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REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

31 August 1983

DETERMINATION OF FAECAL COLIFORMS IN BIVALVES BY MULTIPLE TEST TUBE METHOD

REFERENCE METHODS FOR MARINE POLLUTION STUDIES No. 5 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. 5 Rev. 1
CORRIGENDA

- NOTE of paragraph 6.1: "seawater" should read "test dilution"
- paragraph 9.5: "(9.2.2) from" should read "(9.2.2) a drop from"
- paragraph 9.5, fourth section should read: "Repeat this operation (9.5) with the tubes from the presumptive test (9.3) which have become positive during the 24-48 hours interval and record the results in table 2, item 7, under (2d)"
- section 11, part 4.5: "____%S" should read "____‰"
- section 11, part 6.1: "6.1 MacConkey at 36°C" should read "6.1 Lactose broth at 36 C"
- section 11, part 7: "MacConkey Brilliant" should read "MacConkey or Brilliant"

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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.

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

The method described is suitable for the determination of faecal coliforms (3) in bivalve (shellfish) specimens from temperate and tropical seas. It is designed to be used in sanitary surveillance of sea-food.

Faecal coliforms are specific indicators, exhibiting a high positive correlation with faecal contamination from warm-blooded animals. Filter-feeding shellfish concentrates coliforms from its marine environment. The concentration of faecal coliforms in edible shellfish tissue gives an indication of the potential health hazard to consumers of shellfish due to pathogens of faecal origin which may have been present in the marine environment surrounding the shellfish.

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 (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 coliforms are aerobic and facultatively anaerobic, Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas, both at 36°C and at 44.5°C in less that 24 hours. At 44.5°C they produce indole in tryptone water containing tryptophan.

4. PRINCIPLES

After the shellfish have been washed and brushed in the laboratory, their soft tissue is separated under sterile conditions from the shell and transferred to a sterilized blender flask where the soft tissue is macerated and diluted by nine times its weight with phosphate buffer (or 0.1% peptone water). In this way a solution is obtained which contains 1 g mussel flesh per 10 ml of homogenate.

From this homogenate a first multiple test tube dilution series containing lactose broth is set up and incubated at 36 ± 1°C (presumptive test).

After 24 hours, one drop of all positive test tubes is transferred into a second multiple test tube dilution series containing MacConkey broth (or brilliant green broth) and incubated at 44.5 ± 0.2°C (first confirmed test).

At the same time, one drop of all positive test tubes is transferred into a third multiple test tube series containing tryptone water and incubated also at 44.5 ± 0.2°C (second confirmed test).

The frequency of positive reactions in the test tubes is used for the calculation of the most probable number (MPN) of faecal coliforms in the analytical test sample.

5. APPARATUS AND GLASSWARE

5.1 Thermoisolated plastic boxes (camping equipment) with cooling pads or similar cooling units for transport and keeping live mussel specimens.

5.2 Water incubator for 36 ± 1°C and for 44.5 ± 0.2°C.

5.3 Autoclave, max 2 atm, electric or gas.

5.4 Drying oven for sterilization of glassware and equipment at 160°C.

5.5 pH meter, precision ± 0.1 pH units.

5.6 Stainless steel forceps.

5.7 Balance for media preparation, precision ± 10 mg.

5.8 Refrigerator, 4 ± 2°C.

5.9 Vibrator for mixing liquids in culture tubes.

5.10 Ehrlenmeyer flasks of borosilicate glass for media preparation, capacity 500 and 1000 ml.

5.11 Borosilicate glass bacteriological culture tubes with autoclavable racks.

5.12 Small borosilicate glass tubes ("Durham vials").

5.13 Total volume (blow-out) borosilicate glass pipettes of 1, 5, 9 and 10 ml capacity for transfer of culture media in test tubes, with stainless steel containers for sterilization.

