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REVIEW OF INTERCALIBRATION EXERCISES ON MICROBIOLOGICAL METHODS FOR COASTAL WATER QUALITY MONITORING

In co-operation with

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INTRODUCTION

The pilot phase of the Coordinated Mediterranean Pollution Monitoring and Research Programme, MED POL Phase I, was approved in 1975 by the Contracting Parties to the Barcelona Convention as the scientific and technical component of the Action Plan.

Among the specific objectives of the MED POL Phase I, the following two can be highlighted: 1) to formulate and carry out a coordinated pollution monitoring and research programme and 2) to assist national research centers in developping their capabilities to participate in the programme (UNEP, 1983a).

One of the seven pilot projects initially considered in the MED POL Phase I was the MED POL VII Project, entitled Coastal Water Quality Control, jointly coordinated by UNEP and WHO. The general objective of this project was to obtain statistically significant data, scientific information and technical principles which are required for the assessment of the present levels of coastal pollution as it concerns human health.

The results gathered by national centers participating in the MED POL VII Project have allowed an assessment of the state of microbial pollution of the coastal waters of the Mediterranean, while at the same time they have highlighted the need to ensure that microbiological data collected by national centers is reliable and comparable. The need for a quality control and intercalibration programme was one of the most relevant conclusions of the MED POL VII Project.

In 1979, after examining the status of the work conducted by Mediterranean centers in cooperation with United Nations organizations, the Contracting Parties recommended that a long-term pollution monitoring and research programme should be formulated by UNEP, as Secretariat of the Barcelona Convention. A 10-year programme was formulated by UNEP, in cooperation with the organizations supporting the MED POL Phase I, which was approved by the Contracting Parties in 1981, as the MED POL Phase II (UNEP, 1983b).

The general long-term objective of the MED POL Phase II was to further the goals of the Barcelona Convention by assisting the Parties to prevent, abate and combat pollution of the Mediterranean Sea Area and to protect and enhance the marine environment of the Area.

In accordance with Article 10 of the Barcelona Convention and its related protocols, one of the specific objectives of the MED POL Phase II is to establish complementary or joint programmes for pollution monitoring in the Mediterranean Sea Area as well as to establish a pollution monitoring system for the Area (UN, 1978).

To fulfill the basic principles considered in its development, the MED POL Phase II specifies that sampling and analytical techniques used in the monitoring will be based on mandatory reference methods, and

that all national research centers will participate in the continuing intercalibration of sampling and analytical techniques or in data quality control programmes (UNEP, 1983b).

Furthermore, the MED POL Phase II includes an assistance component, according to which a quality control programme will be a part of MED POL Phase II, to ensure the highest degree of quality and comparability of data. Weaknesses detected through the quality control programme will be corrected through additional training and technical assistance, whenever necessary.

It is in this context that a series of 6 intercalibration exercices on microbiological methods for coastal water quality monitoring have been conducted in the Mediterranean, between 1982 and 1985, under the joint coordination of UNEP and WHO, and the collaboration of Mediterranean research centers.

The considerable number of experimental results obtained, and the relevance of the conclusions reached during the technical sessions of these intercalibration exercices, should be of great benefit for the advancement of the microbiological component of the monitoring programmes being conducted by Mediterranean countries, and thus deserve a detailed review and evaluation.

OBJECTIVES

The general objective of this study is to review and evaluate the experimental results obtained and the conclusions reached during the series of six intercalibration exercices on microbiological methods for coastal water quality monitoring, conducted in the Mediterranean region from 1982 to 1985.

The specific objectives of this study are the following:

1. To discuss the practical difficulties encountered during the execution of the intercalibration exercices, particularly those concerned with the preparation and analysis of seawater samples of unknown microbial concentrations.

2. To identify the analytical steps which need to be further specified and standardized, as to ensure they do not introduce significant variations in the microbiological analysis of coastal waters.

3. To determine the degree and the affecting factors of the precision of the membrane filtration method and the multiple-tube method when analyzing the microbiological quality of coastal waters.

4. To examine the influence that culture media and culture conditions have on the results of microbiological analyses of coastal waters.

5. To evaluate the degree of comparability of the results obtained by the membrane filtration method and the multiple-tube method when analyzing the microbiological quality of coastal waters.

6. To discuss the practical interest of using proposed statistical methods for interpreting the microbiological results of intercalibration exercices.

7. To recommend possible alternatives for the continuation of the intercalibration exercices and the quality control programme included in MED POL Phase II.

INTERCALIBATION EXERCICES

Six intercalibration exercices on microbiological methods for coastal water quality monitoring have been organized in the Mediterranean region under the joint coordination of UNEP and WHO. Although the first exercice, held in Rome in November 1982, was considered both as a summary review of work conducted during MED POL Phase I and as a preliminary activity of the five exercices which were planned in the framework of MED POL Phase II, the considerable number of results obtained and the relevance of the conclusions reached during the Rome intercalibration exercice have recommended its inclusion in this study.

Table 1 shows the list of Mediterranean institutions responsible for the organization of each intercalibration exercice, as well as the timetable and working languages of the exercices.

The nationalities and number of participants in the series of intercalibration exercices are shown in table 2. The average number of participants in each intercalibration exercice has been 25; between 30 and 80% of the participants have come from the country hosting the exercice. In total, 148 scientists have participated in the six intercalibration exercices: 137 from the Mediterranean region and 9 from other regions of the world.

Table 1. Intercalibration exercices on microbiological methods for coastal water quality monitoring held in the Mediterranean region, under the joint coordination of UNEP and WHO.

Organizing Institution	Date	Working Languages
Istituto Superiore di Sanità. Roma, Italy.	22-26 September 1982	English and French
Escuela Técnica Superior de Ingenieros de Caminos, Canales y Puertos. Barcelona, Spain.	7-11 November 1983	French
Environmental Pollution Control Project. Athens, Greece.	25-29 June 1984	English
Institut Pasteur de Tunis. Tunis, Tunisia.	12-16 November 1984	French
Institute of Oceanography and Fisheries. Split, Yugoslavia.	15-20 April 1985	English
Laboratoire Départemental de Santé Publique. Marseille, France.	18-23 Novembre 1985	French and English

Table 2. Participants in the intercalibration exercices on microbiological methods for coastal water quality monitoring held in the Mediterranean region, under the joint coordination of UNEP and WHO.

