Long-term Programme for Pollution Monitoring and Research
in the Mediterranean Sea
(MED POL Phase II)

MICROBIOLOGICAL METHODS FOR COASTAL WATER QUALITY MONITORING

Second report on a joint WHO/UNEP meeting

Athens
25-29 June 1984

WORLD HEALTH ORGANIZATION
Regional Office for Europe
Copenhagen, 1984

Original: English

ICP/CEH 001/m01
4862I

Note

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, quoted or translated without the agreement of the World Health Organization Regional Office for Europe. Authors alone are responsible for views expressed in signed articles.

CONTENTS

PREFACE

Within the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II), national monitoring programmes in most Mediterranean countries have either become operational or are in the process of formalization. These programmes include regular microbiological and related monitoring of coastal recreational and shellfish-growing areas.

In order to promote standardization of methodology, four reference methods, covering the major microbiological indicator organisms for the monitoring of coastal recreational waters, shellfish-growing waters and shellfish flesh were developed by the United Nations Environment Programme (UNEP) and WHO. These methods form part of a comprehensive series being prepared by UNEP's Regional Seas Programme Activity Centre (RS/PAC) in collaboration with the relevant United Nations specialized agencies. They are designed not only to cover all the monitoring parameters approved by the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols when formally adopting the MED POL Phase II programme, but also to enable their utilization, to the extent possible, in regions other than the Mediterranean.

The four microbiological reference methods developed jointly by UNEP and WHO were as follows:

- determination of total coliforms in seawater by the membrane filtration culture method;
- determination of faecal coliforms in seawater by the membrane filtration culture method;
- determination of faecal streptococci in seawater by the membrane filtration culture method;
- determination of faecal coliforms in bivalves by the multiple test tube method.

All four methods were developed and tested during the first half of 1982 and were reviewed at a WHO/UNEP joint Consultation Meeting on Methods for Monitoring selected Pollutants in Sewage Effluents and Coastal Recreational Waters, held in Rome from 24 to 26 November 1982. Following such review, the methods were issued in their final form in 1983. During the same year, work was started by WHO and UNEP on the development of reference methods for the first three parameters listed above, using the multiple test tube method. These methods are expected to be ready for review and testing in late 1984.

The Rome consultation meeting was combined with a preliminary intercalibration exercise, held at the same venue from 22 to 26 November 1982, at which participants from selected Mediterranean laboratories performed determinations of the three microbiological parameters (TC, FC and FS) and a series of intercalibration exercises in a number of Mediterranean countries was planned, with the intention of involving scientists from the home country engaged in the MED POL programme and also a number of scientists from other Mediterranean countries similarly engaged. This was designed to improve comparability of results and quality control at both country and intercountry levels.

The first exercise in the series was held in Barcelona from 7 to 11 November 1983, and was preceded by an interlaboratory testing exercise, using the same reference methods, in Catalonia in the summer of 1983. The present exercise, the second in the series, was organized by WHO and UNEP in collaboration with the Environmental Pollution Control Project (Ministry of Physical Planning, Housing and the Environment), Athens, from 25 to 29 June 1984, and was similarly preceded by a national exercise held at the same institution in March 1984.

The main objectives of the present exercise were to enable participants to conduct actual laboratory determinations of bacterial parameters using the same samples of seawater, utilizing the reference methods listed above, and, for comparison purposes, conducting parallel tests using the multiple test tube method.

The main objectives of the consultation meeting, held in conjunction with the exercise, were the following:

- to review the results of the laboratory exercise, as well as of previous ones;
- to review the results of the national exercise of March 1984;
- to review the revised draft guidelines for monitoring the quality of coastal recreational and shellfish-growing areas, jointly developed by WHO and UNEP as part of the Reference Methods series;

- to make recommendations on future intercalibration exercises, as well as on any other related matter being conducted or planned within the framework of MED POL Phase II.

Representatives of (a) Greek institutions participating in the relevant aspects of the MED POL Phase II monitoring programme, (b) institutions in Cyprus, Egypt, Israel, Malta, Turkey and Yugoslavia and (c) institutions participating in the West African Regional Action Plan, were invited to attend the exercise and consultation meeting. In addition, the following international organizations were invited to send representatives: Food and Agricultural Organization of the United Nations (FAO), Intergovernmental Oceanographic Commission (IOC), United Nations Development Programme (UNDP), United Nations Educational, Scientific and Cultural Organization (UNESCO), World Meteorological Organization (WMO) and International Atomic Energy Agency (IAEA).

1. Opening of the meeting (agenda item 1)

The meeting took place at the Environmental Pollution Control Project, Athens, from 25 to 29 June 1984. It was attended by 26 temporary advisers from Greece and five other Mediterranean countries. There was one representative of UNEP and one from WHO/EURO. A list of participants is given in Annex 3.

Dr L.J. Saliba, Senior Scientist, Mediterranean Action Plan, World Health Organization Regional Office for Europe, opened the meeting on behalf of the Regional Director, Dr Leo A. Kaprio. He mentioned the deep involvement of WHO in the Mediterranean Action Plan, within the framework of which this activity was being organized, and stressed the importance of ensuring a high quality for recreational and shellfish-growing areas in the Mediterranean, which required continuous monitoring and comparability of individual results to enable the drawing-up of an accurate overall picture of the situation. He also expressed WHO's appreciation of the work undertaken and the facilities provided by the Environmental Pollution Control Project both for the laboratory exercise and the consultation meeting. He mentioned the appropriateness of the present venue, as the host institution had been deeply involved both in the health-related aspects of MED POL Phase I and in a comprehensive WHO-executed project.

Mr A. Manos, Coordinator of the Mediterranean Action Plan, United Nations Environment Programme, addressed participants on behalf of the Executive Director of UNEP, Dr Mostafa K. Tolba. He recalled the commitment of the coastal states to protect and enhance the Mediterranean Sea area and their obligation under the 1976 Barcelona convention to endeavour to establish a pollution monitoring system for this area. UNEP, working in close cooperation with the relevant specialized agencies of the United Nations system, had developed a strong scientific component (MED POL) which aimed at identifying the sources and effects of major pollutants. In so doing, the scientific tools and methods had to be perfected and harmonized, in order to provide governments and the public with reliable and comparative information on the state of pollution of the Mediterranean. This intercalibration exercise being conducted in Athens and the others in the same series, were essential steps towards that important goal.

Dr G. Kardassis, Director of Environment in the Greek Ministry of Physical Planning, Housing and the Environment, welcomed participants on behalf of the Minister, Mr A. Tritsis, who augured a fruitful and successful activity. He stated that Greece, as a country with an outstanding historic role for the development of civilization in the Mediterranean basin, considered all activities focusing on the protection of the environment of this basin as very important. Monitoring of coastal water quality was vital for ensuring suitability of such waters for bathing and other activities. A knowledge of the limits of natural resources was a prerequisite to their exploitation linked with environmental protection, and actions and measures had to be based on a full and correct picture of the ecosystem involved. This activity would contribute greatly to the attainment of such a picture with regard to Mediterranean coastal waters.

