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Annual Report of the Council for 1987

Presented at the Annual General Meeting of the Association of Public Analysts at Manchester on 21–23 April 1988 by the Honorary Secretary, Mr. M. Barnett.

Few, if any, new initiatives came from central government sources in 1987, which could reasonably be regarded as a year of consolidation for the Association.

House of Lords Select Committee—Science and Technology in Local Government

In last year's Report of Council, the Association expressed its regret that some of the most important of the Select Committee recommendations were unlikely to come into operation. Nevertheless the Association, mindful of the Committee's comments about the standards of Public Analyst laboratories, has considered the means by which a system of "peer review" could be operated. This, it will be recalled, was recommended by the Select Committee to ensure that equivalent standards of service apply within all public analyst laboratories and to promote the best possible practices.

The publication, last year, of the Association's document "A Protocol for Analytical Quality Assurance in Public Analyst Laboratories" has been a great help in this respect and members have generally found the procedures extremely helpful in ensuring and testing the reliability and precision of their day to day laboratory operations. At the same time, it must be recorded that full quality assurance systems add considerably to the cost of analysis if they are carried out properly. It is therefore vital that similar assurance expectations be required of non-public analyst laboratory services lest the Association be placed in a competitive environment where comparisons are made on dissimilar bases.

Continuing Competence

In similar vein, and related quite distinctly to peer review, the Association has this year been asked to consider steps to ensure that its members keep abreast of developments in those scientific activities related to its normal functions. In this context the Association has had opportunity to have discussions with, and to make comment to, the Royal Society of Chemistry which it is understood will be giving advice on continuing competence to practising chemists in general.

Completion of the Internal Market

Last year's report drew attention to the Association's concern, in concert with that of the local authorities and other enforcement officer groups, at the

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implications of the proposals for processes leading to the completion of the internal market. In particular, the proposals seen so far for a system of inspection and food control are unsatisfactory in certain respects, and the Association has continued to make its observations through Members of the European Parliament. No further initiatives have been developed in 1987, and comments on progress have been scant.

In the early part of the year, the Association became aware that a Select Committee of the House of Lords was giving further consideration to the means of inspection of foodstuffs. The Association made written statements to the Select Committee, and although supporting oral evidence was offered, insufficient time was available for this to be given. At the time of writing of this report, no feedback was available.

During 1987 a Commission document was published which indicated the current situation regarding the monitoring of foodstuffs in the various Member states. At the same time, the action programme for co-operation at Community level was suggested, and there were proposals for inspectorate and analytical staff from the various parts of the EEC to visit each other's countries for mutual discussion and investigation. The Association will take advantage of the possibility of sending one or more of its members to other parts of the EEC for discussion with counterpart officers. It is hoped that the work within this area will be financed by the Commission.

Review of the Food Act

In December 1984, the Government had produced a Consultation Document on a review of food legislation, in which response from interested organisations was invited. The Government has now examined the responses, and towards the end of 1987 issued a press release indicating that further consultation would take place with various organisations on a number of topics, which included:

the extension of enforcement from the retail level to the manufacturing or production level;

the introduction of a "due diligence" defence for certain offences under food legislation;

the tightening of controls over contaminated or unfit food;

the need for controls on the development of "novel" foods and other technological changes; and

the extension of the powers to make regulations on the production of milk to that coming from animals other than cows.

The Association has already made its comments to the Ministry of Agriculture, Fisheries and Food on changes of enforcement to production levels. There can be no question that food enforcement in factories will demand a high degree of scientific investigation, and there will be a need for multi-disciplinary teams for proper inspection processes. It remains to be seen what proposals the Ministry makes when further documentation is produced.

Milk and Dairies Sub-committee

The Milk and Dairies Sub-committee has continued to work with the BSI Technical Committees on the production of standards for milk and dairy products. It has also been involved with the Ministry of Agriculture, Fisheries and Food following the adoption of the EEC Council Directive 85/397/EEC on Intra-community Trade in Heat Treated Milk.