Country of Participant	Number of participants at the exercice of						Total number of participants
	Rome	Barcelona	Athens	Tunis	Split	Marseille	
Algeria	-	1	-	-	-	1	2
Cyprus	-	-	1	-	1	-	2
Egypt	1	-	-	-	-	1	2
France	3	1	-	2	-	16	22
Greece	1	-	19	-	-	1	21
Israel	2	-	2	-	1	1	6
Italy	8	-	-	2	-	-	10
Lebanon	-	-	-	-	1	-	1
Libya	-	-	-	-	1	-	1
Malta	1	-	1	-	-	1	3
Monaco	-	1	-	-	-	-	1
Morocco	1	-	-	4	-	1	6
Spain	3	22	-	-	-	1	26
Tunisia	1	1	-	14	-	1	17
Turkey	-	-	1	-	2	-	3
Yugoslavia	2	-	2	2	9	1	16
Other	5	2	-	2	-	-	9
Total	28	28	26	26	15	25	148

MICROBIOLOGICAL METHODS

The basic objective of this series of intercalibration exercices on microbiological methods for coastal water quality monitoring was to unable participants to make bacteriological determinations in identical samples of seawater and shellfish, using the Reference Methods jointly prepared by UNEP and WHO. In addition, the exercices had the objective of obtaining further information on the comparability of the membrane filtration method and the multiple-tube method for the determination of total coliforms, faecal coliforms and faecal streptococci in seawater samples.

The knowledge and experience gained by Mediterranean scientists, both during routine monitoring activities and while participating in this series of intercalibration exercices, have resulted in a continuous revision and updating of the Reference Methods for determination of microbial indicators by the membrane filtration technique, whose most significant changes will be discussed in this study.

As the Reference Methods for determination of microbial indicators by the multiple-tube method were available later in the series of intercalibration exercices, it has been necessary to conduct most of the microbial determinations by means of interim methodologies of this analytical method.

In order to identify the activities actually carried out and the analytical methods adopted during each intercalibration exercice, the types and numbers of analyses conducted, as well as the culture media and culture conditions used in each exercice, have been summarized in table 3.

As table 3 indicates, the intercalibration exercices held in Rome and Barcelona were devoted to the determination of faecal indicators in seawater by the membrane filtration method. The actual comparison between the membrane filtration method and the multiple-tube method has been essentially carried out during the exercices held in Athens, Tunis and Marseille. The result obtained during the exercice held in Split only allowed for an intercalibration of the membrane filtration method.

Apart from minor changes in the analytical methods used during the intercalibration exercices, the most significant modification in culture media has been the one relative to faecal streptococci: while the KF-Streptococcus agar was the media adopted during the first three exercices, the M-Enterococcus agar has been the media chosen for the last three exercices. The reasons for this change will be discussed in a subsequent section of this study.

Table 3. Analyses carried out and analytical methods adopted during the intercalibration exercices on microbiological methods for coastal water quality monitoring held in the Mediterranean region, under the joint coordination of UNEP and WHO.

Intercalibration Exercice	Type of Indicator	Type of Sample	Analytical Method	Culture Media	
				MF	MPN
Rome	FC	3 seawater samples	MF	M-FC agar 44ºC	-
	FS	3 seawater samples	MF	KF-Streptococcus agar 36ºC	-
Barcelona	TC	9 seawater samples	MF	M-Endo agar 37ºC	-
	FC	9 seawater samples	MF	M-FC agar 44,5ºC	-
	FS	9 seawater samples	MF	KF-Streptococcus agar 37ºC	-
Athens	TC	2 seawater samples	MPN	-	Lactose broth 36ºC BGB broth 44ºC
	FC	2 seawater samples	MF/MPN	M-FC agar 44,5ºC	Lactose broth 36ºC EC medium 44ºC Indole 44ºC
	FS	2 seawater samples	MF/MPN	KF-Streptococcus agar 36ºC	Azide Dextrose broth 36ºC EVA broth 36ºC
Tunis	TC	3 seawater samples	MF/MPN	M-Endo agar 37ºC	Lactose broth 37ºC
	FC	3 seawater samples	MF/MPN	M-FC agar 44ºC	Lactose broth 37ºC BGB broth 44ºC Indole 44ºC

Table 3 (continued)

	FS	3 seawater samples	MF/MPN	M-Enterococcus agar 37ºC	Rothe broth 37ºC Litsky broth 37ºC
	FC	1 shellfish sample	MPN	-	Lactose broth 37ºC BGB broth 44ºC Indole 44ºC
Split	TC	3 seawater samples	MF	M-Endo agar 37ºC	-
	FC	3 seawater samples	MF/MPN	M-FC agar 44,5ºC	Lactose broth 36ºC MacConkey broth 44,5ºC Indole 44,5ºC
	FS	3 seawater samples	MF	M-Enterococcus agar 37ºC	-
	FC	1 shellfish sample	MPN	-	Lactose broth 37ºC MacConkey broth 44,5ºC Indole 44,5ºC
Marseille	FC	2 seawater samples	MF/MPN	M-FC agar 44,5ºC	A-1 44ºC Indole 44ºC
	FS	2 seawater samples	MF/MPN	M-Enterococcus agar 37ºC	Rothe broth 37ºC Litsky broth 37ºC
	FC	1 shellfish sample	MPN	-	Lactose broth 37ºC BGB broth 44,5ºC Indole 44,5ºC

STATISTICAL INTERPRETATION

The experimental results of the six intercalibration exercices have been analyzed and interpreted by means of appropiate statistical methods.

The microbial concentrations obtained by different analysts, when applying the same analytical method on identical samples, have been evaluated by means of a log-normal probability distribution. It is well known that the log-transformed values of the microbial concentrations obtained on replicate analyses of identical samples follow a normal probability distribution, and thus allows their interpretation by common statistical techniques.

A log-normal probability distribution can be completely defined by its mean and its standard deviation.

The mean value of a log-normal distribution gives an estimation of the central tendency of the experimental results. In the particular case of replicate microbiological analyses, the mean value of the log-normal distribution gives the best estimate of the microbial concentration of the sample analyzed. The geometric mean of the microbial concentrations is equivalent to the mean of the log-normal distribution.

The standard deviation of a log-normal distribution gives an estimation of the dispersion of the experimental results with respecto to their mean value. In the particular case of replicate microbiological analyses, the standard deviation of the log-normal distribution is the best estimate of the precision of the analytical method used for determining the microbial concentration of the sample analyzed.

The degree of precision of a series of replicate determinations is commonly expressed by a confidence interval of the experimental values. The 95% confidence interval is the most frequently used; as its name reflects, the 95% confidence interval designates the range of experimental results which would include, on the average, 95% of the results contained in an unlimited number of sets, each containing a considerable number of experimental values. The smaller is the 95% confidence interval of the experimental determinations, the more precise is the analytical method.

