COMMISSION OF THE EUROPEAN COMMUNITIES

environment and quality of life

Report of a feasibility study on the distribution and use of simulated water samples for comparative bacteriological analysis

1978

EUR 6037 EN

COMMISSION OF THE EUROPEAN COMMUNITIES

environment and quality of life

Report of a feasibility study on the distribution and use of simulated water samples for comparative bacteriological analysis

by

G.I. BARROW & D.C. MILLER
Public Health Laboratory, Royal Cornwall Hospital (City), Infirmary Hill,
Truro, Cornwall, TR1 2HZ

R.D. GRAY & G.H. LOWE
Public Health Laboratory, Clytha Square,
Newport, Gwent, NPT 2TZ

Environment and Consumer Protection Service
Directorate General 'Employment and Social Affairs'

1978

EUR 6037 EN

Published by the
COMMISSION OF THE EUROPEAN COMMUNITIES
Directorate-General
'Scientific and Technical Information and Information Management'
Bâtiment Jean Monnet
LUXEMBOURG

LEGAL NOTICE

A bibliographical slip can be found at the end of this volume

Printed in Belgium

ISBN 92-825-0530-8 Catalogue number : CD-NO-78-018-EN-C

C O N T E N T S

INTRODUCTION

A Technical Seminar commissioned by the Health and Safety Directorate for comparison of some of the media and methods used in member states of the European Economic Community (EEC) for the bacteriological examination of drinking water was held at the Pasteur Institute at Lyon in 1975 (Vial, 1976). The results obtained were subsequently discussed at a meeting of Technical Experts at Luxembourg where it was agreed that the Seminar had been very useful in permitting exchange of information and ideas as well as in pin-pointing some of the difficulties inherent in comparing different media and methods employed in different countries. At this meeting, a small group of experts was asked to formulate proposals for future work, and these were later discussed. The proposals included the distribution of simulated water samples for bacteriological analysis as currently used for quality control purposes in the Public Health Laboratory Service (PHLS) in Britain for coliform organisms and Eschericia coli (faecal coli). The Commission agreed that a feasability study

of this method should be carried out among member states during 1976 - 77. This report describes briefly the nature of the study, the work done and the results obtained. Despite some limitations, the study confirms that the distribution of simulated water samples for bacteriological analysis is both feasible and practicable among countries within the EEC. We suggest that such comparative studies should be continued and later expanded to include the distribution of other indicator organisms as well as various culture media with detailed instructions for their preparation and use. In this way, considerable harmonization could well be achieved, not only of the media and methods used but also of the way in which results are recorded, interpreted, and reported.

MATERIAL & METHODS

THE STUDY

For this feasability study, arrangements were made for three distributions of specimens each containing known numbers of coliform organisms during 1976. The specimens consisted of stable suspensions of concentrated viable bacteria in a modified glutamate medium containing a preservative. These were distributed in bijoux bottles together with detailed instructions for the preparation and examination of the simulated water samples on specified dates. All EEC laboratories which participated in the Technical Seminar at Lyons were asked to examine these simulated samples by their normal routine media and methods. For comparison, a similar number of PHLS laboratories in the United Kingdom were also asked to examine and report similarly on the same samples. On each occasion, the issuing laboratory prepared and examined five samples of each specimen on

the specified dates by both multiple tube and membrane filtration methods after postal distribution and return within the United Kingdom. These formed the basis of the "expected" results. In order to record the information and results, standard forms were devised for use by all laboratories; despite their apparent simplicity, however, it is clear that these forms were not fully understood by some participants, possibly because they normally record and report their results in a different way. Although customs clearance for these specimens could not be guaranteed, we were assured by the relevant authorities that the postal arrangements were satisfactory and that, given normal conditions, there should be no undue delays. In addition, arrangements were made to transcribe the results for statistical analysis by computer at the Epidemiological Research Laboratory, Colindale. Preliminary and final reports on the results obtained were sent to all participants after each distribution.

SIMULATED WATER SAMPLES

These were prepared and distributed as previously described by Gray and Lowe (1976) for laboratory quality control purposes in the United Kingdom. Briefly, varying numbers of selected strains of Esch. coli and of Klebsiella aerogenes were added to lactose- and indicator-free improved formate glutamate medium (Gray 1964) containing 1.8% boric acid as a preservative. The specimens were given arbitrary descriptions such as "rural unchlorinated water" and "shallow well water", etc., consistent with their content of organisms. These were kept at room temperature in the dark until the date for examination. Detailed instructions for preparing these simulated water samples for bacteriological analysis on the dates

specified were given with each distribution (see Appendix A). Essentially, this consisted of adding the specimens to a stated volume of sterile deionized water so that the prepared samples theoretically should then contain calculated numbers of viable organisms with the bacteriostatic effect of boric acid diluted out. The importance of thoroughly shaking the samples to ensure homogenous distribution of the organisms during all stages of preparation and examination was stressed.

After reconstitution of the simulated water samples by dilution of the bacterial suspensions, all laboratories were asked to examine them by their usual methods - multiple tubes, membrane filtration or both - as though they were normal routine samples of water and to record and express their results in terms of numbers of coliform organisms and/or Escherichia coli (faecal coliforms) present per 100 ml. on the forms provided (see Appendix B). It was appreciated that because of the many variations in media and techniques used among different EEC countries, direct comparison of results would not be possible at this stage, although some idea of their range would be obtained. It would also yield useful information on the analytical methods as well as the confirmatory tests and incubation times and temperatures used.

STATISTICAL ANALYSIS

All the results received for each distribution were transcribed where possible for analysis by computer. Because the bacterial content of each sample was different, it was not possible to give combined total laboratory values, and the results of the three distributions must therefore be considered separately. Preliminary

and final reports on each distribution were sent to all participants, and these are included in this paper as Appendices C, D, and E.

For each specimen in each distribution, the issuing laboratory kept 2 bottles and posted 3 other bottles of the concentrated suspensions of organisms to another laboratory for subsequent return by post. All 5 of these samples were prepared and examined on the specified dates by (1) the multiple tube (MT) method (1 x 50ml; 5 x 10ml; 5 x 1.0ml and where necessary 5 x 0.1ml) with minerals modified glutamate medium incubated for 48 hours at 37°C. and (2) by the membrane filtration (MF) method with 0.4% enriched teepol broth (Oxoid) using 100ml each respectively for coliform organisms at 37°C with pre-incubation at 25°C for 4 hours and for E. coli at 44°C with pre-incubation for 6 hours at 30°C (Report 1969). The maximum and minimum numerical values thus obtained for each organism in each sample by either method were regarded as the "expected" results. In practice, individual laboratory values for any sample within a range of twice the maximum and half the minimum values of those "expected" were arbitrarily regarded as "satisfactory".

With each method (MT and MF), the range, the mean and standard deviation values were determined for the results for each sample from (a) the issuing laboratory (b) all EEC laboratories (c) all PHLS laboratories and (d) both EEC and PHLS laboratories, thus allowing some comparisons to be made. These values are shown in Appendices C, D, and E.

RESULTS

Much of the information obtained has already been summarized in the preliminary and final reports issued after analysis of the

results of each distribution (see Appendix C, D, and E). On each occasion, the samples were sent to a total of 27 EEC laboratories and 21 constituent laboratories of the PHLS in the United Kingdom. Each laboratory was identified by a code number known only to itself, and to us. In general, the response from participants was very good, although some replies were received too late for inclusion in the analyses of the relevant distributions. In addition, for various reasons, the actual number of laboratories which did report varied slightly with each distribution.

Distribution of Samples. The concentrated bacterial suspensions for preparation of the simulated water samples were dispensed in bijoux bottles. These were packed in approved cardboard boxes and despatched by "letter" post. Those for EEC laboratories were marked "Air Mail" and "EEC Quality Control Trial - 5ml. Water Samples for Analysis - Net Weight 100gm" on Customs/Douane labels. No leakages or breakages occurred during transit and the majority of both EEC and PHLS laboratories received the specimens well before the dates specified for starting the bacteriological analyses. It is therefore evident that, under normal circumstances, the distribution of such samples by post for bacteriological examination is entirely satisfactory.

Delay in starting Tests. In each distribution, for various reasons a few laboratories started the analyses after the specified date, but this did not appear to affect the expected results.

Media. As shown in Appendices C, D, and E, several media were used. In general, most PHLS laboratories used the multiple tube method with commercial (Oxoid) minerals modified glutamate medium (PHLS, 1969) based on Gray's (1964) improved formate lactose glutamate medium. Although relatively few PHLS laboratories used the membrane filtration method, all except one employed Oxoid 0.4% enriched Teepol broth (Report, 1969). The majority of EEC laboratories used both multiple tube and membrane filtration methods, although it is not yet known whether these methods are employed together as a routine. EEC laboratories used either glutamate media, MacConkey or lactose broth for multiple tube tests; for membrane filtration, however, TTC Tergitol agar was the medium most frequently employed.

Laboratory Reports. Although cumulative analysis of the collective results reported by each laboratory was not possible, considerable difficulties were experienced in collating and interpreting many of the actual reports. Some of these difficulties were technical in nature, some were due to misunderstanding of the report form, and others arose because the results were recorded in such a way that they could only be interpreted by us with difficulty. They included:

(i) A few laboratories clearly did not follow the instructions and shake the bottles thoroughly to ensure homogenous distribution of the organisms during each stage of preparation of the samples.

(ii) Variation in the times and temperatures of incubation - for example some laboratories incubated for coliform organisms at 30°C and others gave multiple tube results for coliform organisms after incubation for only 24 hours at 37°C.

(iii) Some laboratories used pre-incubation at lower temperatures with both the multiple tube and membrane filtration methods for coliform organisms and Esch. coli.

(iv) Many different volumes of water were examined, ranging from 3 x 100ml to 5 x 1.0ml for multiple tube tests; in contrast for membrane filtration, some laboratories calculated results per 100 ml from the examination of as little as 1.0 ml of the water samples.

(v) Some laboratories used multiple membranes with several volumes of water and either averaged the results or gave more than one set of results. One laboratory used as many as 18 different membranes for each sample.

(vi) Some laboratories identified completely the organisms present, whereas some others did not use any confirmatory tests for either multiple tube or membrane filtration results.

(vii) Some laboratories gave Esch. coli results after incubation of multiple tubes at 37°C for only 24 hours.

(viii) Some laboratories incubated tubes directly at 44°C for Esch. coli.

(ix) The choice of statistical tables varied with the sets of tubes and volumes of water used.

and (x) Similarly some laboratories reported inadequate numerical results for coliform organisms and/or Esch. coli (e.g. 18+ or 180+) due to insufficient numbers of tubes used in the tests.

Despite these difficulties, we were able to interpret the majority of the reports received. The ranges of results "expected" by MT and MF methods for each sample in each distribution are shown in Table 1. The numbers of EEC and PHLS laboratories which obtained numerical results within these "expected" ranges are shown in Tables 2 and 3. These also show the numbers of laboratories outside these limits but which were arbitrarily regarded as "satisfactory" in that their results were within ranges from half the minimum to twice the maximum values of those "expected". In fact, the greater majority of these results were very close to the "expected" values. For convenience, the coliform results within the "expected" and "satisfactory" ranges are shown in Table 2 and those for Esch. coli (faecal coli) in Table 3. It should be noted, however, that this artificial separation of organisms and results is only for clarity. It is evident that, whatever the media and techniques used, more than 82% of all the laboratories obtained acceptable results for all specimens.

DISCUSSION

Inter-laboratory calibration programmes involving the distribution of water samples for analysis are well established in the physico-chemical field, but until recently the difficulties inherent in its application to microbiology were thought to be too great for practicability. However, a satisfactory method using simulated water samples containing stable bacterial suspensions has recently been developed by Gray and Lowe (1976) for microbiological laboratory quality control purposes by the Public Health Laboratory Service in the United Kingdom. The present feasability study was undertaken primarily as a trial to determine whether the distribution of such

samples was suitable for comparative bacteriological analytical work among member states of the EEC. It is clear from the results of the three distributions in this study that it is in fact both feasible and practicable.

