.2 day: _____

8. Confirmatory tests carried out

   8.1 MacConkey: _____ 8.2 Brilliant green: _____

9. Number of colonies per individual filter

Dilution	ml of original sample filtered	Number of total coliform colonies per filter in replicate dilution		
		1st	2nd replica	3rd

10. Number of total coliforms per 100 ml

dilutions	n/100 ml

11. Test results (total coliforms/100 ml sample):

mean	range	stand. deviation	coef. var. (%)

12. Anomalies observed in the test procedure:

13. Full address of the institution which carried out the test:

_____

_____

_____

_____

_____

14. Name(s) and signature(s) of the person(s) who carried out the test:

_____

_____

Date: _____

LIST OF REFERENCE METHODS FOR MARINE POLLUTION STUDIES

UNEP/WHO : Guidelines for monitoring the quality of coastal recreational waters. (Draft) Reference Methods for Marine Pollution Studies No. 1, UNEP 1982.

UNEP/WHO : Determination of total coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 2 Rev. 1, UNEP 1983.

UNEP/WHO : Determination of faecal coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 3 Rev. 1, UNEP 1983.

UNEP/WHO : Determination of faecal streptococci in sea-water by the membrane filtration cultrue method. Reference Methods for Marine Pollution Studies No. 4 Rev. 1, UNEP 1983.

UNEP/WHO : Determination of faecal coliforms in bivalves by multiple test tube method. Reference Methods for Marine Pollution Studies No. 5 Rev. 1, UNEP 1983.

UNEP/FAO/IAEA : Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6, UNEP (in preparation)

UNEP/FAO/IAEA : Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies No. 7 Rev. 1, UNEP 1983.

UNEP/FAO/IAEA : Determination of total mercury in selected marine organisms by flameless atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 8 Rev. 1, UNEP 1983.

UNEP/FAO/IAEA : Determination of total arsenic in selected marine organisms by flameless atomic absorption spectrophotometry. (Draft) Reference Methods for Marine Pollution Studies No. 9, UNEP 1983.

UNEP/FAO/IAEA : Determination of total selenium in selected marine organisms by flameless atomic absorption spectrophotometry. (Draft) Reference Methods for Marine Pollution Studies No. 10, UNEP 1983.

UNEP/FAO/IAEA : Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 11 Rev. 1, UNEP 1983.

UNEP/FAO/IAEA : Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. Reference Methods for Marine Pollution Studies No. 12, UNEP 1982.

UNEP/FAO/IAEA : Determination of methylmercury in selected marine organisms. (Draft) Reference Methods for Marine Pollution Studies No. 13, UNEP 1982.

UNEP/FAO/IAEA : Determination of DDTs and PCBs in selected marine organisms. Reference Methods for Marine Pollution Studies No. 14, UNEP 1982.

UNEP/IOC/IAEA : Monitoring of tar on marine beaches. Reference Methods for Marine Pollution Studies No. 15, UNEP (in preparation)

UNEP/IAEA : Determination of DDTs, PCBs, PCCs and other hydrocarbons in sea-water by gas chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 16, UNEP 1982.

UNEP/IAEA : Determination of DDTs, PCBs and other hydrocarbons in marine sediments by gas liquid chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 17, UNEP 1982.

UENP/IOC : Determination of total dissolved cadmium in sea-water by differential pulse anodic stripping voltammetry. (Draft) Reference Methods for Marine Pollution Studies No. 18, UNEP 1983.

UNEP/IOC : Determination of total mercury in estuarine waters and suspended matter by cold vapour atomic absorption spectrophotometry. (Draft) Reference Methods for Marine Pollution Studies No. 19, UNEP 1983.

UNEP/IOC : Monitoring of petroleum hydrocarbons in sediments. Reference Methods for Marine Pollution Studies No. 20, UNEP (in prepartion)

UNEP/WHO : Determination of total coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 21, UNEP 1983.

UNEP/WHO : Determination of faecal coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 22, UNEP 1983.

UNEP/WHO : Determination of faecal streptococci in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 23, UNEP 1983.

UNEP/IOC : Monitoring of petroleum hydrocarbons in sea-water. (in preparation)

UNEP/IAEA : Guidelines for monitoring of estuarine waters and suspended matter. (in preparation)

UNEP/WHO : Determination of faecal coliforms in estuarine waters, suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of phosphorus in suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of nitrogen in suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of BOD_5 and COD in estuarine waters. (in preparation)

UNEP/UNESCO : Determination of total cadmium in estuarine waters and suspended matter. (in preparation)

UNEP/IOC : Determination of basic oceanographic and meteorological conditions. (in preparation)

UNEP/IOC : Determination of standard physical and chemical parameters. (in preparation)

UNEP/WHO : Statistical methods for the evaluation of results from monitoring the quality of coastal recreational and shellfish-growing waters. (in preparation)

UNEP/WMO : Sampling of aerosols and wet precipitation for analysis of chemical pollutants. (in preparation)

UNEP/IAEA : Determination of selected trace metals in aerosols and in wet precipitation. (in preparation)

UNEP/IAEA : Determination of halogenated hydrocarbons in aerosols and in wet precipitation. (in preparation)

UNEP/WMO : Sampling of dry deposition. (in preparation)

UNEP/IAEA : Determination of total mercury in marine sediments by flameless atomic absorption spectrophotometry. (in preparation)

UNEP/IAEA : Determination of total cadmium in marine sediments by flameless absorption spectrophotometry. (in preparation)

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