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

5.14 Graduated borosilicate glass cylinders of 100, 500 and 1,000 ml capacity with glass beakers for cover.

5.15 Stainless steel homogenizer or blender with several blender vessels, sterilizable in a drying oven (5.4) or autoclave (5.3).

5.16 Brush for cleaning shellfish shells.

5.17 Surgeon's scalpels or similar knives for opening mussels.

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 Lactose broth

	strength	
	single	double
Beef extract	3.0 g	6.0 g
Peptone	5.0 g	10.0 g
Lactose	5.0 g	10.0 g
Distilled water (6.7)	1.0 litre	1.0 litre

Preparation: Dissolve ingredients in the distilled water (6.7). pH should be between 6.8 and 7.0, but preferably 6.9 after sterilization (5.7).

Place in an autoclavable test tube rack 3 rows (more in case the expected MPN of faecal coliforms is high) of 5 clean culture tubes (5.11, 9.1) each. Then add inverted vials (5.12) to all culture tubes 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. Into the first row of these culture tubes transfer double strength broth (6.1). Into the second and third rows (and if necessary into successive rows) transfer single strength broth and close the tubes with cotton plugs. Autoclave (5.3) the closed culture tubes at 121°C for 15 minutes. Check if the pH is between 6.8 and 7.0. If not, adjust the pH of the remaining broth and prepare a new test tube series.

NOTE: Double strength broth is only necessary in the first row where 10 ml of test solution is added to the culture tubes to contract dilution. If higher than 10 ml inocula of the seawater sample are used the lactose broth has to be prepared in higher than double strength in order to avoid that the lactose broth is diluted below single strength after the addition of the inoculum.

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 (6.7)	1.0 litre

Preparation: Dissolve ingredients by shaking and adjust pH to 7.1 ± 0.1 with diluted HCl or NaOH. Add 2 ml bromo-cresol purple solution (6.2.2) to the MacConkey broth.

Place in an autoclavable test tube rack 3 rows (more in case the expected MPN of faecal coliforms is high) of 5 clean culture tubes (5.11, 9.1) each. Then add inverted vials (5.12) to all culture tubes and close the tubes with cotton plugs. Autoclave (5.3) the closed culture tubes at 121°C for 15 minutes. Check if the pH is between 7.0 and 7.4. If not, adjust the pH of the remaining broth and prepare a new test tube series.

6.2.2 Bromo-cresol purple solution

Preparation: Dissolve 1 g of bromo-cresol purple in 99 ml of 95% ethanol (6.9).

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 (6.7)	1.0 litre

Preparation: Dissolve the chemicals in one litre of distilled water (6.7). Then add inverted vials (5.12) to all the culture tubes and dispense sufficient medium into the culture tubes so that the inverted vials are at least partially covered after the entrapped air in the vials has been driven out during autoclaving. Close the tubes with cotton plugs. Sterilize by autoclaving 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 ± 0.2. Test the samples of the finished product for performance using control stock cultures (6.10).

6.4 Tryptone water

Tryptose	10.0 g
NaCl	5.0 g
Distilled water (6.7)	1.0 litre

Preparation: Dissolve the ingredients in distilled water (6.7).

Dispense into each of the 5 test tubes in the 3 rows (more in case the expected MPN of faecal coliforms is high) of the dilution series 10 ml of tryptone water. Autoclave (5.3) at 121°C for 15 minutes. The final pH should be between 7.2 and 7.4. If necessary, adjust the pH before sterilization.

6.5 Dilution solutions

6.5.1 Phosphate buffer (pH 7.2) for dilutions

K_2HPO_4	3.0 g
KH_2PO_4	1.0 g
Distilled water (6.7)	1.0 litre

Preparation: Dissolve components and dispense 9 ml in test tubes used for dilutions in the dilution series (9.3) and autoclave (5.3) at 121°C for 15 min.