In the report on the first distribution, the importance of thoroughly shaking the samples during preparation to ensure homogenous distribution of the organisms was emphasized. It was arbitrarily suggested that failure to find less than 20 coliform organisms or E. coli per 100ml in any sample should be regarded as unsatisfactory; this applied to 6 of 27 EEC laboratories and 10 of 21 PHLS laboratories, though no laboratory failed to detect their presence. The second distribution showed that small numbers of organisms gave good and uniform results. The third distribution in which there were similar numbers of one of the organisms in each pair of samples, indicated that a reasonable degree of reproducibility was achieved - particularly with the membrane filtration method among EEC laboratories.

This method is also suitable not only for ongoing quality control work but for prior comparison and evaluation of different media and methods before extensive field trials. Although only coliform organisms and Esch. coli (faecal coli) were used in this study, the same approach can easily be extended to other bacterial indicator organisms and developed to include the distribution of prepared dehydrated media to assess technical performance, especially with small numbers of organisms. Indeed, for drinking water safety, it is more important to use media and methods which will consistently detect the presence of small numbers of coliform organisms and Esch. coli than necessarily give greater comparability with larger numbers. For this reason, not only are frequent laboratory quality control tests essential in water microbiology, but

theyshould be based essentially on samples with small numbers of organisms. For the same reasons, satisfactory evaluation should eventually include the use of stressed or damaged organisms in order to show up small differences more quickly and thus aid any subsequent field work. It is important to appreciate that in practice the nature and quality of the water to be examined may affect the choice of the media and methods used for bacteriological analysis. It seems probable that alternative but comparable cultural methods will continue to be needed in different areas for different reasons: a universal best method or medium, although ideal, is unlikely to be achieved in practice in the forseeable future.

Despite some difficulties of interpretation, the results of this study suggest that, with the simulated water samples distributed the membrane filtration method gave results consistently closer to those expected than the multiple tube method, although Endo medium tended to give low numbers. Indeed, one laboratory failed to detect Esch. coli or coliform organisms in all three samples in the third distribution by the membrane filtration method with Endo broth. This laboratory, however, obtained the "expected" results in duplicate membrane tests on the same samples using enriched teepol broth. The study has been useful in revealing some differences in the details of the methods used and the need for greater uniformity. It has also shown the importance of actual numerical results in any future comparative work on media and method evaluation as well as the necessity for all test results to be accurately recorded in the same way. We suggest that, in addition to complementary research, exchange visits between laboratories and occasional technical seminars, the distribution of simulated water samples for

quality control purposes should be continued and expanded to aid harmonization and ultimate standardization, not only of media and methods, but of the way in which bacteriological results are recorded, interpreted and reported among member states of the EEC.

SUMMARY

A feasability study is described in which simulated water samples were distributed by post on three separate occasions to a total of 27 laboratories within the EEC and 21 laboratories in the PHLS in Britain. Bijoux bottles containing concentrated suspensions of viable coliform organisms and/or Escherichia coli in a modified glutamate medium containing boric acid as a preservative were issued with precise instructions for the preparation of the simulated water samples. All laboratories were asked to prepare the samples on specified dates and then examine them for these organisms by their normal bacteriological methods as though they were routine samples of water and report their results on forms provided. The response was very good and the results of the study indicate that the distribution of such samples is not only practicable but that this quality control approach could be used for ongoing evaluation of techniques and media performance for coliform and other organisms, as well as for harmonization of the way inwhich results are recorded, interpreted and reported. This could usefully supplement other work and occasional technical seminars.

ACKNOWLEDGEMENTS

We are grateful to the Health and Safety Directorate, Commission of the European Communities, Luxembourg, for encouragement and financial support for this work; Mr. W. Fletcher and the staff

of the Epidemiological Research Laboratory, Central Public Health Laboratory, Colindale, for statistical analyses; and the staff of the participating laboratories for their cooperation.

REFERENCES

Gray, R.D. (1964). An improved formate lactose glutamate medium for the detection of Escherichia coli and other coliform organisms in water. J. Hygiene, Cambridge, 62, 495.

Gray, R.D. & Lowe, G.H. (1976). The preparation of simulated water samples for the purpose of bacteriological quality control. J. Hygiene, Cambridge, 76, 49.

Public Health Laboratory Service (1969). A Minerals modified Glutamate medium for the enumeration of coliform organisms in water. Report by the Standing Committee on the Bacteriological Examination of Water Supplies. J. Hygiene, Cambridge, 67, 367.

Report (1969). The Bacteriological Examination of Water Supplies. Reports on Public Health and Medical Subjects, No. 71, London, H.M.S.O.

Vial, J. (1976). Bacteriological Analyses of Drinking Water - Lyons Technical Seminar. Health Protection Directorate, Commission of the European Communities, Luxembourg.

TABLE 1

Range of "Expected" results obtained by the Issuing laboratory from the examination of 5 specimens of each sample.

Distribution	Sample No. (Bottle)	EXPECTED RESULTS							
		MULTIPLE TUBE METHOD				MEMBRANE FILTRATION METHOD			
		Coliform organisms		E. coli (faecal coli)		Coliform organisms		E. coli (faecal coli)	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
1 February 1976	1	50	160	35	90	92	124	42	64
	2	90	180	0	0	115	158	0	0
	3	90	180+	35	90	111	159	46	50
2 April/May 1976	195	1700	5500	1700	5500	1850	2500	1850	2500
	196	35	160	8	35	34	64	2	8
3 December 1976	(A) 231	3	13	3	13	8	14	3	7
	(B) 232	17	35	8	17	11	22	5	13
	(C) 233	13	25	8	25	15	25	3	14

TABLE 2

Number of laboratories recording results for coliform organisms within the "expected" and "satisfactory" range

Distribution	SAMPLE (BOTTLE) No.	LABORATORIES	NUMBER OF LABORATORIES							
			MULTIPLE TUBE METHOD				MEMBRANE FILTRATION METHOD			
			E*	S*	O*	T*	E*	S*	O*	T*
1 February 1976	1	E E C	11	4	2	17	10	9	1	20
		P H L S	10	7	3	20	5	3	0	8
	2	E E C	6	8	3	17	6	14	0	20
		P H L S	9	11	0	20	5	3	0	8
	3	E E C	11	5	1	17	14	6	0	20
		P H L S	16	2	2	20	4	4	0	8
2 April/May 1976	195	E E C	8	4	6	18	7	10	4	21
		P H L S	12	4	4	20	1	2	2	5
	196	E E C	12	2	3	17	15	4	1	20
		P H L S	19	1	0	20	4	1	0	5
3 December 1976	(A) 231	E E C	14	3	0	17	10	7	2	19
		P H L S	16	0	0	16	5	1	0	6
	(B) 232	E E C	6	6	5	17	14	3	2	19
		P H L S	11	4	1	16	4	2	0	6
	(C) 233	E E C	7	7	3	17	12	5	2	19
		P H L S	8	8	0	16	2	3	1	6

E* "Expected' results S* Satisfactory results O* Other results T* Total

TABLE 3

Number of laboratories recording results for Esch. coli (faecal coli) within the "expected" and "satisfactory" range

Distribution	SAMPLE (BOTTLE) No.	LABORATORIES	NUMBER OF LABORATORIES							
			MULTIPLE TUBE METHOD				MEMBRANE FILTRATION METHOD			
			E*	S*	O*	T*	E*	S*	O*	T*
1 February 1976	1	E E C	7	8	2	17	10	9	1	20
		P H L S	12	3	5	20	5	3	0	8
	2	E E C	16	0	1	17	19	0	1	20
		P H L S	20	0	0	20	8	0	0	8
	3	E E C	11	3	3	17	6	12	2	20
		P H L S	12	3	5	20	3	3	2	8
2 April/May 1976	195	E E C	7	3	8	18	7	8	6	21
		P H L S	11	4	5	20	1	2	2	5
	196	E E C	8	5	4	17	8	5	7	20
		P H L S	15	1	4	20	3	1	1	5
3 December 1976	(A) 231	E E C	14	1	2	17	11	7	1	19
		P H L S	13	3	0	16	4	1	1	6
	(B) 232	E E C	6	5	6	17	14	5	0	19
		P H L S	12	2	2	16	4	2	0	6
	(C) 233	E E C	12	2	3	17	15	3	1	19
		P H L S	14	1	1	16	4	1	1	6

E* "Expected" results S* "Satisfactory results O* Other results T Total

APPENDIX A.

Example of instructions for the preparation and examination of the simulated water samples.

PUBLIC HEALTH LABORATORY SERVICE WATER COMMITTEE

E.E.C. WATER QUALITY CONTROL TRIAL (3) - DECEMBER 1976.

NOTES FROM PUBLIC HEALTH LABORATORY, NEWPORT

Three samples are enclosed. They are numbered 231 A, 232 B and 233 C. They should all be regarded as rural unchlorinated water.

INSTRUCTIONS

The bottles should be stored UNOPENED at room temperature in the dark and examined on December 7 for the numbers of coliform organisms and Escherichia coli (faecal coli) ONLY. If received after this date, they should be examined immediately.

PREPARATION OF SAMPLES

It is important that the instructions given are followed precisely. Prepare EACH SAMPLE SEPARATELY as follows:-

1 POUR HALF OF THE CONTENTS OF THE SMALL BOTTLE INTO A LARGER STERILE BOTTLE (25-50ml) MARKED WITH THE SAMPLE NUMBER.

2 THE WATER REMAINING IN THE SMALL BOTTLE SHOULD BE THOROUGHLY MIXED BY SHAKING VIGOROUSLY. THEN ADD THIS WATER TO THE REST OF THE WATER IN THE LARGER BOTTLE.

3 SHAKE THIS BOTTLE VIGOROUSLY BY HAND FOR AT LEAST TWO MINUTES TO ENSURE THOROUGH MIXING OF THE WATER AND THEN ADD 3ml ASEPTICALLY TO 400ml OF STERILE DISTILLED OR DEIONISED WATER IN A STERILE BOTTLE. THIS NOW CONSTITUTES THE SIMULATED WATER SAMPLE.

4 EACH SIMULATED WATER SAMPLE SHOULD BE SHAKEN THOROUGHLY AND THEN EXAMINED BY YOUR USUAL METHOD (MULTIPLE TUBES, MEMBRANE FILTRATION OR BOTH) WITH YOUR USUAL MEDIA.

RECORDING OF RESULTS

Forms on which to record your results have already been sent to you by Dr. G.I. Barrow. The coloured forms are marked 'A', 'B' and 'C'. Please check that the results are recorded on the correct forms.

You will be informed later of (a) the intended results and (b) the actual results obtained in this water quality control trial.

Please return forms BY AIRMAIL to:

Dr. G.I. Barrow,
Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill, Truro TR1 2HZ.
Cornwall. U.K. (Tel. 0872 3029)

APPENDIX B.
Example of Forms for recording laboratory results and other information

Public Health Laboratory Service Water Committee

FORM **A**

E.E.C. WATER QUALITY CONTROL TRIAL (3)

Issued by: *Dr. R.D. Gray, Public Health Laboratory, Clytha Square, Newport, Gwent NP7 2ZZ, U.K.*

BOTTLE NO. 231

Issued November 29, 1976.

Rural unchlorinated water

LAB. IDENTITY CODE NO.

PLEASE RECORD:

Date sample received: *Date examination started:*

Membrane Filtration Method and Results

1. Volume of water filtered through each membrane:_____ No. of membranes _____

2. Media used: _____

		Incubation Time	Incubation Temp
3. Total coliform organisms	_____ per 100 ml.	_____ hr	_____ °C
4. Number of *Escherichia coli* (included in 3) (faecal coli)	_____ per 100 ml	_____ hr	_____ °C

5. Confirmatory tests used: _____

Any comments?: _____

_____

Multiple Tube Method and Results

a) Number of tubes/bottles and volumes of water used.