Environmental Sub-committee

The Environmental Sub-committee has continued to take an active role in representations on the monitoring of Radiation and Radioactivity. Its support has enabled members to participate in a local authority co-ordinating group examining the possibilities for collation of local authority data and for quality assurance schemes for local authority monitoring.

Consumer Hazards Sub-committee

The Association has, during the year, continued to comment on numerous draft proposals for new or amended legislation.

An approach has been made to the British Standards Institution seeking reappraisal of APA involvement on technical committees.

The Department of Trade and Industry has advised that, in future, it will be a requirement for laboratories to have NAMAS accreditation before being approved by the Department for the performance of testing for compliance with Consumer Safety Regulations.

Food Labelling

The Association continues to take a keen interest in trends in food labelling, and is able to provide opinions which are independent of both the trade and the legislature.

Nutritional Labelling

An important topic this year has been the provision of nutritional information on food labels. The Ministry of Agriculture, Fisheries and Food will not enforce nutritional labelling on foodstuffs, but has recently brought out revised guidelines for setting out the information when manufacturers decide to include it.

The major drawback to the ministry's proposed formats is that the consumer will be provided only with contents of fat, protein, carbohydrate and energy, and some more detailed constituents, in grams per 100g or grams per portion of food. Unfortunately the figures have no real meaning unless they are related to the amount of that food which is consumed in the diet.

A more rational approach to nutrition labelling has been proposed by the Coronary Prevention Group, their prescribed formats being based on a banding system which indicates whether a food is high, medium or low in certain constituents.

The view of this Association, however, is that if nutrition labelling is sufficiently important to warrant involvement by Government Departments,

then a programme of public education would be of greatest benefit to the consumer.

Natural Foods

The description "natural" is a marketing plus for any foodstuff, but some manufacturers apply the word, and an ever increasing number of similar words and derivatives, to all types of foodstuffs.

In order to attempt a logical approach to the use of the word, the Association has produced a draft code of practice which sets out a list of basic foodstuffs, together with processes which could be considered to be "natural".

The APA Code formed part of the LACOTS submission to the Ministry, and it was also mentioned in the Report of the Food Advisory Committee on Natural and Negative Claims for Foodstuffs.

Accreditation

The Association reported last year that accreditation had now been accepted as part of its forward planning. The Association has continued to discuss with officers of the National Testing Laboratory Accreditation Scheme the protocol for accreditation, and progress in this area can be recorded.

APPENDIX

STATISTICAL SUMMARY 1987

Total number of samples	264 240
Including:	
Foods (including complaint samples and those submitted under Imported Food Regulations)	79 604
Milks (including those examined for antibiotics and those submitted under the Milk [Special Designation] Regulations)	14 335
Drinking Waters	19 054
Mineral Waters	200
Swimming Pools	3 743
Environmental Pollution (water, effluents, tip leachates)	48 999
Environmental Pollution (other samples)	32 452
Feeding Stuffs (Agriculture and Medicines Act and Feed Supplements)	3 499
Fertilisers (all kinds)	900
Consumer Protection and Trade Descriptions Act samples	7 226
Cosmetic Regulations samples	492
Health and Safety at Work Act samples	28 256
Miscellaneous (HM Coroner, Health Authorities etc)	25 401

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Evaluation of Microbiological Methods of Analysis for Natural Mineral Waters. Part 1: *Escherichia coli*—Collaborative Trial

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A collaborative trial to evaluate proposed methods of analysis to detect *E. coli* in natural mineral water was carried out. There were nine participants in the trial, seven of whom tested one sample and two of whom tested two samples. Although the methods used were similar to those described in ISO/DP 9308/1, there were significant differences.

