



DETERMINATION OF FAECAL STREPTOCOCCI IN SEA-WATER
BY THE MEMBRANE FILTRATION CULTURE METHOD

REFERENCE METHODS FOR MARINE POLLUTION STUDIES No. 4 Rev. 1

Prepared in co-operation with



WHO

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. 4 Rev. 1

CORRIGENDA

- section 1, third paragraph, 7th line: "coliforms" should read "streptococci"
- section 2, fourth paragraph, 3rd line: "No. 22" should read "No. 23"
- paragraph 6.1.1: " 7.2 ± 1 " should read " 7.2 ± 0.1 "
- paragraph 8.7: "faecius" should read "faccium"
- section 10: "(table 1)" should "(tables 1 and 2)"

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REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

31 August 1983

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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.

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1. SCOPE AND FIELD OF APPLICATION

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

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

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 1983b). 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.

The group of faecal streptococci normally originate from the intestine of warm-blooded animals and when found in sea-water indicate contamination with faecal material. Recent studies indicate that streptococci, similar to the faecal streptococcal 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 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 (1983a) Determination of faecal coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies, No. 3 Rev. 1, UNEP, Geneva.

UNEP/WHO (1983b) Determination of faecal streptococci in seawater 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

Faecal streptococci are Gram-positive oblong/oval cocci occurring in pairs or in short chains when grown on KF agar containing 2,3,5-triphenyl-tetrazolium-chloride solution (TTC). After an incubation of 48 hours at $36 \pm 1^\circ\text{C}$, the TTC will produce pink to dark red colonies or colonies with dark red centre.

4. PRINCIPLES

From sea-water samples taken under sterile conditions, a dilution series is set up according to the number of faecal streptococci expected in the water sample. Aliquots of this dilution series are filtered through 0.45 μ pore size membrane filters. The membrane filters are placed on the surface of KF agar containing triphenyl-tetrazolium-chloride and incubated at $36 \pm 1^\circ\text{C}$ for $48 \pm$ hours. Pink to red or red centred colonies are considered to be faecal streptococci.

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

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.