Volume (ml) _____

No. tubes etc. _____

b) Media used: _____

_____

Most Probable Number (MPN) per 100 ml. of

		Incubation Time	Incubation Temp.
c) Coliform organisms	_____ per 100 ml.	_____ hr.	_____ °C.
d) *Escherichia coli* (faecal coli) (included in c)	_____ per 100 ml.	_____ hr.	_____ °C.

e) Confirmatory tests _____

Any comments? _____

Please return this form by AIRMAIL as soon as tests are completed to: →

Dr. G.I. Barrow,
Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro TR1 2HZ, Cornwall, U.K.

Enter date posted

Public Health Laboratory Service Water Committee

FORM **B**

E.E.C. WATER QUALITY CONTROL TRIAL (3)

Issued by: *Dr. R.D. Gray, Public Health Laboratory, Clytha Square, Newport, Gwent NPT 2TZ, U.K.*

BOTTLE NO. 232

Issued November 29, 1976.

Rural unchlorinated water

LAB. IDENTITY CODE NO.

PLEASE RECORD:

Date sample received: *Date examination started:*

Membrane Filtration Method and Results

1. Volume of water filtered through each membrane:_____ No. of membranes _____

2. Media used: _____

		Incubation Time	Temp
3. Total coliform organisms	_____ per 100 ml.	_____ hr	_____ °C
4. Number of *Escherichia coli* (included in 3) (faecal coli)	_____ per 100 ml	_____ hr	_____ °C

5. Confirmatory tests used: _____

Any comments? _____

_____

Multiple Tube Method and Results

a) Number of tubes/bottles and volumes of water used.

Volume (ml)	No. tubes etc.

b) Media used: _____

_____

Most Probable Number (MPN) per 100 ml. of	Incubation Time	Temp.
c) Coliform organisms _____ per 100 ml.	_____hr.	_____ °C.
d) *Escherichia coli* (faecal coli) (included in c) _____ per 100 ml.	_____hr.	_____ °C.

e) Confirmatory tests _____

Any comments? _____

Please return this form by AIRMAIL as soon as tests are completed to: →

Dr. G.I. Barrow,
Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro TR1 2HZ, Cornwall, U.K.

Enter date posted

Public Health Laboratory Service Water Committee

FORM **C**

E.E.C. WATER QUALITY CONTROL TRIAL (3)

Issued by: *Dr. R.D. Gray, Public Health Laboratory, Clytha Square, Newport, Gwent NPT 2TZ, U.K.*

BOTTLE NO. 233 Issued November 29, 1976.

Rural unchlorinated water

LAB. IDENTITY CODE NO.

PLEASE RECORD:

Date sample received: *Date examination started:*

Membrane Filtration Method and Results

1. Volume of water filtered through each membrane:_____ No. of membranes _____

2. Media used: _____

		Incubation Time	Temp
3. Total coliform organisms	_____ per 100 ml.	_____ hr	_____ °C
4. Number of *Escherichia coli* (included in 3) (faecal coli)	_____ per 100 ml	_____ hr	_____ °C

5. Confirmatory tests used: _____

Any comments? _____

_____

Multiple Tube Method and Results

a) Number of tubes/bottles and volumes of water used.

Volume (ml) _____

No. tubes etc. _____

b) Media used: _____

_____

Most Probable Number (MPN) per 100 ml. of

		Incubation Time	Temp.
c) Coliform organisms	_____ per 100 ml.	_____hr.	_____ °C.
d) *Escherichia coli* (faecal coli) (included in c)	_____ per 100 ml.	_____hr.	_____ °C.

e) Confirmatory tests _____

Any comments? _____

Please return this form by AIRMAIL as soon as tests are completed to: ⟶

Dr. G.I. Barrow,
Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro TR1 2HZ, Cornwall, U.K.

Enter date posted

APPENDIX C.

Preliminary Report - Distribution No. 1.

PUBLIC HEALTH LABORATORY SERVICE

(Headquarters Office: Colindale Avenue, London NW9 5EQ)

TRURO
3029

PUBLIC HEALTH LABORATORY,
ROYAL CORNWALL HOSPITAL (CITY),
INFIRMARY HILL,
TRURO, CORNWALL.

7th April, 1976.

Dear Participant,

E.E.C. Water Quality Control Trial

The results of the first distribution of simulated samples of water for bacteriological examination are now being analysed and a full report will be sent to you about the end of April. Meanwhile, the following preliminary information may be of interest:

1. In general, this exercise went smoothly. The postal services were satisfactory and most E.E.C. laboratories received the samples within a few days.

2. Results were received from 25 of 27 laboratories in the E.E.C. and from all of 21 laboratories in the U.K.

3. The results appear to be reasonably uniform, with only a few outside the expected limits.

4. The minimum and maximum results obtained by the issuing laboratory using both membrane filtration and MPN methods were as follows:

Bottle Number	1	2	3
Coliform organisms	50-160	90- >180	90- >180
Escherichia coli (faecal coli)	35-90	0	35-90

The second set of simulated water samples, comprising two bottles, will be distributed during the week beginning 26th April, for bacteriological examination on May 4. Coliform organisms and/or faecal coli should be present in these samples and we would like to know the ACTUAL NUMBERS you find using your usual techniques and media. Any technical or other difficulties experienced with the preparation and examination of these samples, should be recorded on the forms under "Any Comments?"

Yours sincerely,

Dr. G.I. Barrow.

APPENDIX C.

Final Report - Distribution No. 1

PUBLIC HEALTH LABORATORY SERVICE

(Headquarters Office: 24 Park Crescent, London, W1N 4DA)

TRURO
3029

PUBLIC HEALTH LABORATORY,
ROYAL CORNWALL HOSPITAL (CITY),
INFIRMARY HILL,
TRURO, CORNWALL.

E.E.C. WATER QUALITY CONTROL TRIAL

REPORT ON DISTRIBUTION NO.1 (FEBRUARY, 1976)

1. LABORATORIES A total of 45 laboratories participated: 21 P.H.L.S. laboratories in the U.K. and 27 other E.E.C. laboratories. Of these, 7 used the membrane filtration method, 17 used the multiple tubes method and 21 used both methods.

2. TIME SPECIMENS IN TRANSIT

Days to Receipt	No. of Laboratories
1	16
2	7
3	9
4	4
7 - 13	5
14 - 20	1
Not stated	3

3. DELAY IN STARTING TESTS

Days late starting	No. of Laboratories
0	38
2	2
3	2
7+	1
Not stated	2

Delays in transit or in starting the examinations did not affect the results.

4. MEDIA

Multiple tubes:

Media used	No. of Laboratories
MacConkey Broth	5
Lactose Broth	7
Purple MacConkey Broth	4
Glutamate media	22

Membrane filtration:

Media used	No. of Laboratories
Endo (agar)	2
Tergitol	4
TTC Tergitol agar	11
MF Endo Medium (broth)	2
Membrane enriched Teepol broth	8
MFC Broth	1

Although statistically there is no significant difference, it is not possible to evaluate media performance from this distribution because of (a) lack of detailed information (b) variations in the volumes of samples used for examination, (c) inadequate confirmation of presumptive positive reactions, and (d) differences in the way in which some results were reported.

5. SIMULATED WATER SAMPLES These were prepared by the addition of known organisms to lactose-free IFLG medium containing boric acid as a preservative so that after distribution and dilution, the simulated test sample should contain calculated numbers of organisms. Bottles 1 and 3 contained a mixture of selected strains of Escherichia coli and Klebsiella aerogenes; and Bottle 2 contained K. aerogenes only. Five samples of each bottle, including 2 stored and 3 postal, were examined by the Issuing Laboratory according to the instructions issued, and the expected results are based on these findings. The maximum and minimum results are as follows:

Total no. of organisms per 100 ml.	BOTTLE NUMBER					
	1		2		3	
	MPN	MF	MPN	MF	MPN	MF
Coliforms	50-160	92-124	90-180	115-158	90-180+	111-159
E. coli (faecal coli)	35-90	42-64	-	-	35-90	46-50

MPN = Multiple Tube Method MF = Membrane Filtration

6. RESULTS The results reported from all laboratories are shown in Tables 1 and 2 and in Figures 1, 2 and 3.

Findings: No statistically significant difference was found either in the average results or in the spread of results for coliform organisms or E.coli (faecal coli) between P.H.L.S. and other E.E.C. laboratories for any bottle (Table 1). The results from all laboratories for each bottle were therefore pooled for further analysis.

Coliform organisms: No significant differences were found in the average results or the spread of results obtained by all laboratories compared with the expected results for any bottle (Table 2). Some laboratories, however, obtained consistently lower results using membrane filtration with certain media (notably laboratories 500 and 017).

Escherichia coli (faecal coli)

Bottle 1: No difference was found between the results from all laboratories and the expected results either on average or in the spread (Table 2).

Bottle 2: This bottle did not contain E. coli. One laboratory, however, reported 1 E.coli per 100 ml. using the multiple tube method, but not by membrane filtration.

Bottle 3: The average results from all laboratories agreed with the expected results. The membrane filtration method, however, appeared to have a significantly greater spread (Table 2) because the expected results were all close together.

7. CONCLUSIONS In general, the Tables and Figures indicate reasonable comparability of results. On average, the membrane filtration method and the multiple tube method gave similar results although when compared with the expected results, the membrane filtration method was in all cases significantly more accurate. It is arbitrarily suggested that for any bottle, the finding of less than 20 Coliforms or E. coli per 100 ml. should be regarded as unsatisfactory. This could be due to (a) medium used (b) technique, or most probably (c) insufficient shaking to ensure homogenous distribution of organisms at each stage of preparation and examination of the samples.

This distribution has been useful in revealing a number of areas of non-uniformity in techniques used and in reporting results, which will be taken into account in planning future distributions.

G.I. BARROW

Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro TR1 2HZ,
Cornwall, U.K.

27th May, 1976

TABLE 1

E.E.C. WATER TRIAL 1

Method	Bottle	Laboratories	COLIFORMS				E. COLI			
			No. of reports	No. per 100 ml.			No. of reports	No. per 100 ml.		
				Mean	S.D.	Range		Mean	S.D.	Range
Membrane filtration	1	E.E.C.	21	102.4	25.2	50-170	20	53.5	17.3	29-90
		P.H.L.S.	7	103.6	31.6	59-160	7	44.4	13.1	27-62
	2	E.E.C.	21	110.0	49.2	27-280				
		P.H.L.S.	7	101.4	26.6	64-134				
	3	E.E.C.	21	122.6	27.5	52-170	20	50.0	17.2	11-75
		P.H.L.S.	7	115.0	44.2	55-174	7	36.7	21.9	6-55
Multiple tubes	1	E.E.C.	19	149.2	84.4	24-350	19	72.2	54.0	20-240
		P.H.L.S.	19	125.5	67.7	18+-250	19	64.2	61.8	6-250
	2	E.E.C.	19	120.6	90.3	25-350				
		P.H.L.S.	19	175.3	87.7	50-350				
	3	E.E.C.	19	132.5	85.0	9-350	19	50.7	40.5	13-160
		P.H.L.S.	19	122.1	58.5	35-250	19	53.1	46.3	6-160

TABLE 2

E.E.C. WATER TRIAL 1

Method	Bottle	Laboratories	No. of results	No. of coliforms per 100 ml		No. of E. coli per 100 ml	
				Mean	Standard deviation	Mean	Standard deviation
Membrane filtration	1	All labs	28†	102.7	26.3	51.1	16.6
		Issuing laboratory	5	104.8	14.4	55.8	9.9
	2	All labs	28†	107.9	44.4	0	-
		Issuing laboratory	5	131.8	16.7	0	-
	3	All labs	28†	120.7	31.7	46.5	19.0
		Issuing laboratory	5	126.4	19.1	48.0	1.4
Multiple tubes	1	All labs	38	137.3	76.4	68.2	57.4
		Issuing Laboratory	5	96.0	39.7	71.0	26.6
	2	All labs	38	147.9	92.1	0*	-
		Issuing laboratory	5	150.0	34.6	0	-
	3	All labs	38	127.3	72.2	51.9	43.0
		Issuing laboratory	5	154.0	37.2	55.0	20.6