Dr G. Manes, Public Health Officer, addressed participants on behalf of the Greek Ministry of Health and Welfare, and particularly on behalf of Dr T. Stephanou, Director of Public Health. He referred to marine polluting agents mostly from the medical and epidemiological viewpoints and stressed the increasing threat to public health through exposure of bathers to polluted seawater and through consumption by the public of shellfish harvested from contaminated areas. He similarly stressed the importance of sewage receiving secondary treatment and chlorination, thus ensuring a substantial reduction of pathogenic bacteria and viruses. He expressed the importance of all relevant national authorities involved in various ways acting in full coordination and cooperation under uniform legislation. An ideal situation would be common international legislation to be adopted by all the countries in the region. The Greek Ministry of Health and Welfare augured a complete success to the meeting.

Dr A. Mourmouris, Greek National Coordinator for MED POL, addressed participants and expressed the satisfaction and pleasure of the Ministry of Physical Planning, Housing and the Environment at hosting the present exercise and in continuing its collaboration with WHO and UNEP. This exercise was considered as a good opportunity for exchange of experience and scientific cooperation on concrete environmental matters of general interest, as well as being indispensable for ensuring comparable results. She stated that Greece had amply proved its active interest in MED POL by its contribution to both the first and second phases. In the Greek national intercalibration exercise, convened in Athens from 12 to 16 March 1984, both the MF and MPN methods were used. In the current exercise, apart from the three MF methods recommended so far by the Contracting Parties, the host institution had also provided for parallel determinations using the MPN technique, for which the relevant reference methods had not yet been finalized by WHO/UNEP. This was done as several Mediterranean institutions had been using the latter for several years and the Contracting Parties' current recommendation did not exclude alternative methods, provided these gave comparable results. The present exercise would therefore assist in establishing comparability between methods as well as between individual results.

2. Scope and purpose of the meeting (agenda item 2)

Dr L.J. Saliba explained the scope and purpose of the exercise and consultation meeting. He described the situation emerging from the Rome and Barcelona intercalibration exercises where there was a considerable discrepancy between individual results. He stressed the fact that participants should observe the prescribed methodology during the laboratory sessions to ensure as complete a harmonization as possible from the technical point of view. In spite of all efforts in this direction in previous exercises, the human element had been found important, and there was a need to analyse the reasons for and the means of correcting such variation. In explaining the general organization of the overall activity, including the relationship between the laboratory and meeting sessions, he stated that experience so far had shown that contact between scientists from different laboratories was considerably enhanced when they actually worked together, as distinct from meeting discussions.

The results of the present exercise would have to be very carefully perused. In addition, the two main items for discussion were the results of the Greek national intercalibration exercise of March 1984 and Reference Method No. 1 (Guidelines for monitoring the quality of coastal recreational and shellfish-growing areas) which had been extensively revised since its initial draft presentation in November 1982. Apart from its review by the present meeting, this document was also being submitted to a number of Mediterranean institutions for comments. The revisions arising out of the present meeting would not, therefore, be considered as final.

3. Election of officers (agenda item 3)

Ms S. Sotiracopoulos was elected Chairman, Dr Y. Yoshpe-Purer Vice-Chairman, and Dr N. Krstulovic Rapporteur. Dr L.J. Saliba acted as Secretary to the meeting.

4. Adoption of the agenda (agenda item 4)

The provisional agenda was unanimously adopted.

5. Review of previous intercalibration exercises (Rome, 22-26 November 1982, Barcelona, 7-11 November 1983) and of the national intercalibration held in Greece, (12-16 March 1984) (agenda items 5 and 6)

By unanimous agreement, these two items were considered together.

Dr L.J. Saliba briefly outlined the proceedings of the intercalibration exercises held in Rome (22-26 November 1982) and Barcelona (7-11 November 1983). The former was only a preparatory exercise involving 12 participants, and was organized mainly for the acquisition of experience. In the Barcelona exercise, the first in the current series, 28 participants attended (including 22 from Spain). In spite of the fact that the methodology was identical, there was a considerable discrepancy between individual results. This was partly attributed to the heavy floods which occurred at the time samples were collected. However, it also served to underline the importance of the human element.

Ms S. Sotiracopoulos briefly presented the results of the Greek national intercalibration exercise held in Athens from 12-16 March 1984 (document ICP/CEH 001 m01/7). Representatives of six institutions participated and a comparison of the MF and MPN methods was also made. Samples included (a) high quality seawater, (b) moderately polluted seawater from an area with intermittent pollution, (c) seawater from a polluted area and (d) shellfish from a restricted area. As a result

of the exercise, it was concluded that although the MF and MPN methods for enumeration of TC, FC and FS did not always yield exactly the same results, it could be reasonably assumed that both methods were adequate for establishing the quality of seawater, and should therefore both be recommended for use in the MED POL programme, taking their limitations into account. The summary report of the exercise is attached as Annex 1.

6. Review of draft guidelines on monitoring of coastal recreational and shellfish-growing areas (agenda item 7)

Dr L.J. Saliba briefly introduced document ICP/CEH 001 m01/6. The current version was mainly the result of comments and suggestions made during the consultation meeting held in Rome in November 1982.

The meeting had no comments on the overall lay-out of the document. A number of comments and suggestions were, however, made on specific points.

Definitions: The meeting noted that the requirements for potential bacterial indicators were those previously recommended in the report of the group of experts jointly convened by WHO and UNEP on Health Criteria and Epidemiological Studies related to Coastal Water Pollution (Athens, 1-4 March 1977). It was felt, however, that these should be updated, preferably after reference to a current recognized text. In particular, the meeting considered that potential indicators should:

a) be consistently and exclusively associated with the source of pathogens, and occasionally noxious substances;

b) be present in sufficient numbers without proliferation to provide a reasonable estimation that pathogens could be present and that a health risk may exist.

It was also agreed that in the definition of a water quality protection standard, the main factor to be taken into account was the medical one when determining acceptability, and the definition should therefore be modified accordingly.

Area and problem identification: the meeting agreed that in both fact sheets and maps providing descriptions of monitoring areas and sampling stations, only relevant items should be compulsory.

Sampling (general): it was considered by a number of participants that examination of many samples with a few important parameters was preferable to examination of less samples with more parameters. The need for systematic sampling was emphasized, and the meeting agreed that the actual sampling period (i.e. time of day) in the same station should not vary by more than 2-3 hours. The actual optimum sampling time depended on a number of factors. Two approaches were possible in the determination of the best time: (a) coincidence with peak use and (b) correlation with the effects of intermittent-flow sources, such as sewage outlets. Selection of sampling time would depend on what particular approach was adopted in the light of information required. It was agreed that the standardization of all sampling stations would not be possible in practice, though efforts should be made to achieve the optimum. The importance of sampling personnel having the fullest possible liaison and coordination with the laboratory responsible for analytical determinations (especially where different organizations were involved) was stressed by the meeting.