6.5.2 Peptone water for dilutions

Preparation: Dissolve a sufficient amount of peptone to obtain a 0.1% solution of peptone in distilled water (6.7). Dispense 9 ml in test tubes used for dilution series (9.3) and autoclave (5.3) at 131°C for 15 minutes.

6.6 Kovac's indole reagent

Paradimethyl amino-benzaldehyde	5.0 g
Amyl alcohol	75.0 ml
Conc. HCl	25.0 ml

Preparation: Dissolve the benzaldehyde in amyl alcohol and add HCl. The reagent should be yellow.

6.7 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 (6.10).

6.8 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 (6.10).

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

6.9 95 per cent ethanol per analysis.

6.10 Stock culture of E. coli.

7. SAMPLING

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

8. PREPARATION OF TEST SAMPLE

Weigh the sterilized blender vessel (9.2.1). Select 10 bivalves at random from the specimen samples collected at each sampling station (7). (For example, a mytilus of 4 cm shell length contains a soft part of about 0.5 g fresh weight (FW), hence 10 mussels of this size should yield about 5 g FW.) Before opening the shells, carefully clean them with a brush (5.16) and alcohol (6.9). Then hold the closed bivalve with sterilized forceps (9.2.3) for a short time over a flame in order to dry the outside of the shells.

Cut the bivalve open with a sterilized knife or scalpel (5.17, 9.2.3) by inserting the knife into the opening from which the byssus extrudes and cut the posterior adductor muscle by turning the knife as indicated in figure 1. Then cut in the other direction and open the mussel with sterilized forceps (9.2.3).

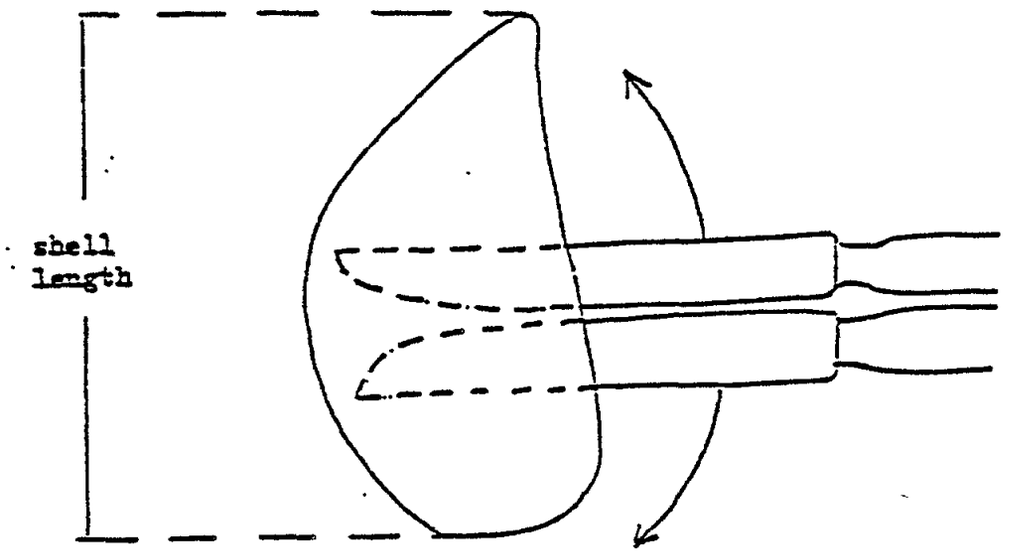

FIGURE 1 : CUTTING THE TWO ADDUCTOR MUSCLES

Do not try to break the mussel open with the knife. If the two muscles are cut the mussel will open easily.