† One laboratory reported a coliform result only

* One laboratory reported 1 per 100 ml

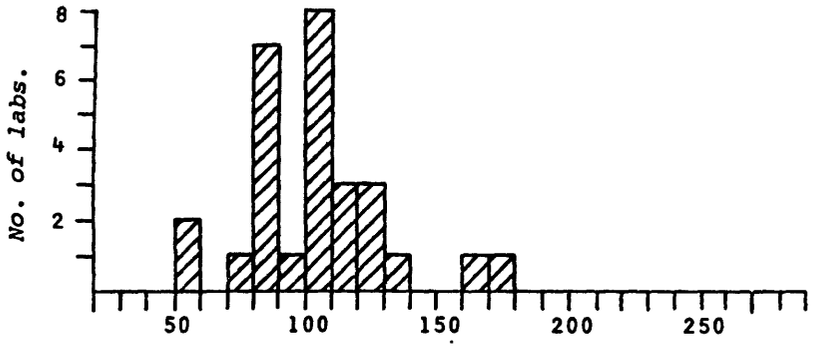
BOTTLE 1

FIG. 1

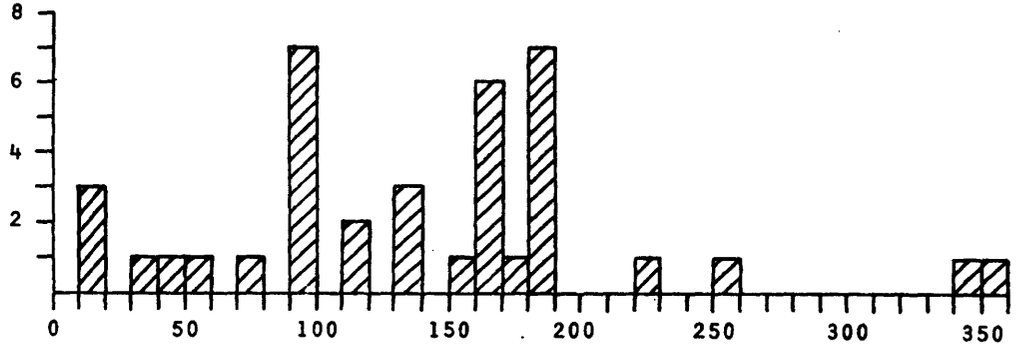
F.F.C. WATER TRIAL 1

C O L I F O R M S

MEMBRANE FILTRATION METHOD

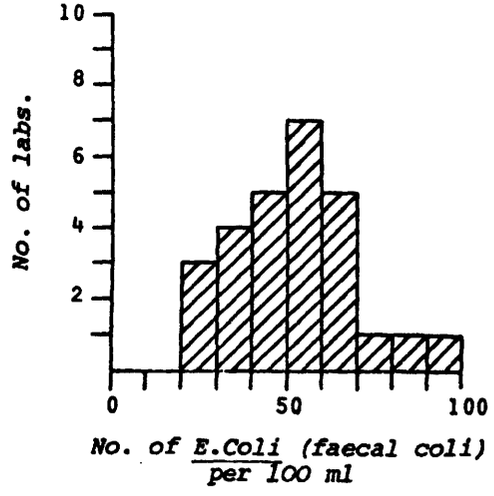


MULTIPLE TUBE METHOD

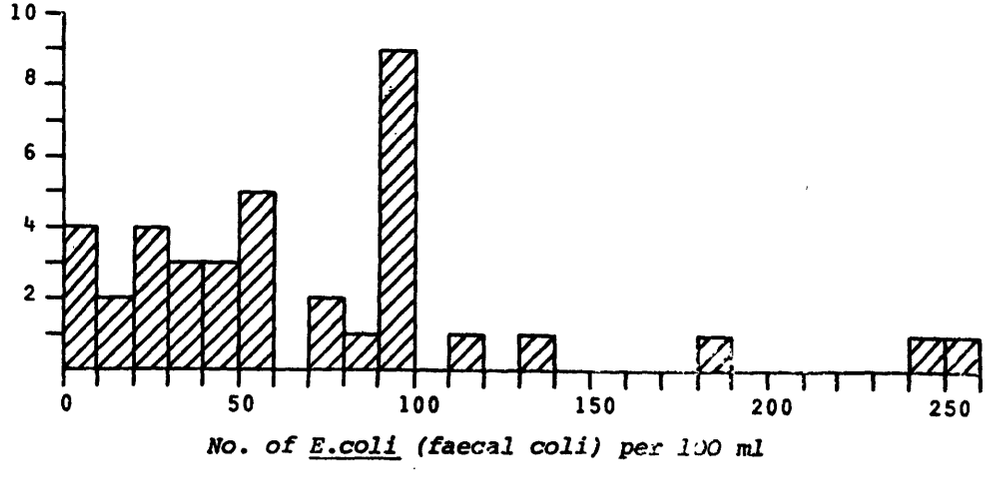


E. C O L I

MEMBRANE FILTRATION METHOD



MULTIPLE TUBE METHOD



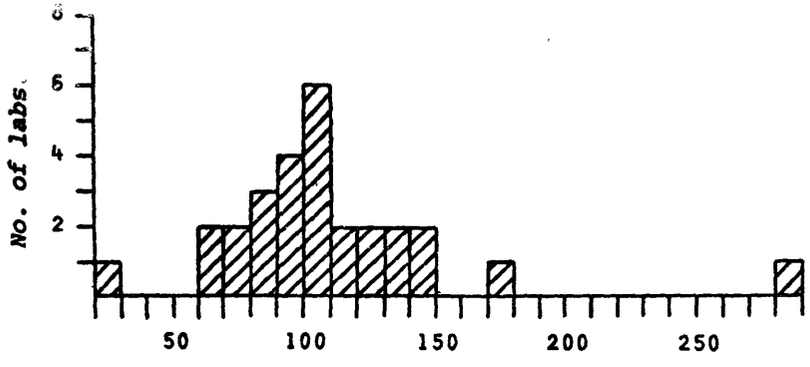
BOTTLE NO. 2

FIG. 2

E.E.C. WATER TRIAL I

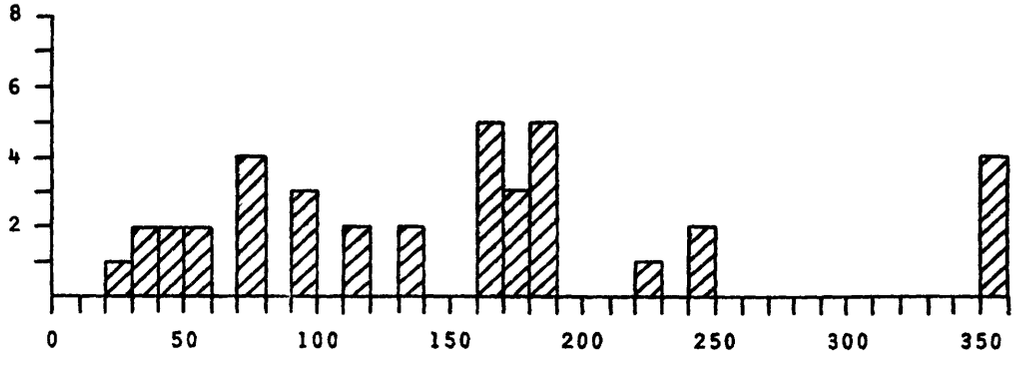
C O L I F O R M O R G A N I S M S

MEMBRANE FILTRATION METHOD



No. of coliform organisms per 100 ml

MULTIPLE TUBE METHOD



No. of coliform organisms per 100 ml

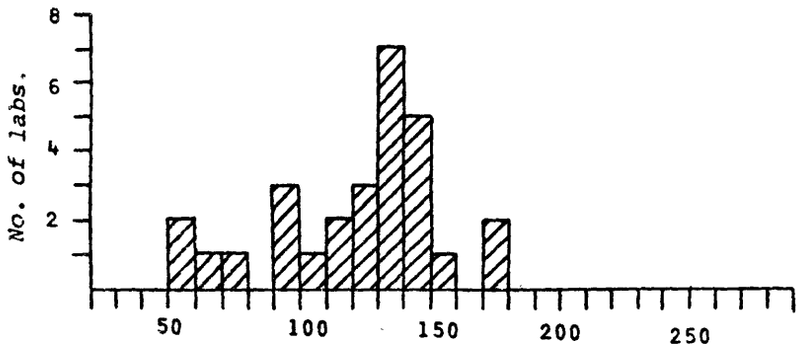
BOTTLE NO. 3

FIG. 3

E.E.C. WATER TRIAL I

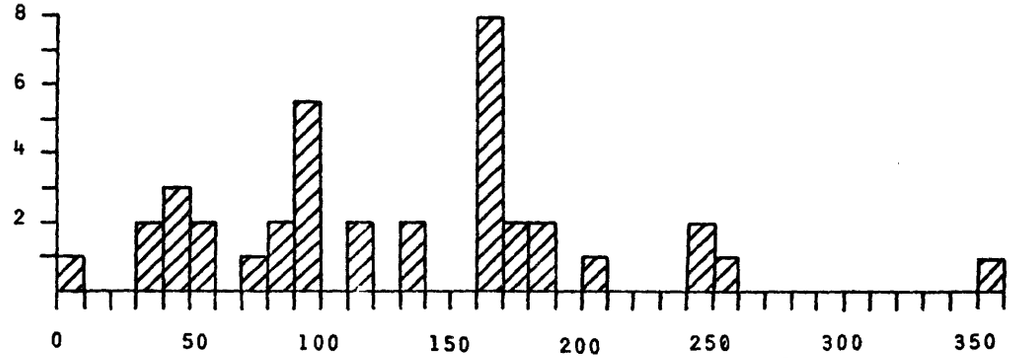
C O L I F O R M S

MEMBRANE FILTRATION METHOD



No. of coliform organisms per 100 ml.

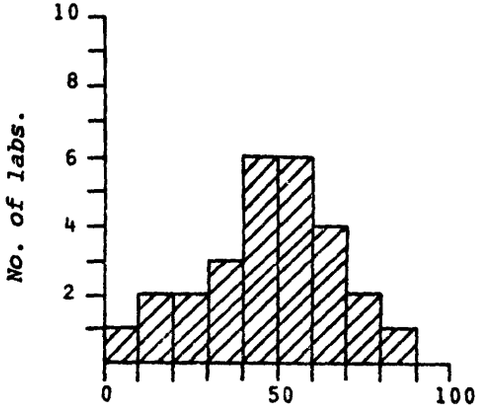
MULTIPLE TUBE METHOD



No. of coliform organisms per 100 ml.

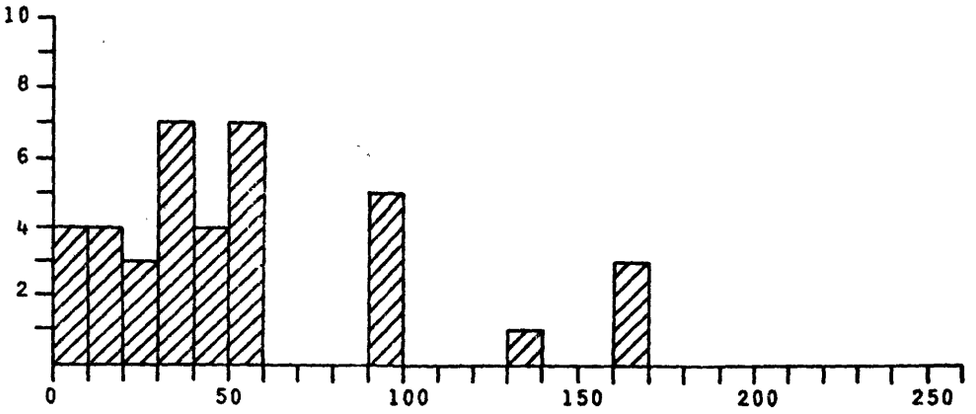
E. C O L I

MEMBRANE FILTRATION METHOD



No. of E.coli per 100 ml.