The 95% confidence interval of a log-normal distribution can be estimated by adding and substracting 1,96 times the standard deviation to the mean value. These calculations have to be carried out in terms of log-transformed data. To obtain the extreme values of the confidence interval in terms of microbial concentrations, it is necessary to calculate the antilogarithm of the values previously estimated.

In this respect, it has to be pointed out that the 95% confidence interval for the log-transformed data would be defined by the mean logarithmic value, plus or minus 1,96 times the standard deviation of the log-normal

distribution. The corresponding 95% confidence interval for the original microbial data would be defined by the antilogarithm of the mean value, multiplied or divided by the antilogarithm of 1,96 times the standard deviation of the log-normal distribution.

The statistical method used to estimate the mean and the standard deviation of the sets of replicate determinations obtained during the intercalibration exercices has been that described in the Annex 3 of the final report of the intercalibration exercice held in Barcelona (WHO/UNEP, 1985a).

The statistical method referred to above is based on a graphical estimation of the log-normal probability associated to a set of experimental microbial concentrations, by interpolation of a straight line to the experimental values drawn on a normal probability paper. The interpolation allows for rejection of aberrant readings, on the basis of statistical criteria.

This statistical method has been included into a Reference Method for evaluation and interpretation of data from microbiological monitoring of coastal recreational waters and shellfish-growing areas which, under the joint coordination of WHO and UNEP, will be published in the coming months.

Finally, the comparison of the experimental results obtained by different microbiological methods has been carried out using common statistical methods for hypothesis testing (Gibra, 1973), on the basis of the statistical parameters previously estimated.

R E S U L T S A N D D I S C U S S I O N

During the execution of the MED POL VII Project, the principal investigators of 30 Mediterranean laboratories had recommended that an intercomparison of analytical methods for determination of microbial indicators should be carried out at a regional level in the Mediterranean, to ensure comparability of results (WHO/UNEP, 1981). However, a comprehensive intercomparison of the membrane filtration method and the multiple-tube method could not be finalized during the MED POL Phase I, which ended in 1981.

At the same time, the first Reference Methods for determination of microbial indicators were being prepared under the joint coordination of UNEP and WHO. By 1982, four Reference Methods were available, which covered the determination of total coliforms, faecal coliforms and faecal streptococci in seawater by the membrane filtration method and of faecal coliforms in shellfish by the multiple-tube method. These Reference Methods were the first to be prepared in accordance with the provision of MED POL Phase II concerning sampling and analytical techniques.

ROME INTERCALIBRATION EXERCICE

In early 1982, a number of Mediterranean laboratories were requested by UNEP and WHO to test the four Reference Methods available, under their local environmental conditions, and also to carry out an inter-laboratory exercice on the comparison of the membrane filtration method and the multiple-tube method, for monitoring the microbiological quality of coastal waters.

The results of these activities would be evaluated by the Istituto Superiore di Sanità in Rome, during a consultation meeting to be held 22-23 November 1982. The consultation meeting would be followed, 24-26 November 1982, by a joint intercalibration exercice of the proposed Reference Methods, through the analysis by participants of identical seawater samples, at the laboratory facilities of the Istituto Superiore di Sanità in Rome.

The technical sessions organized by the Istituto Superior di Sanità in Rome represent thus two different approaches to the intercomparison of microbiological methods: while the first session marks the culmination of the work initiated by Mediterranean laboratories during the MED POL VII Project, in which each laboratory tested independently the two analytical methods, the second session represents the preliminary steps of the approach to be adopted in the series of intercalibration exercices, in which microbiologists from different laboratories would analyze identical samples, using the two analytical methods, under identical laboratory conditions.

It is therefore appropiate to discuss separatedly the work carried out during the two technical sessions held at the Istituto Superiore di Sanità in Rome.

Inter-Laboratory Exercice

Although the comparative analysis of the membrane filtration method and the multiple-tube method covered a wide range of topics, the differences between the contributions and methodologies adopted by the five participating laboratories rendered the process of synthesis and comparability rather difficult (WHO/UNEP, 1983).

Nevertheless, a statistical analysis of the results reported by the participating laboratories allowed to formulate the following conclusions:

1. In general, a highly significant correlation was found between the results obtained by the multiple-tube method and those provided by the membrane filtration method, when considering the same parameter and the same seawater sample.

2. Intra-station contamination variability was observed to range up to a maximum of three orders of magnitude in some cases. A variability of one to two orders of magnitude was very frequent.

3. The microbial concentrations provided by the membrane filtration method and the multiple-tube method show a highly significant correlation when the values are higher than 10 FC/100 ml.

4. Although consistent variations of the contamination level may be appropiately detected by both methods of analysis within the 1-100 FC/100 ml, agreement between corresponding results is limited if small variations are considered.

5. Contaminated and non-contaminated sites can be appropiately distinguished and classified by both methods of analysis, if a reasonable number of analytical determinations are available, the single datum is not usually significant for this purpose.

6. The differences between the two methods appear to be negligible from the general point of view. However, the problem as to which is the most reliable method may occur when particular studies are required, such as evaluation of sites which are on the borderline of bacteriological acceptability.

Reports of Participating Laboratories

In addition of the basic data used for the inter-laboratory exercice, the reports submitted by seven Mediterranean laboratories include some of the most detailed and illustrative studies on the methodological aspects governing the evaluation of the microbiological quality of coastal waters. In summary, they can be considered an excellent synthesis of the scientific work initiated within the Mediterranean region under the MED POL VII Project.

The results and discussions contained in these seven reports clearly highlight two facts: 1) the analytical determination of microbial indicators still requires further study and development, to ensure that experimental results appropiately reflect the microbial concentrations present in the samples, and 2) analytical determination of microbial indicators needs to be standardized and submitted to a continuous quality control programme, to ensure that results obtained by different analysts are reliable and comparable.

Although the simplicity of their analytical determination and the widespread character of the microbial indicators may lead to believe that they can be reliably measured by well established methods, without the need for a continuous quality control programme, the experimental work conducted by those Mediterranean laboratories clearly illustrates the fallacy of these two frequent opinions.

Considering the interest and importance of those experimental studies to interpret the results of the series of intercalibration exercices which were subsequently carried out, their most relevant conclusions have been summarized as follows:

1. A comparative study, carried by El-Sharkawi and co-workers (WHO/UNEP, 1983), of the multiple-tube method, the LES membrane filtration method and the double layer membrane filtration method for the determination of faecal coliforms shows that the multiple-tube method gives the least counts as compared to the other two methods.