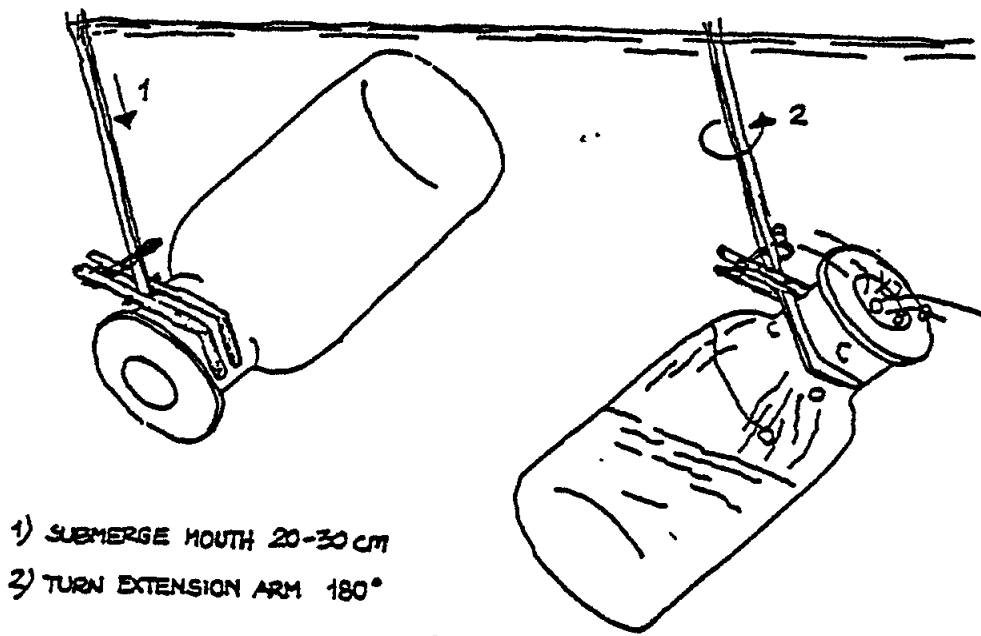


FIGURE 1 : SUBSURFACE SAMPLING WITH EXTENSION ARM

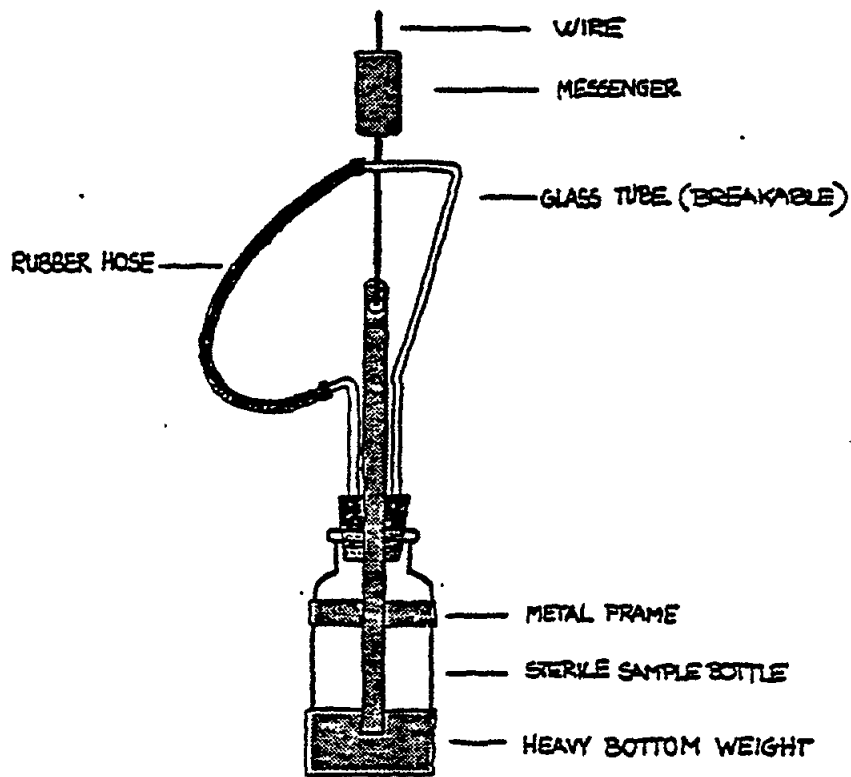


FIGURE 2 : SAMPLER FOR STERILE SUBSURFACE SAMPLING

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 $\pm 1^\circ\text{C}$, preferably unbreakable plastic type, to be used for checking temperature in plastic boxes (5.4).

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 $36 \pm 1^\circ\text{C}$.

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 $4 \pm 1^\circ\text{C}$.

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 500 ml and 1 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 tubes ("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), or membrane filter of similar diameter fitting the filtration apparatus.

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 Filtration apparatus for preparing sterile solutions.

5.27 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 of media and solutions the actual needs have to be established and amounts must be chosen accordingly.

6.1 KF Streptococcus Agar

6.1.1 Agar base

Proteose peptone No. 3 or polypeptone	10.0	g
Yeast extract	10.0	g
Sodium chloride	5.0	g
Sodium glycerophosphate	10.0	g
Maltose	20.0	g
Lactose	1.0	g
Sodium azide	0.4	g
Brom cresol purple	0.015	g
Agar	20.0	g
Distilled water	1000	ml

Preparation: Mix the reagents with the distilled water in a sterile flask then heat in a boiling water bath to dissolve the agar. Cool to 50°C, then add immediately before use to every 1000 ml of agar base (6.1.1) 10 ml of a 1% solution of 2,3,5-triphenyl-tetrazolium-chloride (6.1.2) under sterile conditions. The pH should be 7.2 \pm 1 after heating, if necessary adjust pH with 10% sodium carbonate. After the agar has solidified in Petri dishes (4-5 ml of medium per each Petri dish) invert the Petri dishes so that moisture cannot accumulate on the cover, then store them in a refrigerator.

The medium may be stored at 45 to 50°C for up to 4 hours before the plates are poured. Poured plates can be stored in the dark for up to 30 days when kept

at 2 - 4°C. Check for sterility by incubating a blank KF agar plate containing no membrane filter.

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) hinder the diffusion of nutrients to the colonies on the MF.