MULTIPLE TUBE METHOD



No. of E.coli per 100 ml.

APPENDIX D.

Preliminary Report - Distribution No. 2

PUBLIC HEALTH LABORATORY SERVICE

TRURO
3029

PUBLIC HEALTH LABORATORY,
ROYAL CORNWALL HOSPITAL (CITY),
INFIRMARY HILL,
TRURO, CORNWALL.
TR1 2HZ

30th June, 1976

Dear Participant,

E.E.C. Water Quality Control Trial

The results of the second distribution of simulated samples of water for bacteriological examination are now being analysed and a full report will be sent to you in due course. Meanwhile, the following preliminary information may be of interest:-

1. In general, this exercise again went smoothly. The postal services were satisfactory and all but one of the E.E.C. laboratories received the samples within a few days.

2. Results have been received from 24 of 27 laboratories in the E.E.C; 86 other results have also been received from the Microbiology Quality Control Scheme.

3. The results appear to be generally fairly uniform, but a few were outside the expected limits.

4. The minimum and maximum results obtained by the issuing laboratory, using both membrane filtration and MPN methods, were as follows:

Organism	Bottle No. 1 195	Bottle No. 2 196
Coliform organisms	1,700-5,500	34-160
Escherichia coli (faecal coli)	1,700-5,500	2-35

The third set of simulated water samples will be distributed later in the year, possibly towards the end of August, but you will be notified of this before the actual distribution.

Yours sincerely,

Dr. G.I.Barrow

E.E.C. WATER QUALITY CONTROL TRIAL

REPORT ON DISTRIBUTION NO.2 (APRIL/MAY 1976)

1. A total of 46 laboratories participated: 21 British laboratories and 25 other E.E.C. laboratories. One laboratory (504) has however been omitted from this analysis because the report forms were incomplete and the results could not be interpreted. Of the 45 laboratories, 7 used the membrane filtration method only, 19 used the multiple tube method only and 19 used both.

2. TIME SPECIMENS IN TRANSIT (E.E.C. laboratories only)

Days to Receipt	No. of Laboratories
1	2
2	2
3	5
4	6
7 - 13	5
14 - 20	1
Not stated	3

3. DELAY IN STARTING TESTS (E.E.C. laboratories only)

Days late starting	No. of Laboratories
0	18
2	2
3	2
7+	1
Not stated	1

Delays in transit or in starting the examinations did not appear to affect the results.

4. MEDIA

	Media used	No. of Laboratories E.E.C.	British
Multiple tubes:	MacConkey Broth	2	1
	Lactose Broth	6	0
	Purple MacConkey Broth	5	2
	Glutamate media	5	17
Membrane filtration:	Endo (agar)	2	0
	TTC Tergitol agar	15	0
	Endo (broth)	1	1*
	Membrane enriched Teepol broth	3	4
	MFC Broth	0	1*

*same Lab.

It is again not possible to evaluate media performance yet because of (a) insufficient information given (b) considerable variations in the actual volumes tested by different laboratories (c) inadequate confirmation of presumptive reactions, and (d) difficulty in understanding some of the results and comments given on the forms. However, all the information gained will be summarized and reported after the 3rd distribution.

5. SIMULATED WATER SAMPLES. These were again prepared by the addition of known organisms to lactose-free Improved Formate Glutamate medium containing boric acid. Bottle No. 195 contained Escherichia coli only; and Bottle No. 196 contained a mixture of E. coli and Klebsiella aerogenes. The expected results are again based on the maximum and minumum results

obtained from the examination of 5 samples of each bottle by the Issuing laboratory. These were as follows:

Organism	Total number per 100 ml			
	Bottle 195		Bottle 196	
	MPN	MF	MPN	MF
Coliforms	1700 - 5500	1850 - 2500	35 - 160	34 - 64
E. coli (faecal coli)	1700 - 5500	1850 - 2500	8 - 35	2 - 8

MPN = Multiple Tube Method MF = Membrane Filtration

6. RESULTS. Some difficulty was experienced in collating the results from this distribution, and for the analysis.

(1) where coliform or E. coli counts were given as '>y', the numerical result has been regarded as 'y'.

(2) the results for any sample have been excluded if either the coliform or E. coli count was not reported.

(3) the results from one laboratory (507) for Bottle 195 have been excluded because of discrepancies in the report.

(4) one laboratory (525) did not report on Bottle 196.

(5) one laboratory (014) did not give membrane filtration results for Bottle 195.

Taking these factors into account, the results are shown in Table 1 and Figures 1 - 3.

Findings:

1. There was no statistically significant difference in the average results obtained by the E.E.C. laboratories, the British laboratories and the Issuing laboratory for either of the bottles by either the multiple tube or membrane filtration method.

2. On average the membrane filtration and the multiple tubes method gave the same results. However, the spread of the membrane filtration results was significantly less than the spread of the multiple tubes results in all but three cases. For both the coliform and E. coli counts on bottle 196 by the British laboratories there was no significant difference in these spreads and for the E. coli count on bottle 196 by the E.E.C. laboratories the spread of the multiple tubes results was just significantly smaller.

3. In general, there was no significant difference between the spread of the results from the E.E.C. laboratories and that of the issuing laboratory. With membrane filtration, however, the spread of the results from the E.E.C. laboratories was significantly greater than that of the issuing laboratory for the coliform count of bottle 195 and the E. coli count of bottle 196.

4. In most cases the spread of the results of the British laboratories was significantly greater than that of the issuing laboratory. For the multiple tube results of bottle 196 there was no significant difference.

5. In most cases there was no significant difference in the spread of the results of the British laboratories and that of the E.E.C. laboratories. However for both the multiple tubes coliform and E. coli count for bottle 195 and for the membrane filtration coliform count for bottle 196 the spread of the British results was significantly greater.

6. Bottle 195 contained E. coli only, so as would be expected there was no difference between the average results of the coliform and E. coli counts or in the spread of these results.

7. COMMENTS. As expected, bottle 195 containing large numbers of E. coli yielded a wide range of numerical results. No laboratory, however, failed to detect the presence of E. coli by membrane filtration, although 3 E.E.C. laboratories failed to confirm its presence by the multiple tube method; these 3 laboratories all used purple MacConkey broth. However, 4 other laboratories using this medium obtained satisfactory results. It is interesting to note that surprisingly good results were obtained with bottle 196 which contained a mixture of small numbers of organisms: only 2 laboratories failed to find E. coli.

It is probably more important not to fail to detect the presence of small numbers of coliform organisms and/or E. coli than to obtain greater comparability with large numbers of organisms.

G.I. BARROW

Public Health Laboratory
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro, TR1 2HZ,
Cornwall, U.K.

November, 1976

Table 1

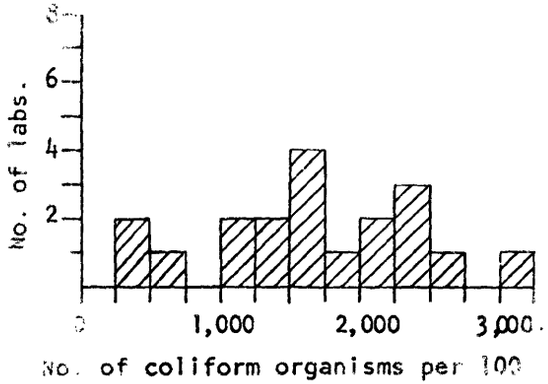
Method	Specimen	Laboratories	No. of results	No. of coliforms per 100 ml.			No. of *E. coli* per 100 ml.		
				Mean	Standard deviation	Range	Mean	Standard deviation	Range
Membrane filtration	195	E.E.C. labs.	19	1536.9	705.4	>300,2500	1384.3	670.9	>300,2400
		British labs.	4	1045.0	727.2	>180,1700	972.3	784.2	>180,1700
		Issuing lab.	5	2220.0	272.9	1850,2500	2220.0	272.9	1850,2500
	196	E.E.C. labs.	19	54.5	13.1	35,85	14.2	13.1	0,48
		British labs.	5	56.6	33.4	22,110	4.4	7.2	0,17
		Issuing lab.	5	50.8	11.8	34,64	4.4	2.9	2,8
Multiple tubes	195	E.E.C. labs.	18	2065.8	2513.7	<2,11000	1816.3	2569.4	0,11000
		British labs.	19	4040.0	5005.5	>180,23000	3956.1	5058.5	35,23000
		Issuing lab.	5	3040.0	1535.6	1700,5500	3040.0	1535.6	1700,5500
	196	E.E.C. labs.	17	58.2	36.8	0,110	8.7	7.6	0,26
		British labs.	19	79.2	41.3	25,160	10.5	6.4	2,30
		Issuing lab.	5	66.0	53.1	35,160	21.2	10.7	8,35