   The LES method proved to be superior and more precise in faecal coliform recovery than the two-layer method. The great efficiency of the LES method may be attributed to the longer resuscitation period on the rich non-selective medium which contains simple carbohydrates. Although the LES method gave higher counts, it required incubation on two different media for two days to obtain the final results.

   The two-layer agar method provides a direct and accurate bacterial count, requires only 24 hours to complete and has the advantage of saving media, tubes, racks and space.

   In summary, the membrane filtration method gives the most convenient results, the LES method gives the highest faecal coliform count, while the two-layer method saves time, media and labor.

2. In a comparative study on the membrane filtration method and the multiple-tube method, Bernard (WHO/UNEP, 1983) reports that the faecal coliforms and faecal streptococci concentrations obtained by both methods are equivalent, with the exception of the analyses of harbor waters. Furthermore, the confidence interval obtained for both microbial indicators is larger when using the multiple-tube method than when using the membrane filtration method.

3. In a comparative study of the recovery of indicator organisms in marine waters, carried out by Yoshpe-Purer (WHO/UNEP,1983), the resuscitation step provided by the two-layer agar method recovered a larger number of faecal coliforms only in a few samples. This result led to the conclusion that the use of the two-layer agar method would not appear justified, particularly in clean beaches, in view of the amount of time and work involved.

   This study also revealed that counts of faecal streptococci were considerably higher at an incubation temperature of 35ºC than at 44,5ºC, and increased significantly with a 2-2,5 hours resuscitation period. In 90% of the samples, the faecal streptococci counts were significantly higher by the direct filtration method at the 35ºC than by the multiple-tube method.

   No explanation could be offered for the enormous discrepancy between the recovery rate of faecal streprococci by the membrane filtration method on KF agar and the multiple-tube method. However, among the colonies that were confirmed, from 10 to 30% were not streptococci, indicating the limited selective character of the KF agar.

   Since the PSE medium has the advantage of even shorter incubation time, and has been reported by some authors to be more selective in some environments, it may be desirable to compare the two media for selecting the most suitable for Mediterranean waters.

4. In a comparison of microbiological methods for monitoring indicator organisms in seawater and sand, carried out by Vila and co-workers (WHO/UNEP, 1983), a highly significant correlation was found between the faecal coliforms counts obtained by the membrane filtration method and the multiple-tube method. The two concentrations appear to be basically equivalent, although the membrane filtration results are somewhat lower than those obtained by the multiple-tube method, particularly when contamination levels are low.

   Log-transformed values were considered, because such transformation allows to obtain a Gaussian-like statistical distribution, thus simplifying the statistical treatment of data.

   All data obtained indicate that the total coliforms, faecal coliforms and faecal streptococci concentrations in the seawater and in the sand samples are generally correlated. The results obtained allow an easy classification of site contamination, when several measurements are considered, and the mean microbial values appear to be the best quality indicator. Single values are scarcely significant for this purpose.

   The repeatability of microbial determinations, as tested by replicate analyses of the same samples, appeared to be characterized by a log-data standard deviation (natural logarithms) of approximately 0,7-0,8, corresponding to a factor of 2 for

original data in the case of total coliforms and faecal coliforms, and slightly better for faecal streptococci.

5. In a comparative study of analytical methodologies for the microbiological analysis of seawater, carried out by Feliú and co-workers (WHO/UNEP, 1983), the total coliforms, faecal coliforms and faecal streptococci counts obtained by the membrane filtration method were not significantly different from those obtained by the multiple-tube method, except in the case of lower concentrations of total coliforms, where the values obtained by the membrane filtration method were higher than those provided by the multiple-tube method.

   The concentrations of total coliforms and faecal coliforms obtained by the membrane filtration method using absorbent pads or agar do not show significant differences except in the case of lower concentrations of total coliforms, when the use of absorbant pads gives slightly better results than when agar is used.

   Under the economic conditions prevailing at the laboratory conducting this study, the average cost of the material and equipment necessary to carry out the analysis of a microbial indicator is slightly higher for the multiple-tube method than for the membrane filtration method. Furthermore, the personal time required to conduct a microbiological analysis by the multiple-tube test method is approximately double than the time required by the membrane filtration method.

6. In a comparative study of analytical methods for the microbiological surveillance of costal waters and sediments, carried out by Jekov and co-workers (WHO/UNEP, 1983), the determination of total coliforms and faecal coliforms was conducted by the multiple-tube method using three alternative culture media: the MacConkey broth, the lactose broth and the brilliant green bile broth.

   The results obtained clearly show the highest capacity of the lactose broth over the two other lactose-containing broths to determine the pollution level of a water sample in term of total coliforms and faecal coliforms.

   The lactose broth seems to provide suitable conditions for the recovery of stressed coliform bacteria, while selective broths, such as MacConkey broth or brilliant green bile broth, seem to pose further difficulties for growth and proliferation of these microbes and thus preclude their detection in the laboratory.

   In summary, the multiple-tube method appears to be superior to the membrane filtration method for determining the pollution level of water, regardless of the type of lactose broth used. Nevertheless, the study points out that the number of samples considered in this case is too small to be able to establish a valid statistical conclusion concerning the superiority of the multiple-tube method over the membrane filtration method.

7. In a contribution to the comparative study of analytical methods for detection of microbial indicators, Fuks (WHO/UNEP, 1983) reports some discrepancies between the results obtained using the membrane filtration method and the multiple-tube method. Higher bacterial counts were obtained in 67% of the samples when analyzed by the membrane filtration method as compared to the results obtained by the multiple-tube method. However, a statistical evaluation of the results only showed significant differences between both methods in one sampling station, for faecal streptococci concentrations.

   Although the membrane filtration method and the multiple-tube method for the enumeration of total coliforms, faecal coliforms and faecal streptococci do not always yield the same results, both methods provide substancially the same information.

As a result of the discussions held in the inter-laboratory exercice and in the reports prepared by participating laboratories, the participants in the meeting agreed on an outline summary of the essential modifications to be introduced in the text of the four Reference Methods considered during the exercice. These modifications, which appear in the Annex 10 of the Rome meeting report (WHO/UNEP, 1983), were concerned with culture media, incubation conditions and colonies identification. Participants expressed their belief that strict adherence to these analytical procedures would improve the reliability and precision of the analytical results.

Joint Intercalibration Exercice

The exercice originally included the determination of total coliforms, faecal coliforms and faecal streptococci by both the membrane filtration method and the multiple-tube method. However, considering the time available during the exercice, it was decided to limit the analyses to the determination of the three microbial parameters by the membrane filtration method. Three water samples were therefore analyzed.

