REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME OCTOBER 1995

Determination of faecal coliforms in bivalves by the multiple test tube (MPN) method

Reference Methods For Marine Pollution Studies No. 5 (Rev.2)

Prepared in co-operation with

WHO

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 (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. 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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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. 5 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. 5 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 coliforms 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.

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 than 24 hours. At 44.5° C they produce indole in tryptone water ncontaining tryptophan.

4. PRINCIPLES

After the shellfish have been washed and brushed in the laboratory, their soft tissue, either with or without their intervalvular fluid, is separated under sterile conditions from the shell and transferred to a sterilized blender where the shellfish sample 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 gram of mussel sample 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 ± o.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 1 and 2 litres.

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 tubes 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 test dilution 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

Tryptone	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 PMN 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% ethanol per Analysis.

6.10 Stock Culture of E. coli.

7. SAMPLING

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

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 grams fresh weigh plus a variable volume of intervalvular fluid, hence 10 mussels of this size should yield about 5 g total weight). 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 valve 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).

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

The microbiological parameters can be determined on either a test sample of shellfish flesh and intervalvular fluid or on a test sample of shellfish flesh only. The option adopted shall be indicated with the results of the analysis.

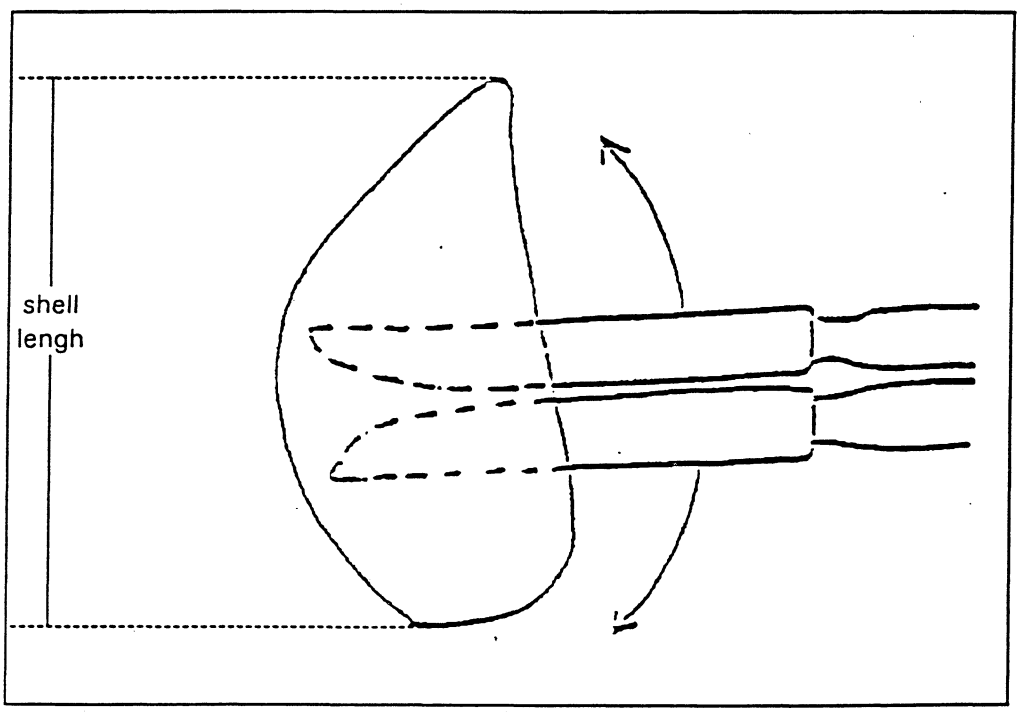

Figure 1. Cutting the two adductor muscles.

8.1 Test Sample of Shellfish Flesh and Intervalvular Fluid

Collect both the shellfish flesh (soft tissue) and their intervalvular fluid into the sterilized blender flask (9.2.1) with sterilized forceps (9.2.3). After transferring the soft parts and the intervalvular fluid of all 10 specimens into the blender vessels, weigh the vessel and determine the weight of the test sample by subtracting the weight of the empty blender vessel from the weight of the vessel plus the soft parts and the intervalvular fluid. Report the weight of the soft parts and the intervalvular fluid obtained from the 10 mussels in table 3, item 5. Then, (in order to arrive at a concentration of 1 g/10 ml) dilute the test sample with 9 times its weight using phosphate buffer (6.5.1) or peptone water (6.5.2). In our example, the test sample is now composed of 5 grams of mussel flesh and intervalvular fluid plus 45 ml of dilution solution, resulting in a total of 50 ml of flesh solution homogenate.