Drain the liquor from the shells, discard it so that it will not be included in the sample and transfer the flesh (soft tissue) into the sterilized blender flask (9.2.1) with sterilized forceps (9.2.3). After transferring the soft parts of all 10 specimens into the blender vessel, weigh the vessel and determine the fresh weight of the flesh sample by subtracting the weight of the vessel plus soft parts from the predetermined weight of the empty blender vessel. Report the weight of the 10 soft parts in table 1, item 5. Then, (in order to arrive at a concentration of 1 g/10 ml) dilute the sample with 9 times its weight using phosphate buffer (6.5.1) or peptone water (6.5.2). (In our example, the sample is now composed of 5 g FW mussel flesh plus 45 ml of dilution solution resulting in a total of 50 ml of flesh solution homogenate).

Homogenize the flesh for 2 minutes. The homogenate now contains 1 g FW sample in 10 ml or 0.1 g FW/ml.

The homogenate represents the test sample (D-1 dilution).

9. TEST PROCEDURE

9.1 Washing of glassware and equipment

All glassware and apparatus (5) should be washed with non-toxic detergent (6.8), first rinsed thoroughly with hot tap water and then rinsed at least three times with distilled water (6.7).

9.2 Sterilization of glassware and equipment

9.2.1 Sterilize the stainless steel blender vessel (5.15) by heating it in a drying oven (5.4) for 3 hours at 160°C or in an autoclave (5.3) for 15 minutes at 121°C.

9.2.2 Place clean pipettes (5.13), complete with a cotton plug in the mouthpiece, into suitable stainless steel containers and sterilize them in a drying oven (5.4) for 3 hours at 160°C.

9.2.3 Sterilize forceps (5.6) and knives or scalpels (5.17) by dipping them into 95% ethanol (6.9) and by flaming them.

9.3 Incubation in lactose broth at 36 ± 1°C for 48 hours (Presumptive test)

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.

Using sterile pipettes (9.2.2) transfer 10 ml of the test sample (8) into five sterilized culture tubes containing double strength lactose broth (6.1). This test tube row contains now 1 g FW/tube (figure 2).

Next transfer with sterile pipettes (9.2.2) 1 ml of the test sample (8) into five sterilized culture tubes containing single strength lactose broth (6.1). This test tube row contains now 0.1 g FW/tube.

For preparing further dilutions transfer with a sterile pipette (9.2.2) 1 ml of test sample (8) (dilution D-1) into a test tube containing 9 ml of phosphate buffer (6.5.1) or peptone water (6.5.2) (dilution D-2). Mix vigorously by hand or with vibrator (5.13). Transfer aseptically (9.2.2) 1 ml of D-2 into each of the five culture tubes containing single strength lactose broth (6.1). This test tube row contains now 0.02 g FW/tube.

If necessary, for further dilutions transfer 1 ml of the D-2 dilution into a test tube containing 9 ml of phosphate buffer (6.5.1) or peptone water (6.5.2) to obtain dilution D-3, etc.

Incubate the series of culture tubes in an air or water incubator (5.2) at 36 $\pm$ 1°C for 48 hours.

After 24 hours, check for gas formation. The gas production is indicated by the broth turning turbid. The observation of gas formation in the Durham vials can be facilitated by slightly tapping on the walls of culture tubes. The appearance of an air bubble must not be confused with actual gas production. If the gas is formed as a result of fermentation, the broth must become cloudy. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial when the fermentation tube is shaken gently. Record the number of tubes showing positive reactions (gas formation after 24 hours of incubation) in table 2, item 7 under (1a).

After 48 hours, check again for gas formation in the same tubes and record the results in table 2, item 7 under (1b).

9.4 Incubation in MacConkey or brilliant green broth at 44.5°C for 48 hours (First confirmed test)

After incubation for 24 hours in lactose broth at 36°C (9.3) a second test tube series (6.2.1) is prepared by transferring with sterile pipettes (9.2.2) one drop from each test tube that provided a positive reading into test tubes situated in identical positions on the tube rack containing MacConkey broth (6.2) or brilliant green broth (6.3).

Incubate this second test tube series at 44.5 $\pm$ 0.2°C in a water incubator (5.2) for 24 hours.