Sampling point locations: the meeting considered that the exact locations of sampling points, including the distance between them, could not be specified from the overall viewpoint, as they would vary with the individual beach, the particular requirements of each of which would have to be determined by the local authorities. Regarding location relative to the shoreline, the criterion should be depth, rather than distance from the shore. The critical part was that immediately near the shoreline, and those areas where the water was 20-30 cm deep should be monitored.

Sampling frequency: it was considered that during bathing seasons, heavily populated beaches should be monitored at least once a week. It was noted that there was no generally accepted definition as to what in quantitative terms constituted a crowded or heavily populated beach. This would have to be determined by local authorities in each individual case.

Minimum monitoring: compulsory oceanographic and meteorological parameters should be limited to those absolutely relevant. In this regard, wind and current direction during sampling times were considered as particularly important and should be recorded. In view of the fact that contact infection could be even more important than intestinal infection through ingestion, it was considered that one pathogen causing infection through contact (selection depending on local circumstances) should be monitored if possible.

Beach surveillance: the meeting considered that most monitoring organizations did not possess the facilities in terms of manpower to conduct the full range of observations listed in the Garber classification, and that in any monitoring programme, only the most important should be compulsory.

Extended monitoring: it was recommended that sampling should be extended to microbial examination of (a) sand on beaches, and (b) sediments. In the latter case, a strict definition should be provided. Sediments could be considered as the sea-bottom material immediately beneath the sampling area, and a suitable sampler capable of collecting the upper layer of sediments should be recommended. In sampling of recreational beaches, double sampling should be practised, with the selection of two particular depths, one frequented mostly by adults and the other by young children and elderly people (who constitute the groups most susceptible to infection).

Most participants agreed that the list of pathogens in this section of the document should generally remain. However, it was suggested that:

- Aeromonas hydrophila should also be included as a parameter;
- fungi were very important as causative agents of contact diseases, and special attention should therefore be accorded to pathogenic species;
- consideration should be given to the inclusion of Campylobacter, on which, however, much basic study was still required;
- there was no justification for retaining Shigella in the list of parameters even for extended monitoring.

The meeting took note of the mandatory and optional parameters adopted by Mediterranean Governments for the monitoring component of MED POL Phase II, and recognized that the actual pathogens and indicators to be determined would vary from country to country, as would the order of priority for each. The following observations were also made:

- Vibrio parahaemolyticus was invariably encountered in areas with no sewage pollution and no correlation therefore existed. However, a number of participants considered it should remain;
- Clostridium perfringens could provide an indication of viruses. It could remain, but with reservations as to its usefulness, as (a) it is not really pathogenic, and (b) its detection in seawater is difficult;
- in considering parasites, Giardia lamblia was at least as important as Entamoeba;
- a number of participants considered that high priority should be accorded to Salmonellas and even in minimum monitoring programmes, if any sample showed more than 500 faecal coliforms per 100 ml, tests for the possible presence of Salmonellas should be performed. Similarly, Vibrios were considered by some participants as close to Salmonellas in overall importance. It was agreed, however, that no general priority ranking could be made;
- a differentiation could possibly be made between (a) pathogens of faecal origin, (b) pathogens of non-faecal origin, and (c) parasites. In this regard, it was recognized that some pathogens would fall between the first two categories;
- the two most important pathogens to determine in bivalves, as distinct from seawater, were Vibrio parahaemolyticus and Salmonellas.

The meeting did not discuss the section on statistical analysis. It was agreed that any comments on this part of the document could be made when the draft was circulated to Mediterranean institutions.

7. Review of results of intercalibration exercise (agenda item 8)

The meeting discussed the results of the intercalibration exercise, and agreed that this could be considered as a success. All results were of very good quality and there was a very significant correlation between the results of individual participants, as well as between these and parallel determinations carried out by the coordinators of the exercise. In addition, there was an equally high correlation between results obtained for both faecal coliforms and faecal streptococci by the MF and MPN methods, such correlation being obtained with both seawater samples (polluted and clean) analysed.

Although it was considered that the results were such as to practically obviate the need for statistical analysis, such analysis was carried out. Practical problems were, however, encountered in view of the relatively small number of samples. In this regard, although the primary aim of the series of intercalibration exercises was to obtain comparable results through harmonization of methodologies, the meeting agreed that in future exercises, consideration could be given to planning the actual programme of work with a statistician, and in particular, to determining the minimum number of samples necessary to satisfy statistical requirements.

The detailed results of the intercalibration exercise and the conclusions drawn therefrom, together with the instructions and forms provided for participants are attached as Annex 2.

8. Future action and recommendations (agenda item 9)

Apart from the observations made regarding the minimum number of samples to be analysed to conform with normal statistical requirements, the meeting also considered that the current duration (five days) of the intercalibration exercises should be extended by at least one day and preferably more. This would enable the more proper observance of the statutory intervals between commencement of the incubation period and reading of the results, and would also provide the necessary time for statistical analysis followed by thorough discussion of the results obtained.

The meeting also recommended that:

- the series of intercalibration exercises on microbiological parameters within the framework of the monitoring component of MED POL Phase II should be continued and extended to include new parameters, especially pathogens;

- in view of the fact that many Mediterranean laboratories were using the multiple test tube (MPN) method, and also in the light of the correlation shown between this and the membrane filtration culture (MF) method, the reference methods utilizing the former technique, currently under development by WHO and UNEP, should be finalized as soon as possible;

- Mediterranean scientists should participate to the fullest extent possible in the research component of MED POL Phase II, especially in those areas leading to the updating of microbiological and related monitoring.

9. Other matters (agenda item 10)

Arising from the results of the intercalibration exercise, the various advantages and limitations of the membrane filtration culture (MF) and the multiple test tube (MPN) methods respectively were discussed by the group. In particular, it stressed that, from experience, the MF method did not provide accurate results when examining heavily-polluted waters with a high number of suspended particles, or highly eutrophicated waters. It was also recognized that both techniques had been originally developed for drinking-water analysis, and their use for seawater posed a number of technical problems. In this regard, the type and quality of media used were important and a number of participants considered that quality control of media should be included in the reference methods, using the same microorganisms.

It was agreed that in Reference Method No. 3 (determination of faecal coliforms in seawater by the membrane filtration culture technique) EC medium should be added to MacConkey and brilliant green at 44.5° C in confirmation tests.

The majority of participants also considered that since the MF and MPN methods would be used interchangeably within the framework of the MED POL monitoring programme, they should be based on an identical principle of identification for selection of the indicator organisms, namely faecal coliforms. The ability to utilize lactose and grow at 44.5° C should be the only microbial metabolic process on which to base identification, since it exists in both methods. As the list for indole production is not included in routine monitoring using the MF technique, it should be omitted from the MPN procedure in order to remove an additional compounding factor which would add to any differences between results obtained by the two methods. In keeping with this, the phrase "produce indole in tryptone water containing tryptophan at 44.5° C" in Reference Method No. 3 should be deleted from the definition.