Participants were devided into six groups of two persons each. Results obtained by individual group were in relative agreement, although the hypothesis of a statistically significant difference among participant could be considered. Moreover, a statistically significant difference could be considered between the two estimates obtained from two different dillution factors.

Considering that this was the first intercalibration exercice organized in the framework of MED POL, the results were considered satisfactory and the experience gained was regarded as the basis on which future intercalibration exercices could be organized. Furthermore, the exercice provided an excellent opportunity for participants to compare their analytical techniques during actual laboratory work, rather than through simple discussion.

The statistical parameters of each of the microbiological determinations carried out have been estimated by means of a log-normal probability distribution, graphically adjusted to the experimental results. These parameters are summarized in table 4.

Table 4 shows that the standard deviation of faecal coliforms and faecal streptococci analyses are approximately 0,45 and 0,18 respectively. This represents that the 95% confidence interval of replicates analyses ranges from 2,4 times higher to 2,4 times lower than the mean faecal coliform concentration, and from 1,4 times higher to 1,4 times lower than the mean faecal streptococci concentration.

These replicate analyses are thus considerably more precise than those reported by Villa and co-workers (WHO/UNEP, 1983) that, characterized by a logarithmic standard deviation of 0,7-0,8, had a 95% condifence interval from 4,4 times higher to 4,4 times lower than the mean value.

BARCELONA INTERCALIBRATION EXERCICE

The exercice included the determination of total coliforms, faecal coliforms and faecal streptococci by the membrane filtration method in nine seawater samples, using the incubation conditions indicated in table 3. Participants were divided into seven groups of three persons each.

The statistical parameters of each of the microbial determinations carried out were obtained during the intercalibration exercice and are summarized in table 5.

A review of the results shown in table 5 reveals a great dispersion of bacterial concentrations, with standard deviation values considerably higher than those obtained during the intercalibration exercice held in Rome and that carried out in Catalonia during the summer of 1983 (WHO/UNEP, 1985a). While standard deviations obtained during the exercice in Rome were in the range of 0,18-0,45, those obtained in Catalonia by a single analyst conducting five replicate analyses of numerous water samples were in the range 0,08-0,11.

Among the possible explanations for the variations observed among individual results in Barcelona, the following were proposed (WHO/UNEP, 1985a):

1. The differences in the bacteriological training of the participants. Results from the first to the third day showed that participants were adjusting to the analytical technique used.

2. The heavy rainfall in the region during the exercice brought a land-based input to the coastal waters, which greatly increased their bacterial load and their suspended matter content. As a result, a large number of dilutions were necessary, bringing a greater risk of error when taking representative volumes of water in the dilution flasks.

Table 4. Statistical parameters of the log-normal probability distributions estimated from the sets of microbiological results obtained by the membrane filtration method at the Intercalibration Exercice in Rome.

Seawater sample	Number of replicate analyses	Faecal coliforms		Faecal streptococci	
		Mean FC/100 ml	Standard deviation	Mean FC/100 ml	Standard deviation
A-1	6	$1,9\ 10^5$	0,47	$4,7\ 10^3$	0,18
B-1	6	3	0,76	5	0,18
C-1	6	10	0,41	10	0,13

Table 5. Statistical parameters of the log-normal probability distributions estimated from the sets of microbiological results obtained by the membrane filtration method at the Intercalibration Exercice in Barcelona.

Seawater sample	Number of replicate analyses	Total coliforms		Faecal coliforms		Faecal streptococci	
		Mean TC/100 ml	Standard deviation	Mean FC/100 ml	Standard deviation	Mean FS/100 ml	Standard deviation
A-1	7	$68\ 10^5$	1,88	$2\ 10^5$	2,42	$57\ 10^3$	0,89
B-1	7	$13\ 10^5$	0,85	$13\ 10^5$	0,25	$32\ 10^3$	0,82
C-1	7	$16\ 10^4$	1,59	$35\ 10^3$	0,63	$16\ 10^3$	0,59
A-2	7	$89\ 10^5$	0,64	$56\ 10^4$	0,84	$32\ 10^3$	0,72
B-2	7	$12\ 10^5$	0,60	$11\ 10^4$	1,34	$35\ 10^2$	0,50
C-2	7	$16\ 10^4$	0,78	$39\ 10^3$	0,57	$18\ 10^3$	0,71
A-3	7	$30\ 10^4$	0,92	$63\ 10^3$	0,58	$10\ 10^3$	0,56
B-3	7	$17\ 10^4$	0,37	$23\ 10^3$	0,80	$50\ 10^2$	0,74
C-3	7	$25\ 10^4$	0,36	$20\ 10^3$	0,87	$24\ 10^2$	1,22

The considerable variation of results among participants, as measured by standard deviations close to and above 1,0, showed how essential it is to perform with great care the homogenization and dilution operations required in the bacteriological analysis of seawater.

ATHENS INTERCALIBRATION EXERCICE

The exercice included the determination of total coliforms by the multiple-tube method and of faecal coliforms and faecal streptococci by both the membrane filtration method and the multiple-tube method. Two seawater samples were analyzed using the incubation conditions indicated in table 3. Participants were divided into five groups of three persons each.

The statistical parameters of each of the microbial determinations carried out have been estimated by means of a log-normal probability distribution, graphically adjusted to the experimental results. These parameters are summarized in table 6.

Table 6 clearly indicates that the standard deviations of the analyses conducted by the multiple-tube method are higher than those associated to the analyses carried out by the membrane filtration method.

The 95% confidence interval for bacterial counts by the membrane filtration method could be approximately defined by 1,15 times above and below the mean value of a series of replicate analyses, which is indeed an excellent degree of precision. On the other hand, the corresponding 95% confidence interval for counts obtained by the multiple-tube method could be defined by 1,5 times above and below the mean value of the replicate analyses, which is still a very acceptable level of precision.

These results support the conclusion that the membrane filtration method provides more precise results than the multiple-tube method. Nevertheless, the continuous and detailed checking carried our during the exercice, with respect to the analytical and counting procedures followed by individual participants, has undoubtedly resulted in excellent levels of precision for both methods of analysis.

The hypothesis testing included in table 6 shows that the mean concentration estimated for faecal coliforms in sample A by the membrane filtration method is significantly higher than the one estimated by the multiple-tube method.

On the contrary, there is no statistically significant difference between the mean faecal streptococci concentrations obtained by both methods of analysis for the seawater sample A.

The microbial concentrations of seawater sample B were too small to allow a meaningful comparison between analytical methods.

Table 6. Statistical parameters and hypothesis testing of the log-normal probability distributions estimated from the sets of microbiological results obtained at the Intercalibration Exercice in Athens.