After 24 hours record the number of tubes showing positive reactions in table 2, item 7 under (2a). Coliforms will develop gas which is trapped in the inverted vials (brilliant green broth) and acid which turns the violet-like colour of the original MacConkey broth into a yellowish colour.

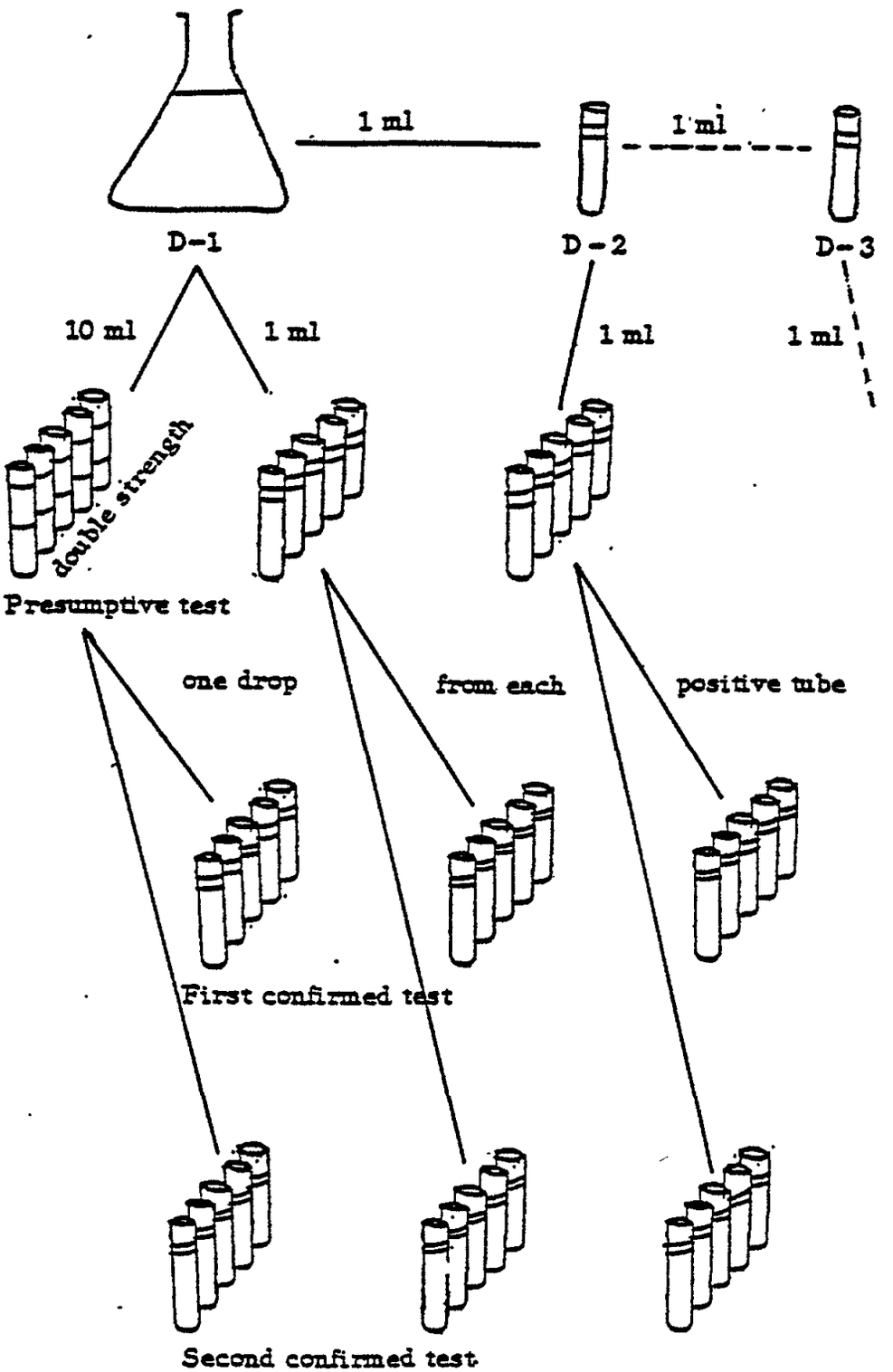

FIGURE 2 : SCHEME FOR THE PREPARATION OF DILUTION SERIES

After incubation for 48 hours in lactose broth at 36°C (9.3) with sterile pipettes (9.2.2) one drop from each previously negative test tube, that after 48 hours provides a positive reading, is transferred into test tubes situated in identical positions on the tube rack containing MacConkey broth (6.2) or brilliant green broth (6.3).

After another 24 hours record the number of tubes showing positive reactions (acid and gas production) in table 2, item 7 under (2b).

9.5 Incubation in tryptone water at 44.5 C for 24 hours (Second confirmed test)

At the same time when the first confirmed test (9.4) is prepared, transfer aseptically (9.2.2) from each test tube which provided a positive reaction after incubation for 24 hours in lactose broth at 36°C (9.4) into the third series of test tubes situated in identical positions of a test tube rack containing tryptone water (6.4).

Incubate this third test tube series at 44.5 $\pm$ 0.2°C in a water incubator (5.2) for 24 hours.

After 24 hours add approximately 1 ml of Kovac's reagent (6.6) into each of the test tubes and shake. Within 10 minutes positive reactions are indicated by a red colour of the amyl alcohol surface layer in the test tubes. Record the results in table 2, item 7 under (2c).