Annex 1

SUMMARY REPORT ON THE GREEK INTERCALIBRATION EXERCISE
Athens, 12-16 March 1984

The activity was held from 12 to 16 March 1984 at the Ministry of Physical Planning Housing and the Environment/Environmental Pollution Control Project - Athens (PERPA). It was attended by 12 scientists from six institutions from Athens, Thessaloniki and Ioannina.

The purpose of the activity was to enable the participants to conduct laboratory determinations of bacterial parameters, using the same samples of seawater and shellfish and following the same methodologies.

Monitoring of the coastal water quality of recreational and shellfish-growing areas is part of the Greek MED POL Monitoring Programme (Phase II) submitted already to the Coordinating Unit of Mediterranean Action Plan (MAP).

A number of reference methods for sampling and analysis of the three bacterial indicator organisms (total coliforms, faecal coliforms, faecal streptococci) have been prepared for use by the Mediterranean Contracting Parties of the Barcelona Convention during the Extraordinary Meeting of April 1984.

During the laboratory sessions, participants made determinations of the three bacterial organisms (total coliforms, faecal coliforms, faecal streptococci) in each of three different seawater samples (seawater of high quality, moderately polluted and polluted) and faecal coliforms in shellfish samples.

For the shellfish microbiological analysis the Most Probable Number (MPN) method was used, since this is the only one that can be applied. (WHO/)UNEP Reference Method, Marine Pollution Studies, No. 5, Rev. 1).

For seawater samples both the MPN and MF techniques were used. As far as the MF technique is concerned, the participants have used the WHO/UNEP Reference Methods, Marine Pollution Studies, No. 2, Rev. 1 (total coliforms), No. 3, Rev. 1 (faecal coliforms) and No. 4, Rev. 1 (faecal streptococci). Regarding the MPN technique different media were used for intercomparison.

- It was found that, using identical samples and standard methodologies, the results had no statistically significant differences.

- It must be noted that both MPN and MF methods may be used for seawater analysis but each method has several advantages, disadvantages and limitations. Consequently, a general use/acceptance of both methods does not seem to be appropriate for all qualities of seawater. So, heavily polluted waters with a high number of suspended particles or highly eutrophicated (algae) waters cannot be examined by the MF technique, while the same holds true for the MPN method for seawater containing toxic substances from industrial effluents.

During the meeting sessions, the participants discussed the progress and results of this laboratory exercise, as well as a number of technical points. The results of the intercalibration exercise held in Rome in November 1982 were also taken into consideration.

Recommendations

The participants agreed upon the following points and recommended:

- to ensure comparability of results and quality control, the MED POL Intercalibration Exercises should be regularly conducted, especially if new parameters are added or new methods are proposed for use;

- the reference methods for the MPN (total coliforms, faecal coliforms and faecal streptococci in seawater) should be completed by WHO/UNEP, reviewed by the Mediterranean scientists and adopted for use as soon as possible;

- scientists should be encouraged to participate in the research component of MED POL, Phase II.

Annex 2

RESULTS OF INTERCALIBRATION EXERCISE
Athens, 25-29 June 1984

Introduction

The purpose of the activity was to enable participants to carry out determinations of microbial concentrations in the same samples of seawater by uniform methodology and to compare results obtained (a) between individual participants for each parameter (total coliforms, faecal coliforms and faecal streptococci) and (b) between the two methods (membrane filtration and multiple test tube) utilized for the latter two parameters.

Organization and methodology

Participants were divided into five groups of three. Two samples of seawater were analysed by each group: sample A from a naturally contaminated beach, and sample B from a clean beach. The original sample containers, each of 10 litres capacity, were thoroughly shaken and in both cases, aliquots of approximately 300 ml of water were poured into each of 5 bottles which served as analysis specimens for the groups of participants.

Every sample was examined for total coliforms (TC) by the multiple test tube (MPN) method and for faecal coliforms (FC) and faecal streptococci (FS) by both the MPN and the membrane filtration (MF) culture methods.

In the case of MF, the methods employed were the reference methods recommended by WHO/UNEP. However, in order to save time and labour (considering the restricted duration of the exercise) and to achieve a better uniformity, the dilutions from each sample were prepared in quantities large enough to suffice for all inoculations and filtrations (45 ml phosphate buffer plus 5 ml of sample water, etc.).

Inoculation and filtration were done on a group basis. However, results were read by each individual in the group as well as by the coordinator of the exercise. Where colony counts on the filters obtained by any individual participant varied by more than 2 to 3 colonies from those of the coordinator, counts were repeated together in order to establish the source of variation. All counts were performed with magnifying glasses and, where necessary, verified with a zoom stereoscopic microscope.

In the case of MPN, the methods employed were as described in "Standard methods for the examination of water and wastewater", 15th edition, 1980 (APHA-AWWA-WPCF). However, confirmatory tests had to be limited to 24 hours (instead of 48) in order to achieve collection of the final data one day before the end of the meeting, thus enabling their proper analysis prior to thorough and detailed discussion during the closing session.

Throughout the exercise, checks were made, to the fullest extent possible, on the procedure and readings of each individual participant during each step, in order to note where errors and deviations could be made which might lead to variations in results greater than those inherent in the methods per se. Attempts were also made to discuss every observation or error made by each individual participant, so that all participants would obtain maximum benefit from the exercise.

The detailed instructions given to participants, as well as the forms prepared for each parameter, are given in Appendix 1. These instructions had to be modified insofar as:

- the originally planned three samples of seawater had to be reduced to two (samples No. 1 and 3)
- analysis of raw sewage and shellfish flesh could not be undertaken.

The above modifications were necessary because of the time factor (i.e. the limited duration of the exercise).

Results and discussion

Individual readings on membrane filters as well as calculations of organism densities based on them are given in Tables 1 and 2 for faecal coliforms and faecal streptococci respectively. These

tables show that individual counting of colonies within each group were in close agreement both between themselves and with readings obtained by the coordinator. There was also a similarly good agreement between readings obtained by different groups, indicating that the dilutions were correctly performed.

The numbers of organisms monitored in each sample by the five groups are summarized in Table 3.

Sample A: Four groups estimated the most probable number (MPN) of TC at between 1.7×10^4 and 3.5×10^4 per 100 ml, and that of FC, at between 1.7×10^4 and 2.4×10^4 per 100 ml. One group estimated 1.6×10^5 per 100 ml for both TC and FC. This was probably due to a clump of bacteria in their sample breaking up by the additional shaking of the specimen before and during dilution. As the confirmatory tests for both organisms are inoculated from the same tubes of the presumptive test in lactose broth and the organisms are usually identical, the result can be considered reasonable.

