REGIONAL SEAS

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

Reference Methods For Marine Pollution Studies No. 22 (Rev.1)

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. 22 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. 22 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 coastal bathing waters of temperature and tropical seas and is designed to be used in sanitary surveillance of bathing beaches.

It is based on the Multiple-Tube Fermentation (MPN) Test and can be employed as an alternative to the Membrane Filtration Culture Method. 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 labour intensive and due to the preconcentration of the bacteria in the sample it is more suitable in situation 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 MF's after incubation.

Faecal coliforms exhibit a highly specific positive correlation with faecal contamination from warm-blooded animals and, therefore, are good indicators for the sanitary quality of coastal waters. Since faecal coliforms die within hours when exposed to sunlight in seawater at temperatures above + 4° C, their presence in seawater indicates only recent contamination by faecal material. Die-away rates (T-90) depend on salinity, temperature, solar radiation, etc. and must be taken into consideration when interpreting the results.

3. DEFINITION

Faecal coliforms are aerobic and facultatively anaerobic, Gram-negative, non-sporeforming rods that ferment lactose while producing acid and gas at 35° C and 44.5° C, in less than 24 hours. They produce indole in tryptone water containing tryptophane at 44.5° C

4. PRINCIPLES

Quantitation of faecal coliforms in seawater samples is accomplished by testing multiple sample portions in a lactose based medium for gas production at 44.5° C. There are two methods: (a) a direct test using A-1 broth with incubation at 44.5° C for 24 hours or (b) the supplemental confirmation of positive lactose tubes in the total coliform procedure.

Five sample portions for each multiple tube dilution (10 ml, 1 ml, 0.1 ml, etc.) are inoculated into individual tubes of A-1 broth (direct test) or tubes of lactose broth (total coliform procedure). The A-1 cultures are inoculated at 44.5 ± 0.2° C for 24 hours, the lactose cultures at 35.0 ± 0.5° C for 48 hours. The direct test (A-1 broth) is read for gas production after 24 hours incubation and faecal coliform density is determined from the combination of positive-negative tubes interpreted in the MPN table (table 1).

When the total coliform procedure is used to obtain a faecal coliform determination, confirm all positive lactose cultures in 24 and 48 hours by transferring a loop-full of culture from each positive tube to either MacConkey or EC broth. Brilliant green broth may be used, however, there may be some loss in detection of stressed faecal coliforms.

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

The frequency of dual confirmed positive reactions (lactose fermentation and positive indole) in the different dilutions 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 Sample bottles of borosilicate glass or autoclavable plastic bottles with screw-cap tops, 200-250 ml capacity, wide-mouthed and with ground-glass stoppers are used to collect marine (seawater) samples.

5.2 Sample rod of non-corrosive material with a clamp to hold the sampling bottle (figure 1).

5.3 Subsurface sampler of the type shown in figure 2, or similar, complete with plastic rope and weight.

5.4 Thermo-insulated plastic container with prefrozen packs of chemical gel (camping equipment) for storage of samples.

5.5 Thermometer, 0 to 50° C, precision ± 1° C, to be used for checking temperature in sample collection containers (5.4). Thermometer, 0° to 50° C, precision ± 0.5° C, to be used for checking temperature in water bath incubator.

5.6 Water incubator for 44.5 ± 0.2° C and air incubator for 35 ± 0.5° C with suitable test tube racks for incubating multiple tube tests (5.15).

5.7 Autoclave, maximum 2 atm., electric or gas.

5.8 Drying oven for sterilization of glass sample bottles and pipettes at 160° C for 2 hours.

5.9 pH meter, precision ± 0.1 pH units.

5.10 Balance for media preparation, precision ± 1 mg.

5.11 Refrigerator with temperature range of 2-8° C for storage of samples to be processed within 24 hours of collection.

5.12 Vibrator (shaker) for mixing sample aliquots in serial dilution (optional).

5.13 Erlenmeyer flasks of borosilicate glass for media preparation, capacity 1 and 2 litres.

5.14 Borosilicate glass bacteriological culture tubes with sufficient capacity to receive 20 ml of medium.

5.15 Small borosilicate glass tubes 6 x 50 mm ("Durham vials") to be inserted in culture tubes (5.15) for detection of gas formation in the multiple test tube.

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

Note: 9 ml capacity pipettes are optional.

5.17 Graduated borosilicate glass cylinders of 100, 500 and 1000 ml capacity with glass beakers, aluminium foil or wrapping paper for cover.