Analytical method	Number of replicate analyses	Faecal coliforms		Faecal streptococci	
		Mean FC/100 ml	Standard deviation	Mean FS/100 ml	Standard deviation
Membrane filtration	5 of sample A	$38\ 10^3$	0,05	$26\ 10^3$	0,08
Multiple-tube	5 of sample A	$22\ 10^3$	0,16	$27\ 10^3$	0,23

Hypothesis testing (5% level of significance) :

Sample A : MF higher than MPN ; No difference

Finally, the majority of the participants recommended that faecal coliforms identification by the multiple-tube method should be based on their ability ot utilize lactose and to grow at 44,5ºC. Since the requirement to produce indole is not included in the routine analysis by the membrane filtration method, this identification criterion should be omitted from the multiple-tube method.

TUNIS INTERCALIBRATION EXERCICE

The exercice included the determination of total coliforms, faecal coliforms and faecal streptococci by both the membrane filtration technique and the multiple-tube method. Three seawater samples and one shellfish sample were analyzed using the incubation conditions indicated in table 3. Participants were divided into eight groups of three persons each.

The statistical parameters of each of the microbial determinations carried out have been estimated by a log-normal probability distribution graphically adjusted to the experimental results. These parameters are summarized in table 7. The results of shellfish analyses were all above the normal detection limit of the method, 2400 faecal coliforms by 100 g, and did not allow their statistical evaluation.

Table 7 does not show a clear indication of the relative degree of precision of the membrane filtration method and the multiple-tube test. In samples A and B, the membrane filtration method has small standard deviation for total coliforms and faecal coliforms, and thus a higher precision level, tant the multiple-tube method. Opposite results were obtained with sample C, and with all the faecal streptococci analyses of the three water samples.

The hypothesis testing included in table 7 shows that, for sample A, the concentration estimated for the three microbial indicators by the multiple-tube test is significantly higher than that estimated by the membrane filtration method. Exactly the contrary occurs for the seawater samples B and C.

Considering the limited influence that the dillution procedure may have on the analysis of samples B and C, due to their relatively low microbial concentrations, it could be concluded that the membrane filtration method does not give significantly lower microbial concentrations that the multiple-tube method, in samples with intermediate or low microbial pollution.

Although the multiple-tube method gives significantly higher results than the membrane filtration method for the seawater sample with considerable microbial pollution, the relatively high standard deviations observed in this case preclude from reaching a definite conclusion.

Table 7. Statistical parameters and hypothesis testing of the log-normal probability distributions estimated from the sets of microbiological results obtained at the Intercalibration Exercice in Tunis.

Analytical method	Number of replicate analyses	Total coliforms		Faecal coliforms		Faecal streptococci	
		Mean TC/100 ml	Standard deviation	Mean FC/100 ml	Standard deviation	Mean FS/100 ml	Standard deviation
Membrane filtration (MF)	8 of sample A	2200	0,31	480	0,32	150	0,85
Multiple-tube	23 of sample A	3200	0,67	1700	0,62	330	0,38
Membrane filtration	8 of sample B	780	0,15	96	0,36	13	0,89
Multiple-tube	23 of sample B	140	0,30	50	0,35	4	0,23
Membrane filtration	8 of sample C	30	0,94	3	1,22	2	1,20
Multiple-tube	23 of sample C	17	0,19	5	0,40	2	0

Hypothesis testing (5% level of significance):

Sample A	MPN higher than MF	MPN higher than MF	MPN higher than MF
Sample B	MF higher than MPN	MF higher than MPN	MF higher than MPN
Sample C	no difference	no difference	no difference

SPLIT INTERCALIBRATION EXERCICE

The exercice included the determination of total coliforms, faecal coliforms and faecal streptococci by the membrane filtration method, and of faecal coliforms by the multiple-tube method. Three seawater samples and one shellfish sample were analyzed using the incubation conditions indicated in table 3. Participants were divided in seven groups of two persons each.

The statistical parameters of each of the microbial determinations carried out have been estimated by a log-normal probability distribution, graphically adjusted to the experimental results. These parameters are summarized in table 8. The results of the analyses by the multiple-tube method, both of the seawater samples and of the shellfish sample, were above the normal detection limit of the method, 2400 colonies by 100 ml, and therefore did not allow their evaluation and subsequent comparison with the results of the other method.

Table 8 does not show a clear indication of the degree of precision of the membrane filtration method. The standard deviation for the three microbial indicators in samples A and C are in the range of 0,12 to 0,40, which can be considered a relatively satisfactory degree of precision. However, the standard deviation for the three microbial indicators in sample B vary from 0,47 to 1,12, considerably higher values than those obtained in the two other samples, and thus indicate an unsatisfactory degree of precision.

The reasons for this discrepancy are most likely related to the dilution procedure required to bring the colony counts to the recommended intervals. The unexpected high pollution level of the samples may have contributed to make the dilution procedure the critical step of the analytical method.

This high level of pollution also resulted in a positive reading on all the inoculated tubes, and therefore prevented from making a comparative study of the two methods of microbiological analysis.

MARSEILLE INTERCALIBRAITON EXERCICE

The exercice included the determination of faecal coliforms and faecal streptococci in two seawater samples by both the membrane filtration method and the multiple-tube method. A shellfish sample was also analyzed for faecal coliforms by the multiple-tube method. The incubations conditions used are indicated in table 3. Participants worked on an individual basis.

Table 8. Statistical parameters of the log-normal probability distributions estimated from the sets of microbiological results obtained by the membrane filtration method at the Intercalibration Exercice in Split.

Seawater sample	Number of replicate analyses	Total coliforms		Faecal coliforms		Faecal streptococci	
		Mean TC/100 ml	Standard deviation	Mean FC/100 ml	Standard deviation	Mean FS/100 ml	Standard deviation
A	7	-	-	$14\ 10^7$	0,18	$28\ 10^6$	0,12
B	7	$33\ 10^5$	0,83	$30\ 10^5$	1,12	$54\ 10^4$	0,47
C	7	$60\ 10^2$	0,40	$28\ 10^2$	0,27	$18\ 10^2$	0,23

The statistical parameters of each of the microbial determinations carried out have been estimated by a log-normal probability distribution, graphically adjusted to the experimental results. These parameters are summarized in table 9.

Table 9 indicates that the standard deviations of seawater analyses are generally lower for the membrane filtration method than for the multiple-tube method. Therefore, the multiple-tube method would provide less precise microbial concentrations than the membrane filtration method. Overall, the precision of both methods can be classified as adequate, considering that the standard deviations vary from 0,16 to 0,39.