With the MF method, however, readings for the five groups ranged from 2.1×10^4 to 3.6×10^4 FC per 100 ml when the numbers of colonies on 1 ml and 0.1 ml filters were averaged, and from 3.5×10^4 to 4.2×10^4 FC per 100 ml when only 0.1 ml filters (which gave 30-50 colonies per filter) were considered.

The five groups estimated the number of FS within the range of 1.1×10^4 to 3.5×10^4 per 100 ml by the MPN procedure and 2.2×10^4 to 2.9×10^4 per 100 ml by the MF (0.1 filter results).

Sample B: All results obtained indicated the clean quality of the water. Most of them showed less than 10 organisms of each group per 100 ml. The actual number of FS was probably less than that indicated by the MF method in Table 3, as four out of the five results were based on 1 to 5 colonies on 10 ml filters.

Conclusions

In general, it can be considered that taking into account all the variations and sources of deviation inherent in each of the methods, the agreement between results of individual groups and between those determined for the two methods (MF and MPN) was very good.

Table 1

Individual readings of FC colonies on membrane filters placed on m-FC agar, incubated at 44.5° ± 0.2 for 24h, and the number of FC per 100 ml of seawater calculated accordingly

Sample A - polluted water

Amount of water filtered		Group I			Gr av.	y*	Group II		Gr av.	Group III	y*	Group IV		Gr av.	y*	Group V
10 ml		>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300
1 ml	(	105	105	108	106	112	95	94	95	290	280	128	114	121	134	70
	)															
	(	110	107	110	109	131	89	86	87	ND	-	139	125	132	132	83
0.1 ml	(	35	32	31	32	38	39	38	38	42	50	35	35	35	33	37
	)															
	(	36	36	36	37	38	39	38	39	ND	-	43	43	43	43	33
0.01 ml	(	6	6	6	6	6	3	3	3	6	7	8	11	10	9	4
	)															
	(	2	2	2	2	3	4	4	4	ND	-	4	4	4	3	5
FSx10^4 /100 ml	a	2.3	2.2	2.2	2.2	2.5	2.4	2.4	2.4	3.6	3.9	2.1	2.1	2.1	2.5	2.1
	b	3.7	3.4	3.4	3.5	3.8	3.9	3.9	3.9	4.2	5.0	3.9	3.9	3.9	3.8	3.5

a - Based on average of readings of 1 ml filters and 0.1 ml filters
b - Based on readings of 0.1 ml filters only. This is the more correct result since it is based on filters with 20-80 colonies on each

Sample B - clean water

Groups I-V: filtered 10 ml, 1 ml, 0.1 ml - there were no colonies on any of them therefore the result is <10 FC/100 ml.

Group V: filtered also 100 ml, there were 2 colonies on one filter and 1 on the other = 2/100 ml.

* y: coordinator's readings

Table 2

Individual readings of FS colonies on membrane filters placed on KF-agar, incubated at $36^{o} \pm 1$ for 48h, and the number of FS per 100 ml of seawater calculated accordingly

Sample A - polluted water

Amount of water filtered	Group I			Gr av.	y*	Group II		Gr av.	y*	Group III		Gr av.	y*	Group IV		Gr av.	y*	Group V	y*
10 ml	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300	>300
1 ml	189	189	186	188	195	192	201	196	192	286	228	217	214	192	208	200	205	-	-
	162	158	159	160	164	149	160	154	150	ND	ND	-	-	170	173	172	171	175	186
0.1 ml	24	23	23	23	24	19	19	19	20	21	22	22	22	36	36	36	37	29	30
	25	24	24	24	24	37	39	38	37	ND	ND	-	-	23	23	23	22	25	26
0.01 ml	6	6	6	6	6	3	3	3	3	2	2	2	2	1	1	1	1	2	2
	1	1	1	1	1	4	4	4	4	ND	ND	-	-	1	1	1	1	5	5
a FSx10^4/100 ml	2.1	2.0	2.0	2.0	2.1	2.3	2.4	2.3	2.3	2.1	2.2	2.2	2.2	2.4	2.4	2.4	2.4	2.4	2.5
b	2.5	2.4	2.4	2.4	2.4	2.8	2.9	2.8	2.8	2.1	2.2	2.2	2.2	2.9	2.9	2.9	2.9	2.7	2.8

a - Based on average of readings of 1 ml filters and 0.1 ml filters
b - Based on readings of 0.1 ml filters only. This is the more correct result since it is based on filters with 20-80 colonies on each

Note: A control was done by Group III to verify whether rinsing between samples was adequate. They inserted sterile filters after the last filtrations of the lowest dilutions, filtered 10 ml of sterile buffer through them, placed the filters on mFC and KF media and incubated with the others. No colonies grew on these filters.

Sample B - clean water

Very few colonies grew on the 10 ml filters and the results are presented in Table 3.

Table 3

Summary of results obtained by the five groups that participated in the intercalibration exercise, Athens (25-29 June 1984)

Two samples of water were monitored by each group (aliquots of the same bulk samples: A - polluted water, B - clean water. TC were monitored by MPN only. FC and FS - by MPN and MF methods.

			Group I	Group II	Group III	Group IV	Group V
TC/100 ml							
MPN	A		$3.5X10^4$	$2.8X10^4$	$1.7X10^4$	$1.6X10^5$	$2.4X10^4$
	B		8	<2	2	9	2
FC/100 ml							
MPN	A		$2.4X10^4$	$1.7X10^4$	$1.7X10^4$	$1.6X10^5$	$2.4X10^4$
MF		a	$2.2X10^4$	$2.4X10^4$	$3.6X10^4$	$2.1X10^4$	$2.1X10^4$
		b	$3.5X10^4$	$3.9X10^4$	$4.2X10^4$	$3.9X10^4$	$3.5X10^4$
MPN	B		8	<2	2	2	2
MF			<10	<10	<10	<10	2
FS/100 ml							
MPN	A		$2.4X10^4$	$3.5X10^4$	$1.7X10^4$	$1.1X10^5$	$2.4X10^4$
MF		a	$2.0X10^4$	$2.3X10^4$	$2.2X10^4$	$2.4X10^4$	$2.4X10^4$
		b	$2.4X10^4$	$2.8X10^4$	$2.2X10^4$	$2.9X10^4$	$2.7X10^4$
MPN	B		2	2	<2	<2	2
MF			10	10	30	330	25

a - Calculation based on average of readings on 1 ml and 0.1 ml filters.

b - Calculation based reading of 0.1 ml filters alone, which is more correct since only these filters had 20-80 colonies.

Appendix 1

INSTRUCTIONS TO THE PARTICIPANTS

Purpose

The purpose of the present intercalibration is for the participants to carry out laboratory determinations of bacteriological parameters in the same samples of seawater and shellfish using prescribed methodologies.

The membrane filtration technique (MF) Annex 1 and the multiple test tube technique (MPN) Annex 2.

Samples

No. 1 Seawater of high quality
No. 2 Moderately polluted seawater from an area with intermittent pollution
No. 3 Seawater from a polluted area
No. 4 Raw sewage
No. 5 Shellfish from a restricted area

Procedure

The sample test amount should be prepared for filtration or inoculation according to the degree of pollution of sample medium using phosphate buffer.