Repeat this procedure (9.5) after in the presumptive test (9.4) after 48 hours new positive tubes have developed. Record the results in table 2, item 7 under (2d).

10. EXPRESSION OF RESULTS

10.1 Calculation of faecal coliforms per gram of shellfish flesh

If the dilutions 1 g, 0.1 g and 0.01 g of flesh per test tube have been used, take the highest number of recorded positive readings from the incubation in MacConkey broth or brilliant green at 44.5°C (9.4) and in peptone water (9.5), i.e. the highest readings from either 2a, 2b, 2c or 2d in table 2, item 7 and find the corresponding most probable number (MPN) from table 1.

When more than three dilutions are employed, the results of only three of these are used in computing the MPN. Select the dilution in which the row of five tubes gives positive readings in all five tubes (no negative readings should exist in lower dilutions) and the two next succeeding higher dilutions from either 2a, 2b, 2c or 2d in table 2, item 7. Determine how many times 10 the highest dilution is smaller than 1 g, find the MPN corresponding to the number of positive tubes in these three dilutions and multiply the MPN found with number of times of 10. Enter this value in the test report (table 2, item 8).

Example:	dilution 1	g:	5 positive tubes	
	dilution 0.1	g:	5 positive tubes	(X)
	dilution 0.01	g:	3 positive tubes	(X)
	dilution 0.001	g:	2 positive tubes	(X)
	dilution 0.0001	g:	1 positive tube	

dilution 10/1 = 10
MPN (532) = 14
(14 x 10)g FW = 140 faecal coliforms/g FW flesh
95% confidence limits: lower (3.7 x 10 = 37)
higher (34 x 10 = 340)

10.2 Precision of results

Select the 95 per cent confidence limits from table 1 and enter them in the test report (table 2, item 8).