The majority of participants isolated and confirmed *E. coli* using Membrane Lauryl Sulphate Broth or Membrane Enriched Teepol Broth followed by Lactose Peptone water and Tryptose water. This combination was shown to give reproducible and repeatable results when approximately 60 *E. coli* colony forming units were present per 250 ml of natural mineral water. Two participants used Lactose Agar with Tergitol 7 and were unable to isolate any *E. coli* from the natural mineral water.

The European Community (EC) Council of Ministers has adopted a Directive relating to the exploitation and marketing of natural mineral waters[1]. This Directive was translated into legislation for England, Wales and Scotland as "The Natural Mineral Waters Regulations 1985"[2] and by similar legislation for Northern Ireland[3]. Amongst the criteria prescribed in the Council Directive are microbiological requirements for waters at source. These criteria include (a) the quantitative determination of the revivable colony count indicative of faecal contamination, and require:

the absence of *Escherichia coli* and other coliforms in 250 ml at 30°C and 44.5°C;
the absence of faecal streptococci in 250 ml;
the absence of sporulated sulphite-reducing anaerobes in 50 ml;
the absence of *Pseudomonas aeruginosa* in 250 ml.

and (b) the determination of the revivable total colony count per ml of water, at:

i. 20–22°C in 72 h on agar–agar or an agar–gelatine mixture, and
ii. 37°C in 24 h on agar–agar.

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Article 11 of the Directive stipulates that agreed methods of analysis to check the microbiological characteristics referred to above shall be developed.

The EC Commission Working Group on Microbiological Methods of Analysis for Natural Mineral Waters is discussing methods which could be incorporated into the Methods of Analysis Directive. That Group is considering two sets of methods, one based on methods originally recommended by Groupement Europeen Source d'Eaux Minerales (GESEM), and the other based on methods originally developed by the International Organisation for Standardisation (ISO). GESEM is the Trade Association of the European Mineral Water Industry; it has developed methods of microbiological analyses for natural mineral waters for its own use and has recommended these methods to the Codex Alimentarius Coordinating Committee for Europe[4]. The ISO has developed a set of methods for use within the potable water industry, the principles of which were considered by the EC Working Group.

Methods which are being discussed in EC Working Groups are assessed within the U.K. by carrying out collaborative trials to validate them. Such trials are organised by the Ministry of Agriculture, Fisheries and Food (MAFF) to assess potential statutory methods of analysis. The results of these trials are normally published as Reports; the aims and objectives of the trials have been given in a previous Report[5]. The natural mineral water methods under discussion within the EC Working Group will be tested as part of this programme, the determination of *E. coli* being the first of such methods to be considered. The results from that trial are now reported.

Development of the Method of Analysis Under Test

One of the methods being discussed by the EC Working Group is the method for *Escherichia coli*. The text being considered by the Group is similar to that given in the ISO Standard "for the detection of coliforms, thermotolerant coliforms and 'presumptive' *E. coli* by membrane filtration"[6]. The text being studied differs from the ISO Standard in that modifications were made to reduce any ambiguities. The modifications are described below:

DEFINITION

The definition for coliforms and thermotolerant coliforms was not included since only *E. coli* was being investigated in this trial.

CULTURE MEDIA

A number of isolation and conformatory media are permitted in ISO/DP 9308/1. These are included in the methods being tested and, in addition, a further confirmatory medium was described; this medium, Schubert's medium, was permitted with the stipulation that, if it were used, only Lactose TTC Agar with Tergitol 7 or Membrane Lauryl Sulphate Broth or Agar could be used as the isolation medium. This addition permitted a combination of the isolation and confirmatory media recommended by GESEM to be tested in addition to those recommended by ISO/DP 9308/1.

RESUSCITATION

A mandatory resuscitation step was prescribed for the trial, in contrast to the procedure given in ISO/DP 9308/1, in which the resuscitation step is optional.

CONFIRMATION

In this trial it was required that 10 colonies, or 20 per cent. of all typical colonies, whichever is the greater number, be subcultured on Nutrient Agar (Oxoid) for 48 h at 30°C before being subjected to the confirmation steps described in the method. This is in contrast to the ISO/DP 9308/1 procedure which does not give any information on how many typical colonies should be confirmed.