Homogenize the flesh and intervalvular fluid for 2 minutes. The homogenate now contains 1 gram of test sample in 10 ml, or 0.1 g sample/ml.

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

8.2 Test Sample of Shellfish Flesh Only

Drain the intervalvular fluid from the shells, discard it so that it will not be included in the test sample and transfer the flesh (soft tissue) into the sterilized blender flask (9.2.1) with sterilized forceps (9.2.3). After transferring only the soft parts of all 10 specimens into the blender vessels, weigh the vessel and determine the fresh weight of the test sample by subtracting the predetermined weight of the empty blender vessel from the weight of the vessel plus the soft parts. Report the weight of the soft parts obtained from the 10 mussels in table 3, item 5. Then, (in order to arrive at a concentration of 1 g/10 ml) dilute the test sample with 9 times its weight using phosphate buffer (6.5.1) or peptone water (6.5.2). In our example, the test sample is now composed of 5 grams of 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 gram of test sample in 10 ml, or 0.1 g sample/ml.

This 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 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 (5.9). 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 0.01 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 ± 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 3, item 8 under (1a).

After 48 hours, check again for gas formation in the same tubes and record the results in table 3, item 8 under (1b).

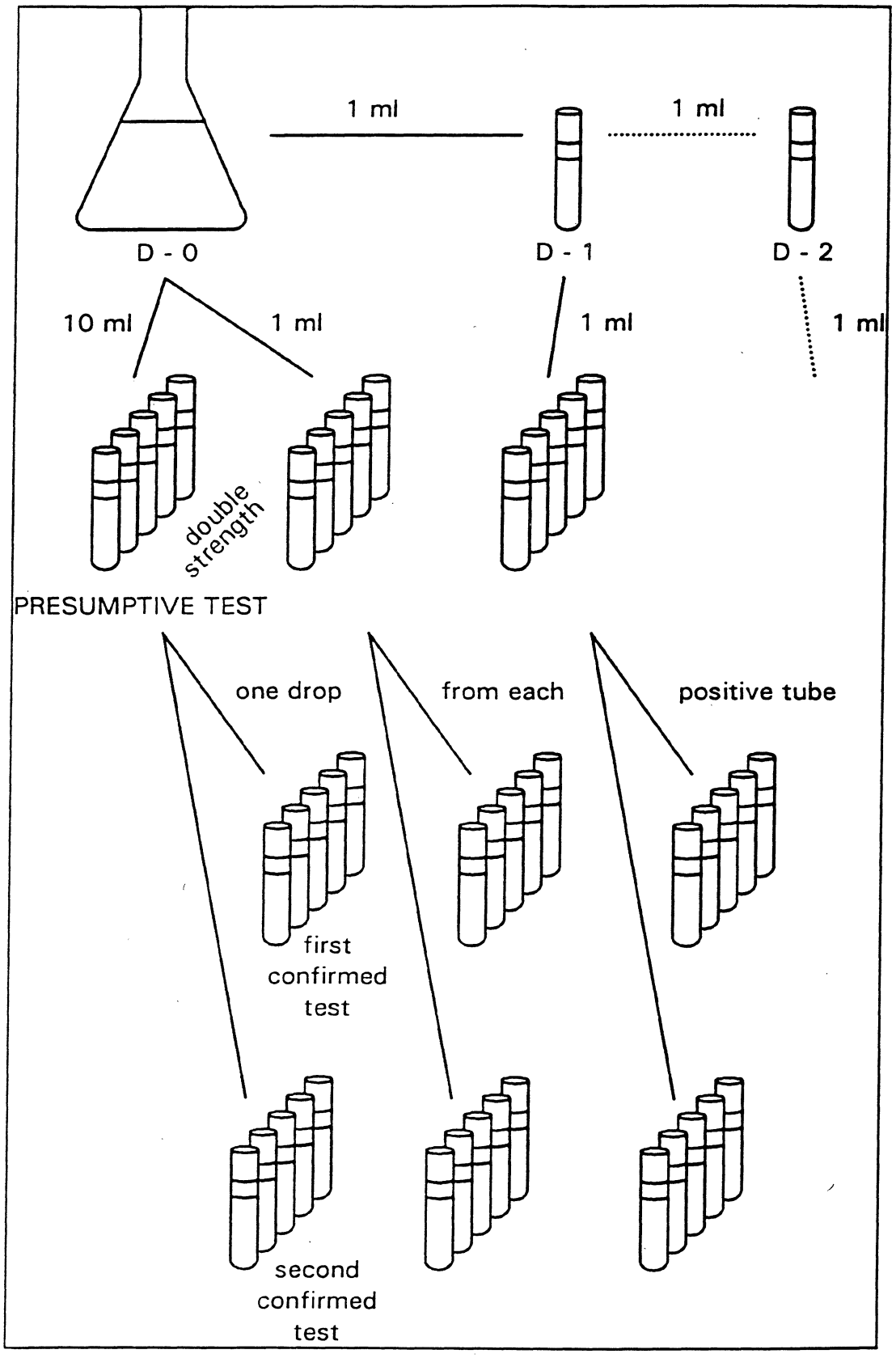

Figure 2. Scheme of preparation of dilution series.

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 ± 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 3, item 8 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.

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 3, item 8 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) a drop from each test tube which provided a positive reaction after incubation for 24 hours in lactose broth at 36° C (9.3) 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 ± 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 3, item 8 under (2c).

Repeat this operation (9.5) with the tubes from the presumptive test (9.3) which have become positive during the 24-48 hours intervals and record the results in table 3, item 8, under (2d).

10. EXPRESSION OF RESULTS

10.1 Calculation of Faecal Coliforms per Gram of Shellfish Sample

If the dilutions 1 g, 0.1 g and 0.01 g of shellfish sample 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 tryptone water (9.5), i.e. the

highest readings from either 2a, 2b, 2c or 2d in table 3, item 8 and find the corresponding most probable number (MPN) from table 2.

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 3, item 8. Determine how many powers of 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 3, item 9).