The adoption of common criteria for counting the colonies grown on the membranes and for interpreting the positive tubes appears to be one of the steps mainly responsable for the levels of precision observed.

Table 9 also shows that the standard deviation of the faecal coliforms analyses by the multiple-tube method is considerably large, indicating that this method of analysis has a relatively low precision. To illustrate this observation, the 95% confidence interval of the faecal coliforms concentration can be estimated as from 0,6 to 820 FC/100 g of flesh. Finally, it has to be pointed out the excellent agreement between the experimental results from the shellfish analyses and the straight line representing the corresponding log-normal distribution.

The hypothesis testing included in table 9 indicates that, for mean microbial concentrations below 100 colonies/100 ml, the membrane filtration method gives significantly higher values than the multiple-tube method in two cases, while the multiple-tube method gives a significantly higher value than the membrane filtration in one case. In another case, there is no significant difference between the two methods.

Considering the range of mean microbial concentrations observed, it could be concluded that both methods of analysis give an equivalent estimation of the microbial content of a seawater sample.

Table 9. Statistical parameters and hypothesis testing of the log-normal probability distributions estimated from the sets of microbiological results obtained at the Intercalibration Exercice in Marseille.

Analytical method	Number of replicate analyses	Faecal coliforms		Faecal streptococci	
		Mean FC/100 ml	Standard deviation	Mean FS/100 ml	Standard deviation
Membrane filtration	18 of sample A	20	0,20	7	0,32
Multiple-tube	18 of sample A	40	0,26	7	0,29
Membrane filtration	18 of sample B	25	0,17	55	0,16
Multiple-tube	18 of sample B	10	0,30	22	0,39
Multiple-tube	18 of shellfish flesh	22(*)	0,80		

Hypothesis testing (5% level of significance)		
Sample A:	MPN higher than MF	no difference
Sample B:	MF higher than MPN	MF higher than MPN

(*) Expressed in FC/100 g of flesh.

DETERMINATION OF FAECAL STREPTOCOCCI

The studies carried out and the experience gained by Mediterranean scientists in the selection of the most adequate culture media for faecal streptococci clearly illustrates the benefits of the work conducted during the MED POL VII Project, and specially during the execution of the intercalibration exercices organized within the framework of MED POL Phase II.

The recommended procedure for faecal streptococci determination was initially established in the Guidelines for Health Related Monitoring of Coastal Water Quality (WHO/UNEP, 1977). The main objectives of the Guidelines was to select and harmonize procedures and analytical methods and thus contribute to the better and more efficient surveillance of beaches, marine waters and shellfish waters. Faecal streptococci determination was based on growth of red to pink colonies within 48 hours at 44ºC on KF-Streptococcus agar.

For unknown reasons, the culture media provided by WHO/UNEP to Mediterranean laboratories participating in the MED POL VII Project was the M-Enterococcus agar. Therefore, the faecal streptococci analyses included in the monitoring programme of coastal recreational waters and shellfish growing waters in the Mediterranean were carried out by incubation on M-Enterococcus agar at 37ºC.

Preliminary results by principal investigators of the MED POL VII Project indicated that the use of KF-Streptococcus agar provides higher colony counts than the M-Enterococcus agar and, in this respect, the KF-medium is superior to the M-Enterococcus medium (WHO/UNEP, 1983). Futhermore, studies from Mediterranean laboratories indicated that counts obtained at 35ºC on KF-Streptococcus agar were considerably higher than those obtained at 44ºC (Yoshpe-Purer, WHO/UNEP, 1983), and a comparative study on membrane filtration method (Havelaar and Engel, 1981) also indicated that the ratio between counts at 37ºC and those at 44ºC varied from 0,9 to 2,5.

An incubation temperature of 36ºC on KF-Streptococcus agar was therefore included into the outline summaries of modifications to be made on the Reference Methods recommended by WHO/UNEP (WHO/UNEP, 1983).

However, a clear disadvantage of the KF-Streptococcus agar was also noted, as 10-30% of the colonies confirmed by one laboratory were not streptococci (Yoshpe-Purer, WHO/UNEP, 1983), and as confirmation of the pinpoint colonies frequently present in large numbers represent group D streptococci in a varrying but sometimes low percentage (Havelaar and Engel, 1981).

During the execution of the MED POL Phase II, it has become apparent that the faecal streptococci concentrations of Mediterranean waters were not adequately determined by incubation on KF-Streptococcus agar. At the Marseille Intercalibration Exercice, the point was raised that the

KF-agar was not a sufficiently selective medium for detection of faecal streptococci, and results in false positives. M-Enterococcus agar was considered a better medium, although it could give rise to false negatives (WHO/UNEP, 1986).

Taking into account that the results from analyses carried out by the multiple-tube method, using the Rothe and Litsky broths, and the membrane filtration method, using the M-Enterococcus agar, did not show a statistically significant difference between them (Feliú, WHO/UNEP, 1983), and that analyses on KF-Streptococcus agar may give a considerable number of false positive colonies, it can be expected that the microbiological evaluation of coastal waters using the KF-Streptococcus agar may suffer from unnecessary interferences with respect to that obtained using the multiple-tube method.

The need to ensure the comparability of the results obtained by different laboratories, and the practical implications that different microbial concentrations may have when assessing coastal waters quality by current microbiological criteria and standards, clearly indicate that a comparative study of the multiple-tube method, using the Rothe and Litsky broths, and the membrane filtration method, using the KF-Streptococcus agar and the M-Enterococcus agar, should be carried out in the Mediterranean region as soon as possible.

CONCLUSIONS

The general objective of this study was to review and evaluate the experimental results obtained and the conclusions reached during the series of six intercalibration exercices on microbiological methods for coastal water quality monitoring, conducted in the Mediterranean region from 1982 to 1985.

From the analysis and discussion carried out the following conclusions can be made:

1. The intercalibration exercices have provided an excellent opportunity, without precedent at a regional level in the Mediterranean, to assess the factors influencing the reliability and precision of the analytical methods for microbiological evaluation of coastal waters.

2. Unforeseen fluctuations of coastal water quality, specially due to land-based discharges by heavy rainfall, have resulted in unexpectedly high concentrations of microorganisms and suspended matter. These conditions have made the experimental results to exceed the detection limits adopted for the analyses and have imposed a greater source of analytical error when transferring representative sample volumes to the dilution flasks.

3. The analytical steps which need to be further specified and standardized are: the sample dilution procedure, the culture media, the criteria for identification and counting of either bacterial colonies or positive tubes, and the basic statistical procedures for interpreting experimental results.