SPECIMEN 195

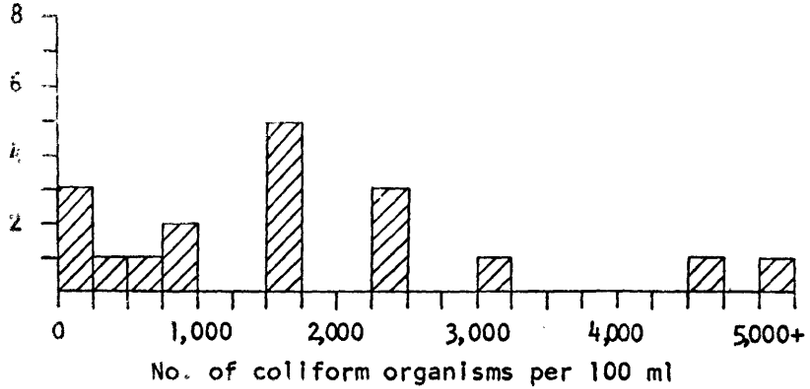
FIG. 1 E.E.C. LABORATORIES E.E.C WATER TRIAL 2

COLIFORMS

MEMBRANE FILTRATION METHOD

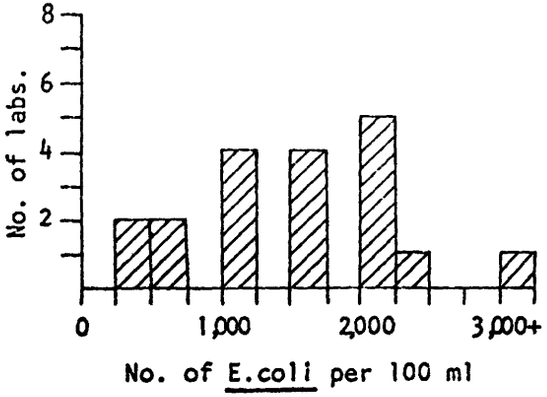


MULTIPLE TUBE METHOD

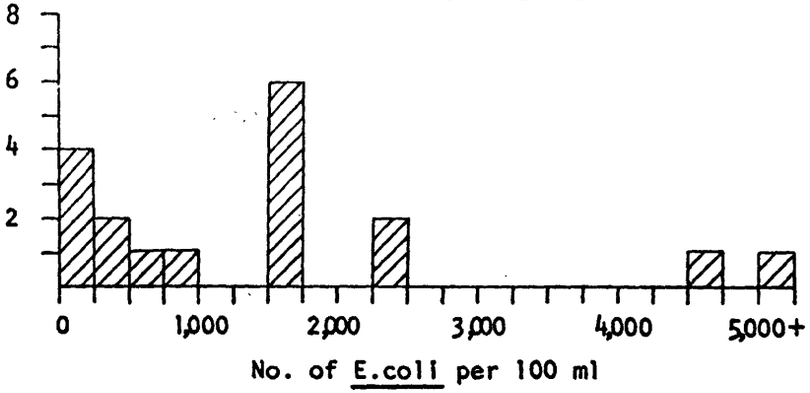


E.COLI

MEMBRANE FILTRATION METHOD



MULTIPLE TUBE METHOD



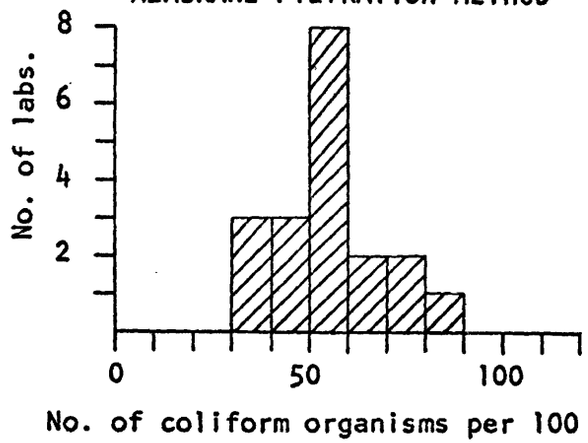
SPECIMEN 196

FIG. 2 E.E.C. LABORATORIES

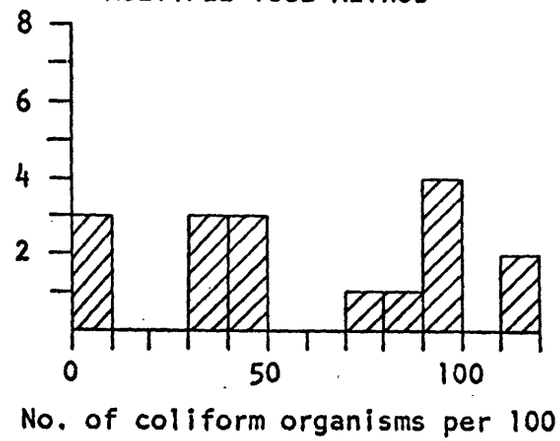
E.E.C WATER TRIAL 2

COLIFORMS

MEMBRANE FILTRATION METHOD

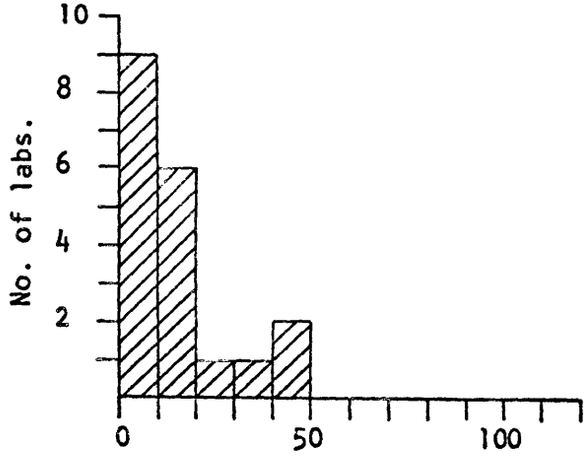


MULTIPLE TUBE METHOD



E.COLI

MEMBRANE FILTRATION METHOD



MULTIPLE TUBE METHOD

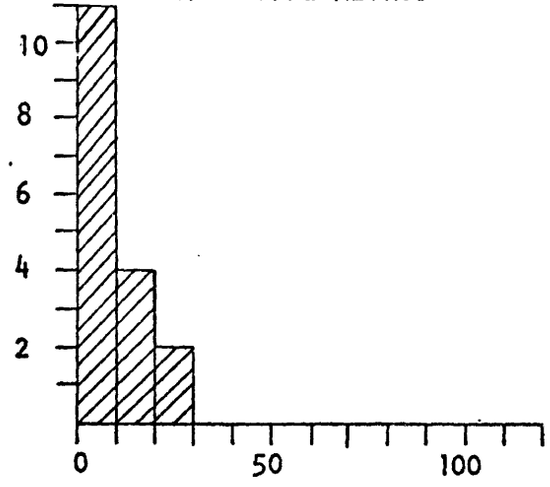


FIG. 3

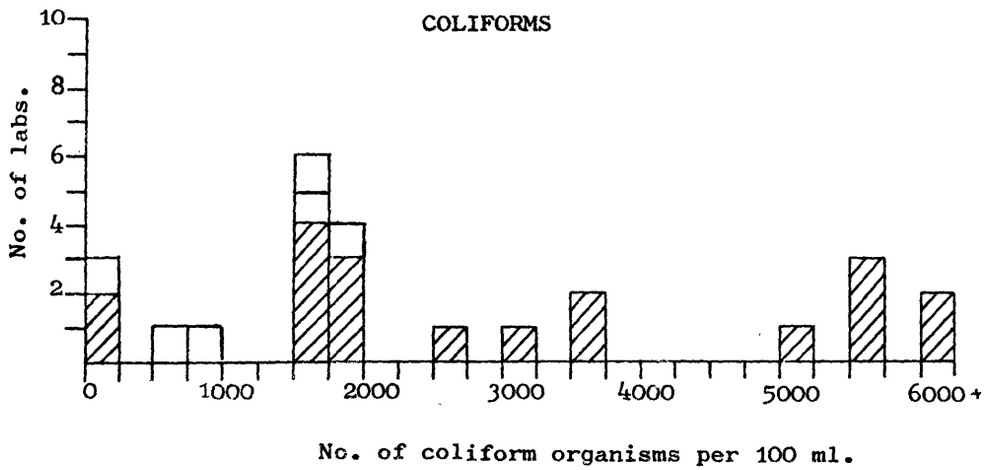
BRITISH LABORATORIES

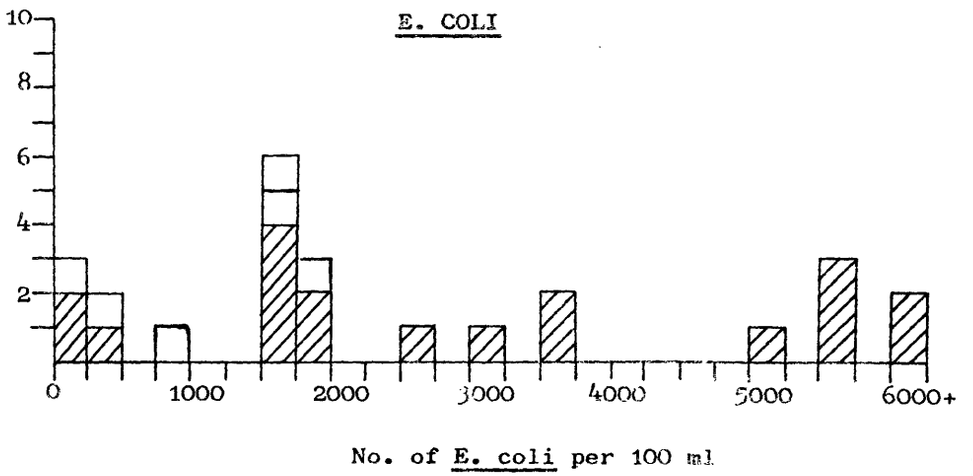
MULTIPLE TUBES ▨

MEMBRANE FILTRATION □

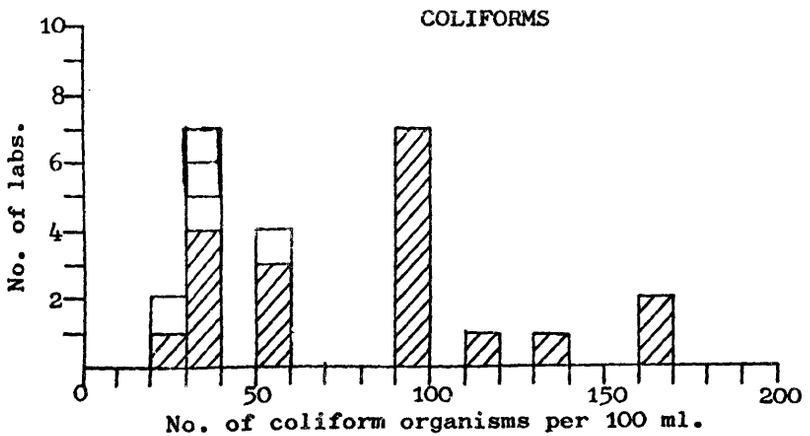
EEC WATER TRIAL 2

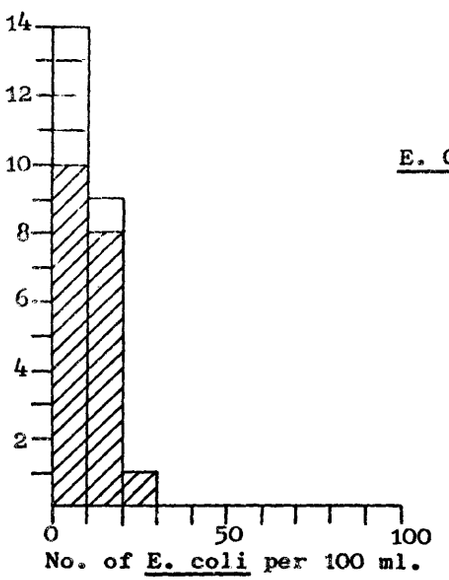
SPECIMEN 195





SPECIMEN 196





E. E. C. WATER QUALITY CONTROL TRIAL

REPORT ON DISTRIBUTION NO. 3 (DECEMBER 1976)

1. A total of 39 laboratories participated: 17 British and 22 EEC laboratories. Of these, 7 used membrane filtration only, 14 used multiple tubes only and 18 used both methods. The report from one EEC laboratory (514) was sent too late to be included in the analysis, but their results were entirely satisfactory and within the expected limits.

2. TIME SPECIMENS IN TRANSIT

	Days to receipt	No. of laboratories
(E.E.C. laboratories only)	1	2
	2	1
	3	4
	4	6
	5	3
	7 - 13	5
	Not stated	1

3. DELAY IN STARTING TESTS

	Days late starting	No. of laboratories
(E.E.C. laboratories only)	Early	1
	0	19
	2	2

Delays in transit or in starting the examinations did not appear to affect the results .

4. MEDIA

	Media used	No. of laboratories	
		E.E.C.	British
Multiple tubes	MacConkey Broth	2	1
	Lactose Broth	6	0
	Purple MacConkey Broth	3	1
	Glutamate Media	5	14
Membrane filtration	Endo (agar)	2	0
	TTC Tergitol agar	13	0
	Endo (broth)	1	1*
	Membrane enriched Teepol broth	3	5
	MFC Broth	0	1*

*same lab.

5. SIMULATED WATER SAMPLES These were again prepared by the addition of known organisms to lactose-free Improved Formate Glutamate medium. All three bottles (A, B and C) contained small numbers of both Escherichia coli and Klebsiella aerogenes. Bottles B and C were distributed from the same bulk preparation and were therefore in effect identical.

The expected results are again based on the maximum and minimum results obtained from the examination of 5 samples of each bottle by the issuing laboratory.

These were as follows:

ORGANISM	TOTAL NUMBER PER 100 ml					
	BOTTLE 231 (A)		BOTTLE 232 (B)		BOTTLE 233 (C)	
	MPN	MF	MPN	MF	MPN	MF
COLIFORMS	3-13	8-14	17-35	11-22	13-25	15-25
E. COLI (Faecal coli)	3-13	3-7	8-17	5-13	8-25	3-14

MPN = Multiple tube method MF = Membrane Filtration

6. RESULTS Again, there were considerable variations in (a) the volumes of water actually tested (b) temperatures of incubation (c) numbers of membranes or tubes used (d) use of confirmatory tests (e) the statistical tables used for MPN results, and (f) in the way the results were reported. All results as reported have been included in the analysis, although from information given on some forms, there are clearly some discrepancies in interpretation.