Selection of sample size and dilution series

Sample test amounts

	Multiple tube (MPN)					Membrane filtration			
Raw sewage	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-2}	10^{-3}	10^{-4}	10
Polluted water	10	1	10^{-1}	10^{-2}	10^{-3}	10	1	10^{-1}	
Clear water	10	1	10^{-1}			100	10	1	
Shellfish	10	1	10^{-1}			N/A			

Preparation of the dilution series

Label all tubes and Petri dishes according to sample number and dilution.

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.

Prepare the dilution series by taking with a sterile pipette after vigorously shaking the sample 1 ml from the original sample and transfer this 1 ml into a test-tube containing 9 ml of phosphate buffer to make the first dilution (D-1).

Agitate the tube vigorously by hand. Continue the preparation of the dilution series by taking 1 ml from the first dilution (D-1) and mixing it in a new test tube containing 9 ml of phosphate buffer in order to obtain the second dilution (D-2) and so on.

Membrane filtration

Begin filtration with the highest dilution in order to avoid contamination from samples containing bacteria of higher concentrations. Use a sterilized filtration funnel for each sample. Place the presterilized MF with flamed sterilized forceps over the porous plate of the filtration apparatus. Carefully place the matching funnel unit over the receptable and lock it in place. Add into the funnel about 20 ml of phosphate buffer solution for filtration. With a sterile 1 ml pipette add 1 ml of last dilution into the phosphate buffer solution in the funnel.

Results

Enter results in appropriate forms

Seawater

1. MF : Faecal coliforms
2. MF : Faecal streptococci
3. MPN: Total coliforms
4. MPN: Faecal coliforms
5. MPN: Faecal streptococci

Shellfish

6. MPN: Faecal coliforms

Calculate results using Tables 1 and 2.

REMARKS

Appendix I, Annex 1

OUTLINE SUMMARIES OF REFERENCE METHODS, AS MODIFIED

Faecal coliforms in seawater (Reference Method No.3)

Method:	Membrane filtration
Medium:	m-FC agar with rosolic acid (not autoclaved)
Incubation temperature:	44.5 ± 0.2°C (water bath)
Time:	24 hours
Colour of colonies:	Blue
Confirmation:	Acid and gas on MacConkey broth 24 hours at 44.5 ± 0.2°C or
Optional:	gas on brilliant green bile broth 24 hours at 44.5 ± 0.2°C and Indol + (Tryptone water incubated for 24 hours at 44.5 ± 0.2°C)

Faecal streptococci in seawater (Reference Method No. 4)

Method:	Membrane filtration
Medium:	KF agar with TTC (not autoclaved)
Incubation temperature:	36 ± 1°C (air incubator)
Time:	48 hours
Colour of colonies:	Pink to maroon
Confirmation:	Bile solubility
Optional:	Growth - at 45°C - at pH 9.6 - in 6.5% NaCl

Faecal coliforms in bivalves (Reference Method No.5)

Method:	MPN
Medium and incubation:	Presumptive test: lactose broth incubated at 36° ± 1°C for 48 hours Confirmed test: MacConkey broth or brilliant green bile broth 2% incubated at 44.5 ± 0.2°C for 48 hours and Tryptone water for Indole test incubated at 44.5 ± 0.2°C for 24 hours.

Appendix 1, Annex 2

OUTLINE SUMMARIES OF REFERENCE METHODS, AS MODIFIED

Total coliforms in seawater

Method:	Multiple Test Tube Technique
Medium:	Presumptive Test. Lactose broth.
Incubation temperature:	36 ± 1°C
Time:	48 ± 3 hours
Medium:	Confirmed test brilliant green bile broth 2%
Incubation temperature:	36 ± 1°C
Time:	48 ± 3 hours

Faecal coliforms in seawater

Method:	Multiple Test Tube Technique
Medium:	Presumptive Test. Lactose broth.
Incubation temperature:	36 ± 1°C
Time:	48 ± 3 hours
Medium:	Confirmed Test E.C. medium
Incubation temperature:	44.5 ± 0.2°C
Time:	48 ± 3 hours

Faecal streptococci in seawater

Method:	Multiple Test Tube Technique
Medium:	Presumptive Test azide dextrose broth
Incubation temperature:	36 ± 1°C
Time:	48 ± 3 hours
Medium:	Confirmed Test EVA broth
Incubation temperature :	36 ± 1°C
Time:	48 ± 3 hours

Figure 1

Scheme of preparation of dilution series
and of the filtration procedure

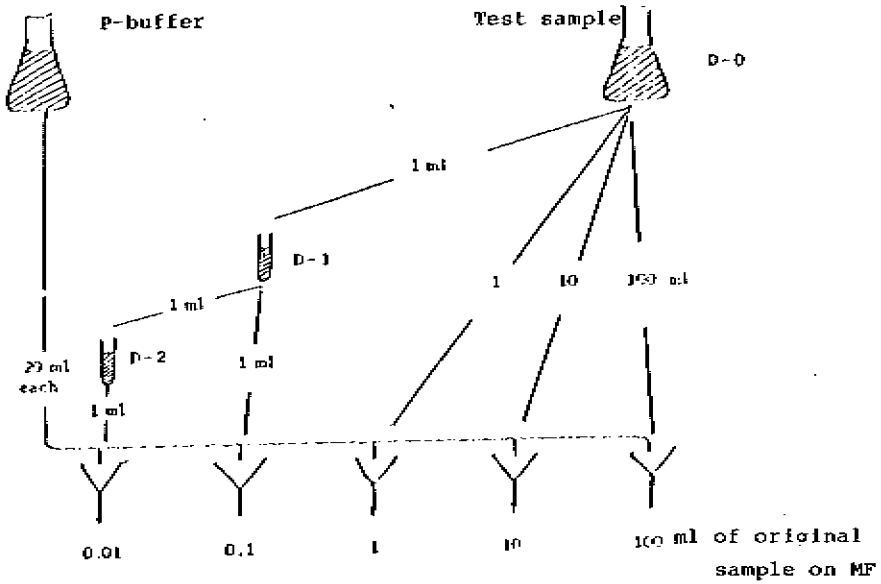
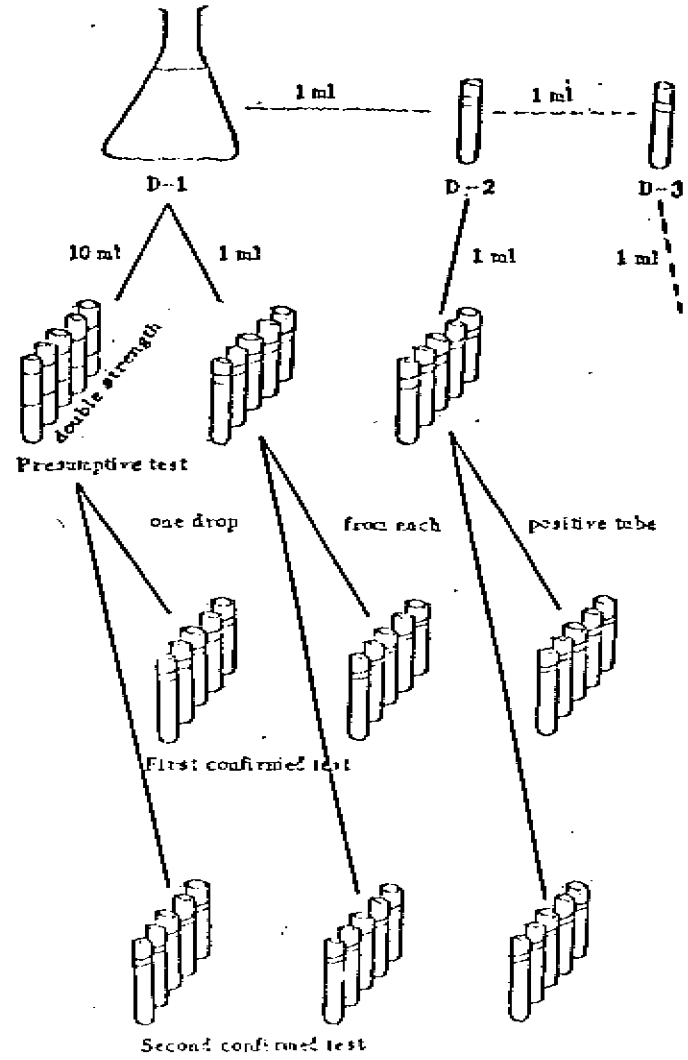


