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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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 ± 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 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