The results of the analyses are shown in Table 1 and Figures 1 - 4.

FINDINGS:

1) Despite one high membrane filtration result (Lab. 522, Bottle A) and one high MPN result (Lab. 519, Bottle B), statistically there was no significant difference in the average results for any sample by either method between E.E.C. laboratories, British laboratories and the Issuing laboratory.

2) Excluding the high membrane filtration result (Lab. 522, Bottle A) and the high MPN result (Lab. 519, Bottle B), statistically there was no significant difference between the spread of results obtained by E.E.C. and British laboratories for any sample by either method. With E.E.C. laboratories, however, the spread of results by membrane filtration was systematically smaller than those obtained by the multiple tube method.

3) With the same two exclusions, statistically there is no significant difference between the spread of results obtained by the British and E.E.C. laboratories and those of the Issuing laboratory, although the spread was generally greater for E.E.C. laboratories.

4) With the same two exclusions, there was no significant statistical difference between the results of the British and E.E.C. laboratories, although in general the British results were slightly closer to those of the Issuing laboratory.

5) At this stage, no attempt has been made to evaluate media performance, but six E.E.C. and two British laboratories failed to detect the presence of coliform organisms and/or E. coli (faecal coli). These organisms were not isolated by the six E.E.C. laboratories from a total of 10 samples, or by the two British laboratories from three samples.

NOTES The three simulated samples in this distribution were deliberately prepared to contain small numbers of organisms, so that the failure of a few laboratories to isolate coliforms and/or E. coli from some of the samples was not unexpected. In fact, only one laboratory (523) failed to detect them in all three bottles by membrane filtration using endo broth medium. This laboratory, however, obtained satisfactory results in duplicate membrane tests with enriched teepol broth.

Since samples B and C were the same, their results should in general be similar. Although precise comparison of such paired results is difficult because of the inherent sampling errors, most of them were satisfactory in that they were generally within the upper and lower 5% statistical confidence limits of the results of the Issuing laboratory. The E.E.C. laboratories obtained consistently good reproducibility of results from bottles B and C by the membrane filtration method.

A report on the information obtained from the three distributions in this feasibility study, together with the conclusions, is being prepared.

G.I. Barrow

Public Health Laboratory,
Royal Cornwall Hospital (City),
Infirmary Hill,
Truro, TR1 2HZ,
Cornwall, UK.

February 1977

TABLE 1

METHOD	SPECIMEN	LABORATORIES	NO. OF RESULTS	NO. OF COLIFORMS PER 100 ML			NO. OF E.COLI PER 100 ML		
				MEAN	STANDARD DEVIATION	RANGE	MEAN	STANDARD DEVIATION	RANGE
MEMBRANE FILTRATION	231	E.E.C. Labs.	18	12.39	11.10	0,48	5.33	3.56	0,13
		British Labs.	6	9.17	1.72	7,12	4.00	3.35	0,7
		Issuing Labs.	5	9.40	2.61	8,14	5.00	1.58	3,7
	232	E.E.C. Labs.	18	19.06	10.78	0,52	9.39	6.23	1,27
		British Labs.	6	14.50	4.72	8,20	9.17	5.71	1,16
		Issuing Labs.	5	17.00	4.64	11,22	9.80	2.95	5,13
	233	E.E.C. Labs.	18	19.61	11.18	0,52	10.56	6.69	0,27
		British Labs.	6	10.83	4.83	2,16	7.83	4.49	0,13
		Issuing Labs.	5	17.40	4.28	15,25	10.40	4.62	3,14
MULTIPLE TUBES	231	E.E.C. Labs.	15	10.13	5.34	3,20	6.60	4.34	0,13
		British Labs.	16	8.31	4.17	3,17	5.75	3.30	1,11
		Issuing Labs.	5	8.40	4.56	3,13	7.00	4.18	3,13
	232	E.E.C. Labs.	15	19.20	31.56	3,130	16.20	32.62	0,130
		British Labs.	16	19.19	8.21	5,35	12.00	5.80	2,25
		Issuing Labs.	5	22.20	7.95	17,35	14.40	3.97	8,17
	233	E.E.C. Labs.	15	13.80	11.48	0,46	9.13	7.02	0,23
		British Labs.	16	23.50	10.32	11,35	13.81	7.12	0,25
		Issuing Labs.	5	17.00	4.90	13,25	14.20	7.12	8,25

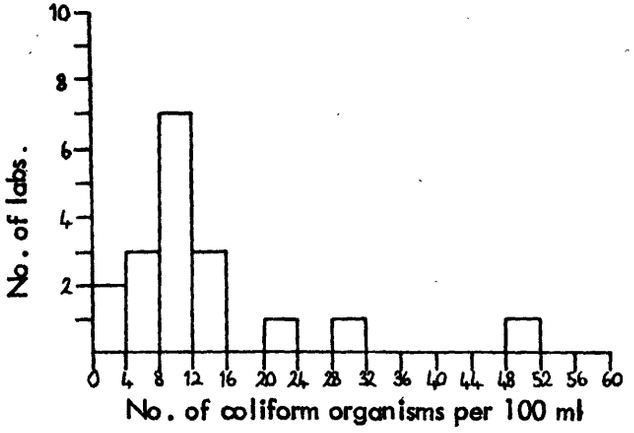
SPECIMEN 231 (BOTTLE A)