Figure 2

Scheme for the preparation of dilution series



MULTIPLE TUBE FERMENTATION TECHNIQUE: Bacterial Density

Table 1. MPN Index and 95% confidence limits for various combinations of positive results when five tubes are used for dilution (10 ml, 1.0 ml, 0.1 ml)

Combination of positives	MPN Index /100 ml	95% Confidence limits		Combination of positives	MPN Index /100 ml	95% Confidence limits	
		Lower	Upper			Lower	Upper
0-0-0	<0	-	-	4-2-0	22	7	67
0-0-1	2	<0.5	7	4-2-1	26	9	78
0-1-0	2	<0.5	7	4-3-0	27	9	90
0-2-0	4	<0.5	11	4-3-1	33	11	93
				4-4-0	34	12	93
1-0-0	2	<0.5	7	5-0-0	23	7	70
1-0-1	4	<0.5	11	5-0-1	31	11	89
1-1-0	4	<0.5	11	5-0-2	43	15	110
1-1-1	6	<0.5	15	5-1-0	33	11	93
1-2-0	6	<0.5	15	5-1-1	46	16	120
				5-1-2	63	21	150
2-0-0	5	<0.5	13	5-2-0	49	17	130
2-0-1	7	1	17	5-2-1	70	23	170
2-1-0	7	1	17	5-2-2	94	28	220
2-1-1	9	2	21	5-3-0	79	25	190
2-2-0	9	2	21	5-3-1	110	31	250
2-3-0	12	3	28	5-3-2	140	37	340
3-0-0	8	1	19	5-3-3	180	44	500
3-0-1	11	2	25	5-4-0	130	35	300
3-1-0	11	2	25	5-4-1	170	43	490
3-1-1	14	4	34	5-4-2	220	57	700
3-2-0	14	4	34	5-4-3	280	90	850
3-2-1	17	5	46	5-4-4	350	120	1000
4-0-0	13	3	31	5-5-0	240	68	750
4-0-1	17	5	46	5-5-1	350	120	1000
4-1-0	17	5	46	5-5-2	540	180	1400
4-1-1	21	7	63	5-5-3	920	300	3200
4-1-2	26	9	78	5-5-4	1600	640	5800
				5-5-5	≥2400	-	-

Source: Standard Methods 15th-Ed. APHA

Table 2: MPN index and 95% confidence limits for various combinations of positive and negative results when five 10 ml portions, five 1 ml potions 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	
1 g	0.1 g	0.01 g	1 g	Lower	Upper
0	0	0	< 0.2		
0	0	1	0.2	<0.05	0.7
0	1	0	0.2	<0.05	0.7
0	2	0	0.4	<0.05	1.1
1	0	0	0.2	<0.05	0.7
1	0	1	0.4	<0.05	1.1
1	1	0	0.4	<0.05	1.1
1	1	1	0.6	<0.05	1.5
1	2	0	0.6	<0.05	1.5
2	0	0	0.5	<0.05	1.3
2	0	1	0.7	0.1	1.7
2	1	0	0.7	0.1	1.7
2	1	1	0.9	0.2	2.1
2	2	0	0.9	0.2	2.1
2	3	0	1.2	0.3	2.8
3	0	0	0.8	0.1	1.9
3	0	1	1.1	0.2	2.5
3	1	0	1.1	0.2	2.5
3	1	1	1.4	0.4	3.4
3	2	0	1.4	0.4	3.4
3	2	1	1.7	0.5	4.6
3	3	0	1.7	0.5	4.6
4	0	0	1.3	0.3	3.1
4	0	1	1.7	0.5	4.6
4	1	0	1.7	0.5	4.6
4	1	1	2.1	0.7	6.3
4	1	2	2.6	0.9	7.8
4	2	0	2.2	0.7	6.7
4	2	1	2.6	0.9	7.8
4	3	0	2.7	0.9	8
4	3	1	3.3	1.1	9.3
4	4	0	3.4	1.2	9.3
5	0	0	2.3	0.7	7
5	0	1	3.1	1.1	8.9
5	0	2	4.3	1.5	11
5	1	0	3.3	1.1	9.3
5	1	1	4.6	1.6	12
5	1	2	6.3	2.1	15
5	2	0	4.9	1.7	13
5	2	1	7	2.3	17
5	2	2	9.4	2.8	22
5	3	0	7.9	2.5	19
5	3	1	11	3.1	25
5	3	2	14	3.7	34
5	3	3	18	4.4	50
5	4	0	13	3.5	30
5	4	1	17	4.3	49
5	4	2	22	5.7	70
5	4	3	28	9	85
5	4	4	35	12	100
5	5	0	24	6.8	75
5	5	1	35	12	100
5	5	2	54	18	140
5	5	3	92	30	320
5	5	4	160	64	580
5	5	5	240		

Form 1

SEAWATER

Number of colonies per individual filter

Dilution		Number of faecal coliform colonies per filter in replicate dilution		
		Replica 1st	2nd	Average
D - 0	100			
D - 0	10			
D - 0	1			
D - 1	0.1			
D - 2	0.01			
D - 3	0.001			
D - 4	0.0001			

Number of faecal coliforms/100 ml sample

Dilutions	n/100 ml

Test result: _____ faecal coliforms/100 ml samples

Anomalies observed in the test procedure:

Team No. _____

Names of participants:

.....