6.1.2 2,3,5-triphenyl-tetrazolium-chloride solution (TTC)

Preparation: Prepare a 1% TTC solution in distilled water (6.4) and sterilize by filtration (5.26) and store in the dark. Discard the TTC solution when it turns red.

NOTE: The TTC solution disintegrates when exposed to light and temperatures higher than 50°C.

6.2 Phosphate buffer (pH = 7.2)

K ₂ HPO ₄	3.0 g
KH ₂ PO ₄	1.0 g
Distilled water (6.4)	1.0 litre

6.2.1 P-buffer for filtration

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

6.2.2 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 at 121°C for 15 minutes.

6.3 Thiosulphate solution

Preparation: Prepare a 10% sodium thiosulphate solution in distilled water (6.4) and sterilize by filtering (e.g. through a sterile membrane filter (8.2.4, 8.2.5)).

6.4 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 streptococci. Before using such water its toxicity should be checked with stock culture (6.7)

6.5 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 (6.7)

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

6.6 95 per cent ethanol per analysis.

6.7 Stock culture of a streptococcus strain from a type collection.

7. SAMPLING

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

7.1 Sampling of surface water

Attach clean sterile 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 sterile 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, 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 sterile 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 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 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 (6.5), first rinsed thoroughly with hot tap water and then rinsed at least three times with distilled water (6.4).

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 (5.27) 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 (6.3) 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 (6.4). 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 re-usable 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 (6.6) 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.