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 0.1 g of sample. The final combination 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 180 faecal coliforms/g (Table 2), and not the MPN value of 140 faecal coliforms/g associated to the 5-3-2 combination.

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

Sample portion g	Positive tubes
1	5
0.1	5. (X)
0.01	3. (X)
0.001	2. (X)
0.0001	1

Dilution factor: 1/0.1 = 10

MPN (5-3-3) = 18 x 10 = 180 faecal coliforms/g of shellfish sample

95% confidence limits:
lower: 4.4 x 10 = 44 faecal coliforms/g
higher: 50 x 10 = 500 faecal coliforms/g

10.2 Precision of Results

Select the 95 per cent confidence limits 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 5 tubes containing			MPN index per 1 g	95% Confidence limits		No. of tubes giving positive reactions out of 5 tubes containing			MPN index per 1 g	95% Confidence limits	
1 g	0.1 g	0.01 g		Lower	Upper	1 g	0.1 g	0.01 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

Table 3. Faecal coliforms in seawater samples.

1. Sampling area
   country: _____
   area: _____
2. Sampling point
   longitude: _____
   description: _____
   code number: _____
   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. Test sample: number of mussels per sample: _____ Weight of sample: _____ g

6. Time of inoculation hour: ___.___ day: ___/___/___
7. Incubations:

	Start of incubation	End of incubation
Lactose broth at 36° C	hour:___.___ day:___/___/___	hour:___.___ day:___/___/___
MacConkey/BG at 44.5° C	hour:___.___ day:___/___/___	hour:___.___ day:___/___/___
Tryptone water at 44.5° C	hour:___.___ day:___/___/___	hour:___.___ day:___/___/___

8. Number of positive reactions:

Portions transferred in g	Lactose		MacConkey or Brilliant Green		Tryptone water	
	24h (1a)	48h (1b)	24h (2a)	48h (2b)	24h (2c)	48h (2d)
10	____	____	____	____	_____	
1	____	____	____	____	_____	
0.1	____	____	____	____	_____	
0.01	____	____	____	____	_____	
0.001	____	____	____	____	_____	
0.0001	____	____	____	____	_____	

9. Test results:
   After 48h in MacConkey, BG or tryptone water: _____ F. col./g
   95% confidence limits: ____;____ F. col./g
   Option adopted:
   Flesh only: _____
   Flesh and fluid: _____

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: _____

12. REFERENCES

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

UNEP/WHO (1988) Guidelines for monitoring the quality of coastal recreational and shellfish-growing waters. Reference Methods for Marine Pollution Studies No. 1, UNEP, Geneva.

WHO/UNEP (1983) Methods for monitoring selected pollutants in sewage effluents and coastal recreational water: Report on a WHO/UNEP joint meeting. Rome 24-26 November 1982. Document ICP/RCE 211(2), WHO regional office for Europe, Copenhagen.

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