.....

.....

.....

Date .....

Form 2

SEAWATER

Number of colonies per individual filter

Dilution		Number of faecal streptococcus colonies per filter in replicate dilution		
		Replica 1st	2nd	Average
D - 0	100			
D - 0	10			
D - 0	1			
D - 1	0.1			
D - 2	0.01			
D - 3	0.001			
D - 4	0.0001			

Number of faecal streptococci/100 ml sample

Dilutions	n/100 ml

Test result: _____ faecal streptococci/100 ml samples

Anomalies observed in the test procedure:

Team No. _____

Names of participants:

.....

.....

.....

.....

Date .....

Form 3

SEAWATER

MULTIPLE TEST TUBE METHOD

TOTAL COLIFORMS

Dilution	ml of sample inoculated	Incubation hours	LACTOSE BROTH 36°C	Br. green 36 1°C
10^0	10	24		
		48		
10	**1**	24		
		48		
10^{-1}	1	24		
		48		
10^{-2}	1	24		
		48		
10^{-3}	1	24		
		48		
10^{-4}	1	24		
		48		
10^{-5}	1	24		
		48		
10^{-6}	1	24		
		48		

Test Results (see table 1)

1) MPN after 48 h in Br. green medium at 36°C

_____ Total coliforms
/100 ml

2) 95% Confidence Limits

Lower	Upper

3) Anomalies observed during the test procedure

Team No. .....

Names of participants:

.....

.....

.....

Form 4

SEAWATER

MULTIPLE TEST TUBE METHOD

FAECAL COLIFORMS

Dilution	ml of sample inoculated	Incubation hours	LACTOSE BROTH 36°C	E.C. MEDIUM 44,5°C
10^0	10	24		
		48		
10	1	24		
		48		
10^{-1}	1	24		
		48		
10^{-2}	1	24		
		48		
10^{-3}	1	24		
		48		
10^{-4}	1	24		
		48		
10^{-5}	1	24		
		48		
10^{-6}	1	24		
		48		

Test Results (see table 1)

1) MPN after 48 h in E.C. medium at 44,5°C

_____ faecal coliforms
/100 ml

2) 95% Confidence limits

Lower	Upper

3) Anomalies observed during the test procedure

Team No. .....

Names of participants:

.....

.....

.....

Form 5

SEAWATER

MULTIPLE TEST TUBE METHOD

FAECAL STREPTOCOCCI

Dilution	ml of sample inoculated	Incubation hours	Azide Dextrose Broth	EVA Broth
10^{0}	10	24		
		48		
10	1	24		
		48		
10^{-1}	1	24		
		48		
10^{-2}	1	24		
		48		
10^{-3}	1	24		
		48		
10^{-4}	1	24		
		48		
10^{-5}	1	24		
		48		
10^{-6}	1	24		
		48		

Test Results (see table 1)

1) MPN after 48 h in EVA medium at 44.5°C

_____ faecal streptococci
/100 ml

2) 95% Confidence limits

Lower	Upper

3) Anomalies observed during the test procedure

Team No. .....

Names of participants:

.....

.....

.....

Form 6

SHELLFISH

MULTIPLE TEST TUBE METHOD

FAECAL COLIFORMS

Dilution	ml of sample inoculated	Incubation hours	Lactose 36°C	Br. green 44.5°C	Tryptone water for INDOL 44.5°C
10^0	10	24			
		48			
10	1	24			
		48			
10^{-1}	1	24			
		48			
10^{-2}	1	24			
		48			
10^{-3}	1	24			
		48			
10^{-4}	1	24			
		48			
10^{-5}	1	24			
		48			
10^{-6}	1	24			
		48			

Test Results (see table 1)

1) MPN after 48 h in Br. Green and Tryptone water at 44.5°C

_____ faecal coliforms / shellfish flesh

2) 95% Confidence limits

Lower	Upper

3) Anomalies observed during the test procedure

Team No. .....

Names of participants:

.....

.....

.....

Annex 3

LIST OF PARTICIPANTS

TEMPORARY ADVISERS

Ms M. Aravantinou (Statistician)
Environmental Pollution Control Project, Athens, Greece

Dr S. Bokan
Zavod za Zastitu Zaravlja, Rijeka, Yugoslavia

Dr Y.G. Goufa
Central Public Health Laboratories, Athens, Greece

Dr N. Dimopoulos
Department of Biology, University of Patras, Greece

Ms E. Ioannou
Ministry of Health, General Analytical Laboratory, Akropolis, Cyprus

Professor V. Katsouyannopoulos
Department of Hygiene, Medical School, University of Ioannina, Greece

Dr V. Krikelis
Enterovirus Laboratory, Institut Pasteur Hellenique, Athens, Greece

Dr N. Krstulovic
Institute of Oceanography and Fisheries, Split, Yugoslavia (Rapporteur)

Dr G. Manes
Director, Public Health Directorate, Ministry of Health and Welfare, Athens, Greece

Dr A. Mates
Ministry of Health, District Public Health Laboratory, Haifa, Israel

Ms A. Mavridou
School of Hygiene, Athens, Greece

Dr A. Mourmouris
Ministry of Physical Planning, Housing and Environment, Athens, Greece

Professor J.A. Papadakis
Department of Bacteriology, Athens School of Hygiene, Athens, Greece

Professor G. Papaevangelou
Athens School of Hygiene, Athens, Greece

Dr M. Papapetropoulou
Department of Hygiene, University of Patras, Greece

Professor J. T. Papavassiliou
Central Health Council, Athens, Greece

Mr F. Portelli
Public Health Laboratory, Department of Health, Valletta, Malta

Dr C. Richardson (Statistician)
Athens School of Hygiene, Athens, Greece

Mr M. Sabatakakis
Ministry of Health and Welfare, Department of Sanitary Environmental Protection, Athens, Greece

Ms S. Sotiracopoulos
Environmental Pollution Control Project, Athens, Greece (Chairman)

Ms D. Spala
Environmental Pollution Control Project, Athens, Greece

Dr G. Stathopoulos
Laboratory of Hygiene, Medical School, University of Thessaloniki, Greece

Dr M. Thalassinou-Tzatzani
Central Public Health Laboratories, Athens, Greece

Dr E. Yianniou
Water Sewage Corporation, Athens, Greece

Ms A. Yilmaz
Middle East Technical University, Department of Marine Sciences, Erdemli, Turkey

Dr Y. Yoshpe-Purer
Director, The Dr A. Felix Public Health Laboratory, Ministry of Health, Tel-Aviv, Israel (Vice-Chairman)

REPRESENTATIVES OF OTHER ORGANIZATIONS

United Nations Environment Programme (UNEP)

Dr F.S. Civili
Marine Scientist, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece

WORLD HEALTH ORGANIZATION

Regional Office for Europe

Dr L.J. Saliba
Senior Scientist, WHO/EURO Project Office, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece (Secretary)