FIG. 1 E.E.C. LABORATORIES

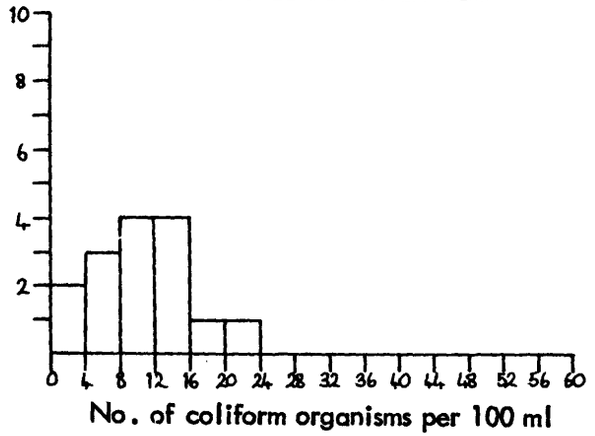
E.E.C. TRIAL 3

COLIFORMS

MEMBRANE FILTRATION METHOD

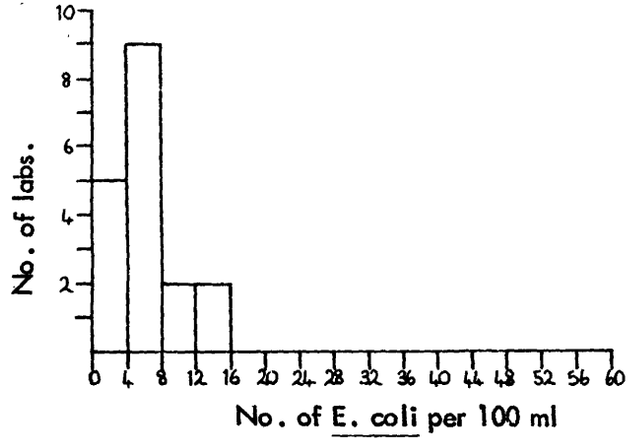


MULTIPLE TUBE METHOD

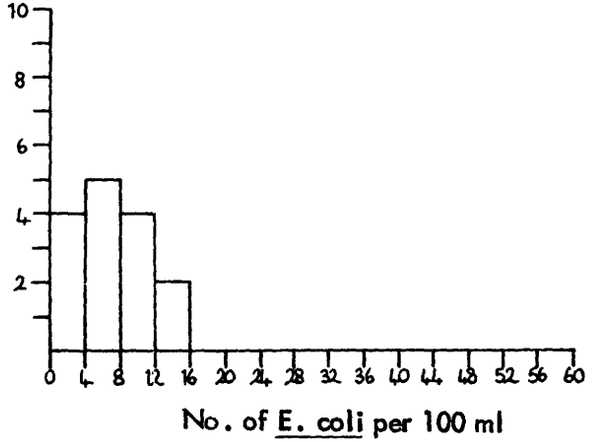


E. COLI

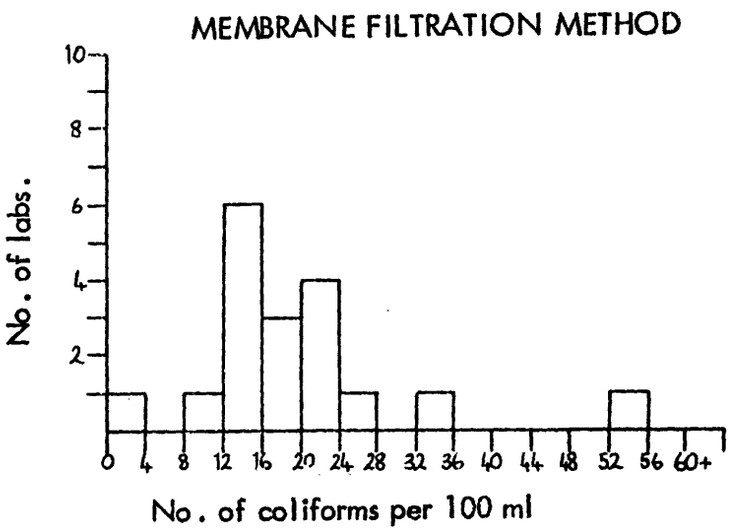
MEMBRANE FILTRATION METHOD

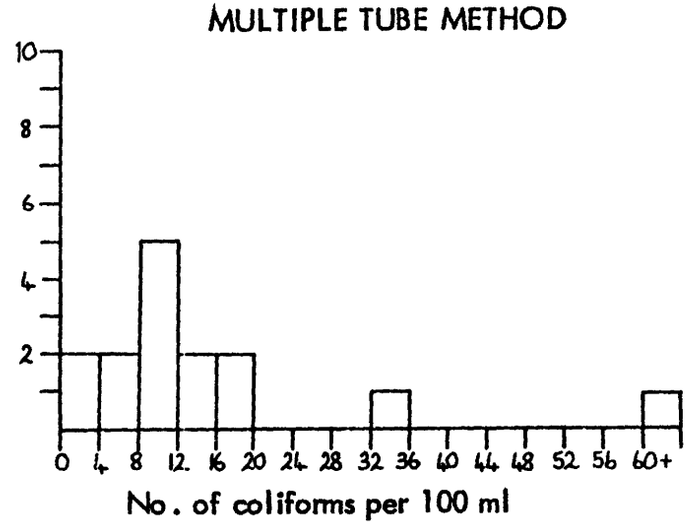


MULTIPLE TUBE METHOD

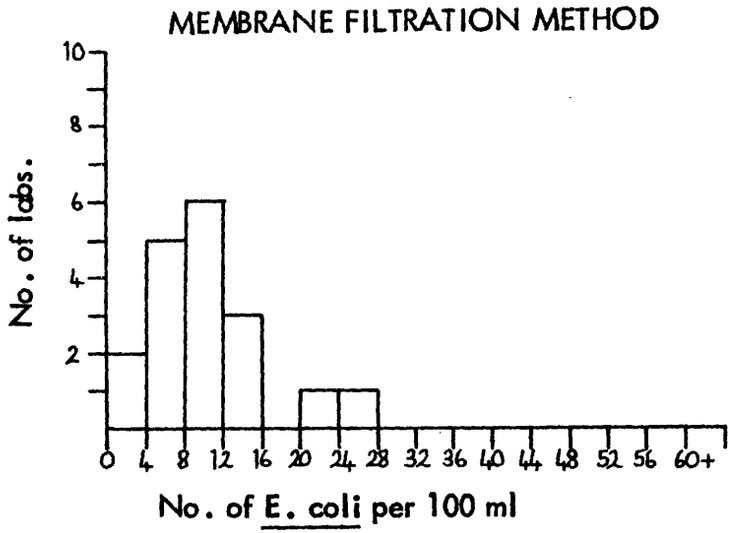


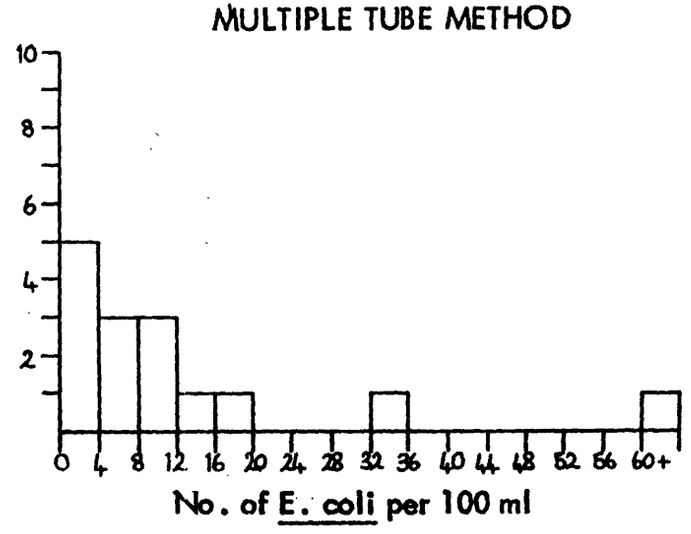
COLIFORMS





E. COLI

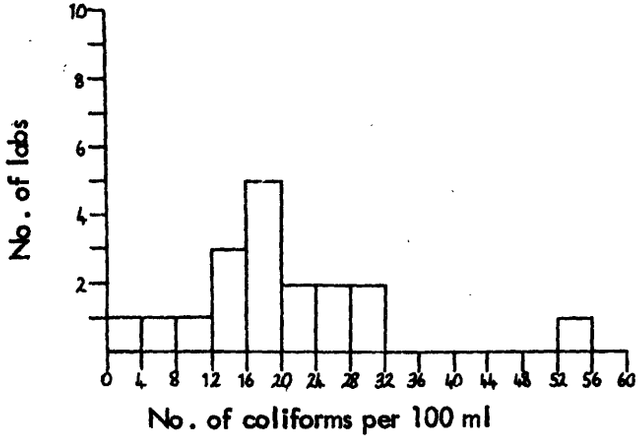




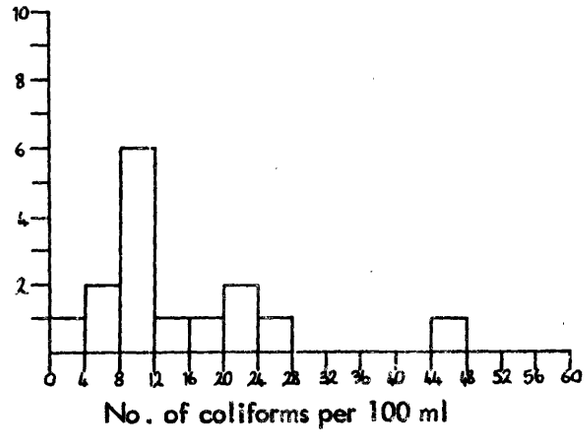
SPECIMEN 233 (BOTTLE C) FIG. 3 E.E.C. LABORATORIES E.E.C. TRIAL 3

COLIFORMS

MEMBRANE FILTRATION METHOD

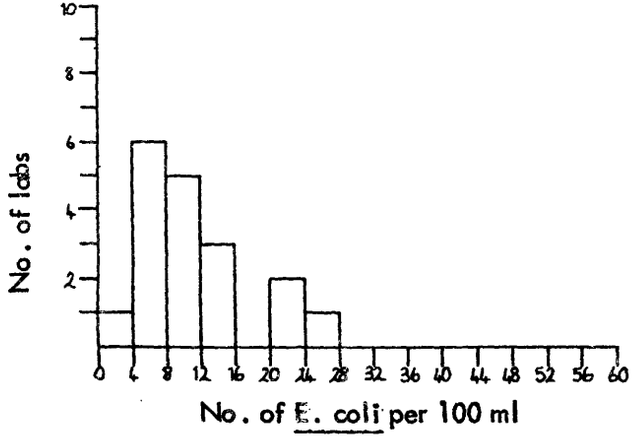


MULTIPLE TUBE METHOD

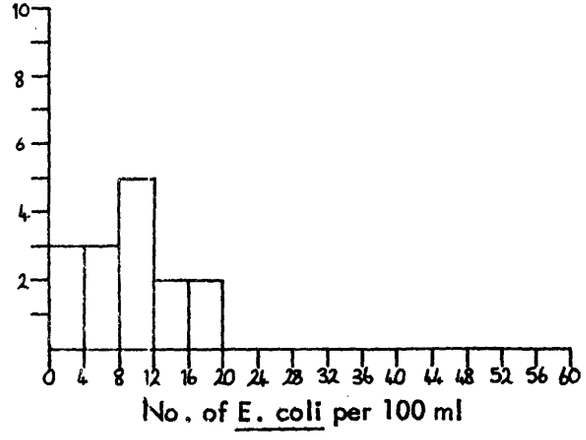


E. COLI

MEMBRANE FILTRATION METHOD



MULTIPLE TUBE METHOD

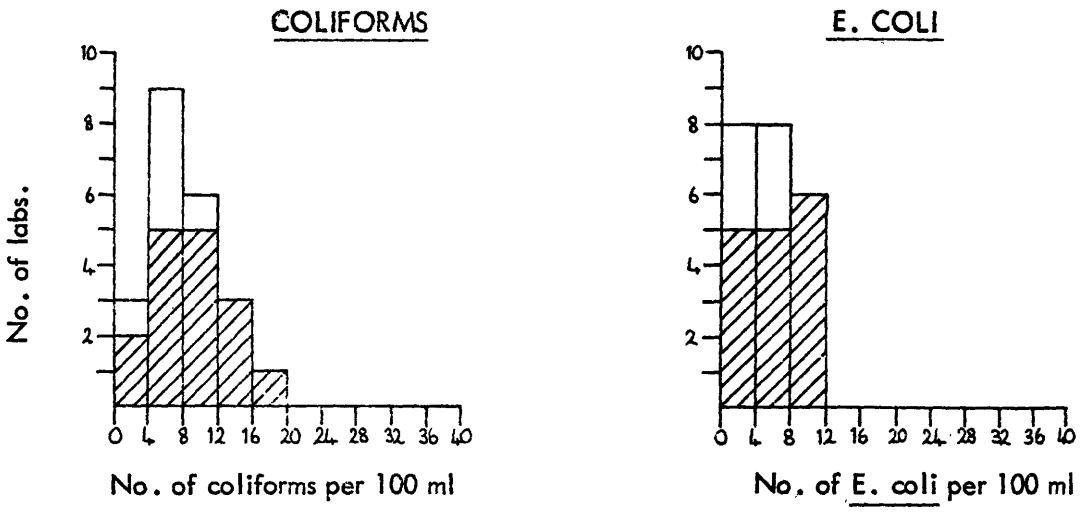


BRITISH LABORATORIES FIG. 4 E.E.C. WATER TRIAL 3

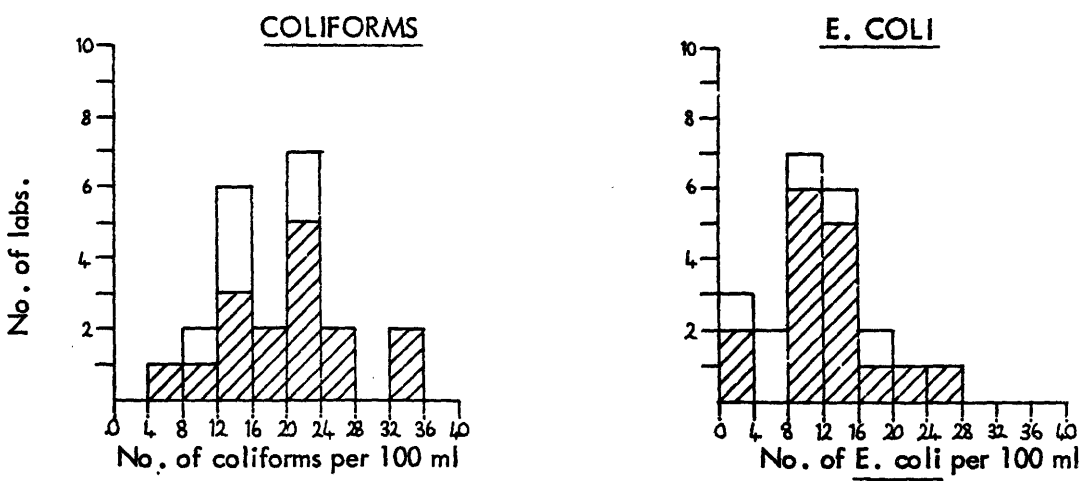
Multiple tube

Membrane filtration

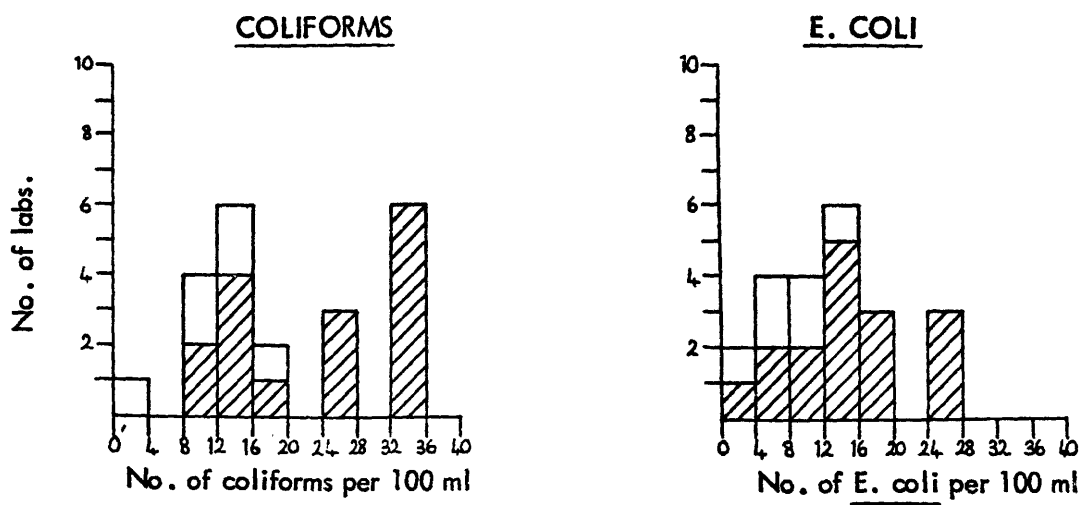
SPECIMEN 231 (Bottle A)



SPECIMEN 232 (Bottle B)



SPECIMEN 233 (Bottle C)



European Communities – Commission

EUR 6037 – Report of a feasibility study on the distribution and use of simulated water samples for comparative bacteriological analysis

G.I. Barrow, D.C. Miller, Royal Cornwall Hospital, Great Britain
R.D. Gray, G.H. Lowe, Public Health Laboratory, Gwent, Great Britain

Luxembourg : Office for Official Publications of the European Communities

1978 – 50 p. – 21.0 x 29.7 cm

Environment and quality of life series

EN

ISBN 92-825-0530-8

Catalogue number : CD-NO-78-018-EN-C

BFR	DKR	DM	FF	LIT	HFL	UKL	USD
365	63	23,25	51,30	9 600	25	6	11.50

A feasibility study is described in which simulated water samples were distributed by post on three separate occasions to a total of 48 laboratories. Concentrated suspensions of viable coliforms and or Escherichia coli were sent to each participating laboratory with precise instruction for the preparation of the simulated water samples. The results of these analyses were very good, and indicate that these methods can be used to the quality control of bacteriological analyses for coliforms.