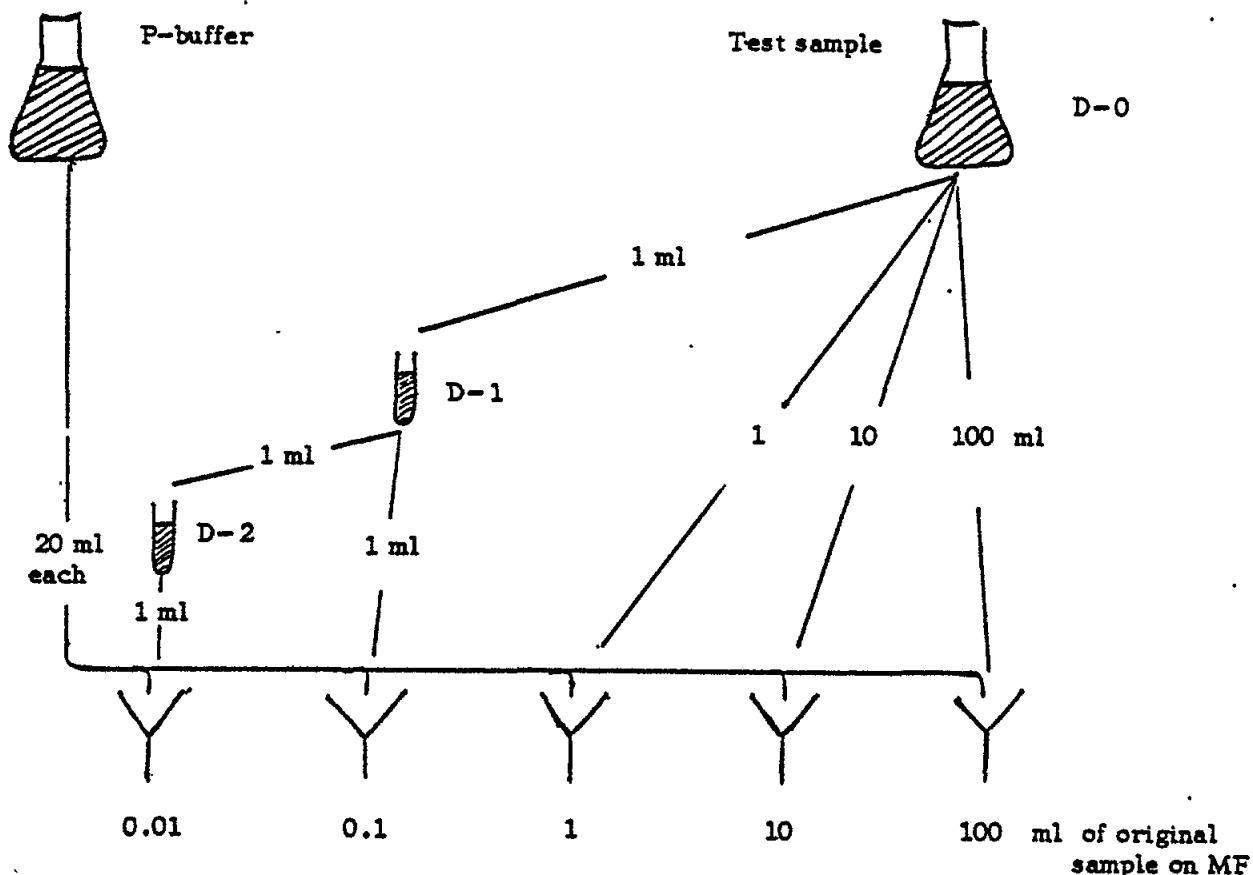


FIGURE 3 : SCHEME OF PREPARATION OF DILUTION SERIES AND OF THE FILTRATION PROCEDURE

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 (6.2.2) 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 (6.2.2) 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 (6.2.1). 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 (6.2.1). Filter with a partial vacuum. Wash the funnel walls two more times with 20 ml of buffer solution (6.2.1). 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) 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 (6.2.1) through the assembled filtration unit.

8.6 Incubation

The Petri dishes (8.5) containing the MFs are sealed and incubated (5.7) immediately for 48 ± 3 hours at $36 \pm 1^\circ\text{C}$. As a sterility check, incubate also one blank (without MF), i.e. a Petri dish containing KF agar (6.1) only.

8.7 Interpretation

Count with a stereomicroscope or similar (5.8) as faecal streptococci colonies only colonies which are pink, dark brownish red, red centred or eventually with a narrow whitish zone around them. The size of these colonies may vary between 0.5 to 3 mm. Report the number of streptococci colonies in the test report (item 8 in table 1).

NOTE: It is a condition for the production of typical reducing colonies that the medium is not overgrown. The reduction of triphenyl-tetrazolium-chloride to a red (triphenyl-formazan) will fail if the colonies are too closely packed. Besides strongly reducing *Streptococcus faecalis* and its variants, the definition (3) comprises also the faintly reducing *Streptococcus faecius*, *S. bovis* and *S. equinus*.

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 (item 8 in table 2).

Calculate the faecal streptococci concentrations of the original sample for each of the replicate results, according to section 9.3 and report the results in the test report (item 9 in table 2).

For each dilution step having the three MF counts between 20 and 200 faecal streptococci 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 (item 10 in table 2).

If the sample does not yield at least 20 colonies per membrane filter in one dilution, prepare a test solution from a stock culture (6.7) and repeat the estimation of precision.

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

9. EXPRESSION OF RESULTS

9.1 Report the number of faecal streptococci colonies on individual MFs after the incubation has been completed. Use only MFs with 20 to 200 colonies (total, including faecal streptococci and non-faecal streptococci colonies). Retain only two significant digits of the counted number of streptococci colonies per filter.

Indicate the obtained results for each filter separately in the test report (table 1, item 8).

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

$$\text{faecal streptococci per 100 ml sample} = \frac{\text{number of streptococci colonies}}{\text{ml of sample filtered}} \times 100$$

Indicate the obtained results for each dilution separately in the test report (table 1, item 9). Report also the results obtained on MFs with less than 20 streptococci colonies per filter. If there are no streptococci colonies on the filter, report the results as "<1 streptococci per 100 ml."

9.3 Compute the number of faecal streptococci per 100 ml sample and report it as the final test result (table 1, item 10). 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 11) 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 (table 1) giving full details in every column.

Table 1 : Test Report on Faecal Streptococci 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: _____
 - 3.3 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 11.
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. Number of colonies per individual filter

dilution	ml of original sample filtered	faecal streptococci 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	_____

9. Number of faecal streptococci/100 ml sample

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

10. Test result: _____ faecal streptococci/100 ml samples

11. Anomalies observed in the test procedure:

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

13. 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 that may influence the test result should be reported under 11.

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. Number of colonies per individual filter

dilution	ml of original sample filtered	Number of faecal streptococci colonies in replicate dilution		
		1st	2nd replica	3rd
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

9. Number of faecal streptococci per 100 ml sample

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

10. Test results (faecal streptococci/100 ml sample):

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

11. Anomalies observed in the test procedure:

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

13. 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 culture 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)
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