Table 1 : 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 -- 5 tubes containing --			MPN Index per	95% Confidence Limits		No. of Tubes Giving Positive Reactions out of -- 5 tubes containing --			MPN Index per	95% Confidence Limits	
1 g	0.1 g	0.01 g	1 g	Lower	Upper	1 g	0.1 g	0.01 g	1 g	Lower	Upper
0	0	0	<0.2			4	2	1	2.6	0.9	7.8
0	0	1	0.2	<0.05	0.7	4	3	0	2.7	0.9	8
0	1	0	0.2	<0.05	0.7	4	3	1	3.3	1.1	9.3
0	2	0	0.4	<0.05	1.1	4	4	0	3.4	1.2	9.3
1	0	0	0.2	<0.05	0.7	5	0	0	2.3	0.7	7
1	0	1	0.4	<0.05	1.1	5	0	1	3.1	1.1	8.9
1	1	0	0.4	<0.05	1.1	5	0	2	4.3	1.5	11
1	1	1	0.6	<0.05	1.5	5	1	0	3.3	1.1	9.3
1	2	0	0.6	<0.05	1.5	5	1	1	4.6	1.6	12
2	0	0	0.5	<0.05	1.3	5	1	2	6.3	2.1	15
2	0	1	0.7	0.1	1.7	5	2	0	4.9	1.7	13
2	1	0	0.7	0.1	1.7	5	2	1	7	2.3	17
2	1	1	0.9	0.2	2.1	5	2	2	9.4	2.8	22
2	2	0	0.9	0.2	2.1	5	3	0	7.9	2.5	19
2	3	0	1.2	0.3	2.8	5	3	1	11	3.1	25
3	0	0	0.8	0.1	1.9	5	3	2	14	3.7	34
3	0	1	1.1	0.2	2.5	5	3	3	18	4.4	50
3	1	0	1.1	0.2	2.5	5	4	0	13	3.5	30
3	1	1	1.4	0.4	3.4	5	4	1	17	4.3	49
3	2	0	1.4	0.4	3.4	5	4	2	22	5.7	70
3	2	1	1.7	0.5	4.6	5	4	3	28	9	85
3	3	0	1.7	0.5	4.6	5	4	4	35	12	100
4	0	0	1.3	0.3	3.1	5	5	0	24	6.8	75
4	0	1	1.7	0.5	4.6	5	5	1	35	12	100
4	1	0	1.7	0.5	4.6	5	5	2	54	18	140
4	1	1	2.1	0.7	6.3	5	5	3	92	30	320
4	1	2	2.6	0.9	7.8	5	5	4	160	64	580
4	2	0	2.2	0.7	6.7	5	5	5	240		

11. TEST REPORT

Fill in the test report (table 2) giving full details in every column.

Table 2: Test Report on faecal coliforms in bivalves

1. Sampling area

   1.1 country: _____ 1.2 area: _____

2. Sampling point (station)

   2.1 type of sampling point (e.g. fish market): _____

   2.2 description of the location of the sampling point: _____

   _____

   2.3 code number: _____ 2.4 longitude: _____

   2.5 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 storage procedure (e.g. + 2°C): _____

   4.3 duration of storage: _____ hours

   4.4 temperature at sampling depth: _____°C

   4.5 salinity at sampling depth: _____‰ S

5. Test sample

   5.1 Number of mussels per sample: _____

   5.2 Weight of all soft parts: _____g FW

6. Incubations

6.1 MacConkey at 36°C

Date and hours of start: _____

Date and hours of end: _____

6.2 MacConkey at 44.5°C

Date and hours of start: _____

Date and hours of end: _____

6.3 Brilliant green at 44.5°C

Date and hours of start: _____

Date and hours of end: _____

6.4 Tryptone water at 44.5°C

Date and hours of start: _____

Date and hours of end: _____

7. MPN in single dilutions

Aliquots transferred	number of positive reactions					
gr	lactose		MacConkey Brilliant		tryptone	
	24 h (1a)	48 h (1b)	24 h (2a)	48 h (2b)	24 h (2c)	48 h (2d)
1						
0.1						
0.01						
0.001						
0.0001						

8. Test results:

MPN after 48 h in MacConkey, brilliant green or tryptone water at 44.5°C

_____ faecal coliform / g shellfish flesh

_____ 95% confidence limits

9. Anomalies observed during the test procedure:

_____

_____

_____

_____

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

_____

_____

_____

_____

11. 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.

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