



REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

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Determination of faecal coliforms in sediments by the pour plate (PP) method

Reference Methods For Marine Pollution Studies No. 47 (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:

WHO/EURO Project Office
Coordinating Unit for the Mediterranean Action Plan
48 Vassileos Konstantinou
P.O. Box 18019
GR-11610 Athens
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. IJLUM: | 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. 47 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.

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1. INTRODUCTION

This recommended method has been 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). It is also being issued separately within UNEP's Regional Seas Programme Activity Centre's Reference Methods for Marine Pollution Studies 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 sediments of coastal bathing waters of temperate and tropical seas and is designed to be used in sanitary surveillance of bathing beaches and shellfish growing areas.

This method allows colonies determination, as the Membrane Filtration Culture Method, but the sample volume cannot be larger than 1 ml because the culture medium must be able to absorb the volume of the material inoculated and solidify without difficulty.

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 and marine sediments. Since faecal coliforms die within hours when exposed to sunlight in sea water, their presence in the sea indicates only recent contamination by faecal material. Die-away rates (T-90) depend, among other parameter, on salinity, temperature and solar radiation, factors that 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 gas in less than 24 hours at 44.5 °C. They produce indole in peptone water containing tryptophane at 44.5 °C. Under the culture conditions described in this reference method, faecal coliforms appear as blue colonies.

4. PRINCIPLES

From sediment samples taken in sterile conditions, a dilution series is set up made according to the quantity of faecal coliforms expected in the sediment sample. A 1-ml aliquot volume of this dilution series are transferred to empty Petri dishes (5.16). Then m-FC agar (6.1) is poured in each Petri dish. Once the agar has solidified, the Petri dishes are incubated at 44.5 ± 0.2 °C for 24 hours. Lactose fermentation will turn faecal coliform colonies into their characteristic blue colour.

Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test using the MacConkey broth or the Brilliant Green broth. In urban bathing areas where sewer outlets discharge rain water as well as wastewater, the confirmative test for indole production is necessary.

5. APPARATUS AND GLASSWARE

- 5.1 Sterile plastic bags (maximum capacity 1 litre).
- 5.2 Core sampling cylinders, metallic or borosilicate glass, of approximately 2-cm diameter and 15-cm length.
- 5.3 Borosilicate glass bottles, 250-ml capacity, to store prepared m-FC agar. These bottles must be sterilized before receiving the agar.
- 5.4 Thermo-insulated plastic boxes (camping equipment), with prefrozen cooling packs or similar cooling units, for transport and storage of sediment samples.
- 5.5 Thermometer, 0 to 50 °C, precision ± 1 °C, preferable of unbreakable plastic type, to be used for checking the temperature in plastic boxes.
- 5.6 Stainless steel homogenizer or blender with several blender vessels; sterilizable in a drying oven (5.10) or in an autoclave (5.9).
- 5.7 Water incubator for 44.5 ± 0.2 °C.
- 5.8 Stereoscopic microscope, magnification of 10 - 50 X, and/or darkfield colony counter.
- 5.9 Autoclave, maximum 2 atmospheres, electric or gas.
- 5.10 Drying oven for sterilization at 160 °C
- 5.11 pH meter, precision $\pm 0,1$ pH units.
- 5.12 Sterile Pasteur pipettes, completed with cotton plugs.
- 5.13 Analytical balance, precision ± 1 mg.
- 5.14 Refrigerator, 4 ± 0.5 °C.

- 5.15 Electric vibrator (shaker) for mixing liquids in dilution tubes.
- 5.16 Borosilicate glass Petri dishes, diameter 8 to 9 cm, with stainless steel containers for sterilization, or plastic disposable pre-sterilized Petri dishes.
- 5.17 Erlenmeyer flasks of borosilicate glass for media preparation (capacity 1 and 2 litres), or media conservation (250 ml).
- 5.18 Borosilicate glass bacteriological culture tubes.
- 5.19 Total volume (blow-out) borosilicate glass pipettes of 1, 10 and 20 ml capacity, with stainless glass 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 spout.
- 5.21 Small borosilicate glass vials ("Durham vials"), 6 x 50 mm, to be inserted in some culture tubes.
- 5.22 Bacteriological transfer loops, made from 22-24 Chromel gauge, nichrome or platinum-iridium. Diameter of the loop: 3 mm.
- 5.23 Heavy wrapping paper.
- 5.24 Aluminum foil (household quality).
- 5.25 Autoclavable wide-mouthed plastic bottles, 200 to 300 ml capacity.
- 5.26 Watertight metal boxes for incubating Petri dishes in a water bath (5.7).
- 5.27 Filter paper.
- 5.28 Water incubator for $44.5 \pm 0.2^\circ\text{C}$.

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