4. It is absolutely essential that the microbiological analysis of coastal waters and shellfish be carried out in strict accordance with the recommended methods. It has to be pointed out that a method of analysis is not only defined by the technical principle for bacterial growth, either the membrane filter or the indution tube, but also by a series of steps that go from the preparation and dilution of the sample to the identification and counting of the colonies or positive tubes.

5. The membrane filtration method gives more precise results than the multiple-tube method. The dilution of the sample before the analysis, and the identification and counting of the colonies or positive tubes, are the two factors mainly influencing the precision of both microbiological methods, specially the membrane filtration method.

6. A careful dilution of the sample and a standardized interpretation and counting of the colonies, when conducting replicate analyses by the membrane filtration method, may result in microbial concentrations whose 95% confidence interval is defined by 1,15 times above and below their mean value. This is an excellent degree of precision. Similar operating conditions with the multiple-tube method may result in a multiplication factor of 1,5.

7. An incorrect dilution process and an inconsistent interpretation and counting of colonies or positive tubes have resulted in considerable low levels of precision for both methods of analysis, with 95% confidence intervals defined by more than an order of magnitude above and below the mean microbial concentration.

8. The culture media used has a large influence on the microbial concentrations obtained by either the membrane filtration method or the multiple-tube method. The use of lactose broth has been shown to be the most effective method for recovering coliform bacteria. The omission of the indole test for the determination of faecal coliforms would make the differentiation criteria of the multiple-tube method equivalent to that of the membrane filtration method.

9. The KF-Streptococcus agar used for faecal streptococci determination by the membrane filtration method gives a considerable number of false positive colonies, and thus introduces a positive interference on analyses conducted by this method. Although it is recognized that there is no culture medium with a satisfactory selective character for these microorganisms, the M-Enterococcus agar should be considered as a more adequate choice, even if it may underestimate the number of microorganisms.

10. The membrane filtration method and the multiple-tube method adopted in these exercices give comparable results when analyzing for total coliforms and faecal coliforms. However, out of 9 comparative analyses, the membrane filtration method gave significantly higher concentrations than the multiple-tube method in 4 cases, while there was no significant difference between the concentrations obtained by the two methods in 2 other cases.

11. The analytical interferences introduced by the dilution of the samples and the interpretation and counting of colonies or positive tubes seem to be responsible for the inconsistencies observed in the comparative study of the two microbiological methods.

12. There was no significant difference between the faecal streptococci concentrations obtained in the analysis of a single seawater sample by the multiple-tube method and the membrane filtration method, using the KF-Streptococcus. However, there is considerable evidence, both in the Mediterranean and elsewhere, that the KF-Streptococcus agar gives significantly

higher concentrations than the multiple-tube method, using the Rothe and Litsky broths.

13. The membrane filtration method and the multiple-tube method give comparable faecal streptococci concentrations in the analysis of seawater samples, using the M-Enterococcus agar and the Rothe and Litsky broths. Out of 5 comparative analyses, the membrane filtration method gave significantly higher concentrations than the multiple-tube method in 2 cases, while there was no significant difference between the concentrations obtained by the two methods in 2 other cases.

14. The objective interpretation of microbiological results still needs to be fully implemented in many Mediterranean laboratories. The routine application of basic statistical principles, as proposed in a forthcoming WHO/UNEP Reference Method, should have greatly helped participating laboratories in evaluating their experimental results and in interpreting the factors governing the precision of their analytical methods.

15. The numerical values obtained for the statistical parameters associated to the microbiological methods considered in these exercices give a clear quantitative indication of the levels of precision that may be reached under different operating conditions. This information should greatly contribute to a better understanding of the factors influencing the precision of the method in each particular case and, therefore, to reach a better quality control of the microbiological analyses.

16. In summary, the analytical determination of microbial indicators for coastal water quality monitoring needs to be further standardized and submitted to a continuous quality control programme, to ensure that results obtained by different analysts are reliable and comparable.

RECOMMENDATIONS

From the results and conclusions obtained during this study, the following recommendations can be made:

1. The intercalibration of analytical methods for microbial indicators should be further continued, to ensure the comparability of results. However, the planning and execution of future intercalibration exercices should be done in such a manner as to avoid the consecuences of unexpectedly high levels of microorganisms and of suspended matter, and to ensure a statistically adequate number of replicate samples.

   Specifically, to ensure the comparability of the faecal streptococci concentrations obtained by the membrane filtration method and the multiple-tube method, a comparative study of the KF-Streptococcus agar, with respect to the Rothe and Litsky broths and the M-Enterococcus agar, should be carried out as soon as possible.

   The practical implications of these results when assessing coastal water quality by current environmental quality criteria and standards make this particular study of the upmost importance.

2. An attempt should be made to carry out an intercalibration exercice in which replicate analyses of seawater are sent to participating laboratories over, at least, a year period. This approach would allow to obtain a large number of results from each laboratory and would ensure that analytical determinations are carried out under local laboratory conditions.

   The experience gained during similar intercalibration exercices, successfully carried out in small areas of the Mediterranean, would greatly contribute to the implementation of this type of exercice at a regional level in the Mediterranean.

3. Comparative studies of analytical methods for microbial indicators should be preferably based on comparisons of chronological series of results obtained for a given sampling station, rather than on comparisons of replicate analyses of water samples from different sampling stations. This approach would allow an evaluation of the comparability of the analytical methods to assess the quality of a sampling station, which is one of the basic objectives of the coastal water quality control programme included in the MED POL Phase II.

4. Current Reference Methods for the analytical determination of microbial indicators should incorporate the modifications recommended during this series of intercalibration exercices. The new texts should be widely distributed, to ensure that Mediterranean laboratories benefit from this regional experience and conduct the analyses in the same standardized manner.

   Revision and updating of the Reference Methods should be made on a timely basis, as to ensure an efficient and continuous progress of the proposed methodology.

5. The upcoming Reference Method on statistical evaluation of microbiological data represents a basic statistical tool for quality control in microbiological laboratories, and therefore should be made available as soon as possible.

   The contents of that Reference Method, together with the material included in this summary report and the results from the monitoring programmes being conducted by Mediterranean laboratories, could be the main subjects to be discussed during a training session of Mediterranean microbiologists, which should be organized with the objective of developing their capabilities in data evaluation and quality control.

6. Additional training of Mediterranean microbiologists should be conducted to ensure that analytical methods are fully understood and strictly applied, specially during dilution of the samples, identification and counting of the colonies or positive tubes, and calculation of results.

   These training sessions, organized as part of the assistance component of the MED POL Phase II, would provide an excellent opportunity for discussing the technical aspects of quality control and comparisons of microbiological results, as well as for the revision and updating of the Reference Methods for microbiological analysis.

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