Collaborative Trial Organisation, Samples, Method and Results

PARTICIPANTS

Nine laboratories (eight U.K. public analysts and one MAFF laboratory) participated in the trial. Two laboratories each received two sets of samples and returned two independent sets of results.

SAMPLES

It was hoped to prepare for each participant a sample of natural mineral water spiked with *E. coli* at a level of approximately 60 colony forming units (cfu) per 250 mls. The preparatory experimental work carried out to establish the procedure for sample preparation is in Appendix I.

Trial samples were prepared at the Strathclyde Regional Chemist's Department, Hamilton Laboratory. They were transported in an insulated cooled box to minimise sample storage variation to a central location for distribution to participants. Two 250-ml samples were then distributed to each participant, who was asked to keep the samples in an insulated container (capable of maintaining its contents at less than 10°C during transport) to take them to their own laboratory. Participants were asked to commence the required experimental work between 10.00 and 11.00 am the day following distribution of samples. The temperature of the samples at the start of analysis was recorded by the participants.

METHOD

The method which participants were required to use is given in Appendix II; it includes the various permitted alternative reagents and media.

RESULTS

The results obtained by each participant, together with the media used and temperature of sample at the time of testing, are given in Table I.

Statistical Analysis of the Results

The results reported were converted to a log 10 basis to enable a normal collaborative trial statistical analysis of the results to be carried out. The converted results are also given in Table I.

TABLE I

DETERMINATION OF *E. COLI* IN NATURAL MINERAL WATER: COLLABORATIVE TRIAL

Laboratory	Isolation medium	Confirmatory medium	Water temp. (°C)	E. coli *cfu/250 ml*	E. coli *cfu/250 ml* (Log_{10})
1A	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	7·3	53, 48	1·72, 1·68
1B	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	9·1	33, 34	1·52, 1·53
2A	Membrane enriched teepol broth	Lactose peptone water and tryptone water	4·0	61, 54	1·79, 1·73
2B	Membrane enriched teepol broth	Lactose peptone water and tryptone water	3·0	61, 63	1·79, 1·80
3	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	4·0	52, 49	1·72, 1·69
4	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	5·4	59, 62	1·77, 1·79
5	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	7·5	82, 77	1·91, 1·89
6 (b)	Membrane lauryl sulphate broth	Lactose peptone water and tryptone water	17·5	58, 36	1·76, 1·56
7 (a)	Lactose agar with tergitol 7	Lactose peptone water and tryptone water	14·0	0, 0	0, 0
8 (a)	Lactose agar with tergitol 7	Lauryl tryptose mannitol broth with tryptophan *and* Brilliant green bile broth	7·0	[4, 31] [Coliforms] 0, 0	[0·6, 1·49] [Coliforms] 0, 0
9 (a)	Membrane lauryl sulphate broth	No confirmation	4·5	[175, 127]	[2·24, 2·10]

Summary of results: Mean (x) 1·74
Repeatability 0·064
Reproducibility 0·329

1. Laboratories 1 and 2 carried out the analysis on two sets of samples.
2. Laboratory 9 was unable to carry out confirmation due to incubator malfunction.
3. Results marked (a) not included in calculation of results.
4. Results marked (b) eliminated by Cochran's test at 1% level.

The results from laboratories 7, 8 and 9 were not included in the statistical analysis of the results; the reasons for this, reflecting the choice of media used by these laboratories, are given in the Section below.

The remaining results were examined for evidence of individual systematic error using Cochran's and Dixon's Tests (at $P < 0{\cdot}5$) progressively by procedures described by the British Standards Institution[7].

REPEATABILITY AND REPRODUCIBILITY

Calculations for repeatability (r) and reproducibility (R), as defined by the procedures given by the British Standards Institution[7], were carried out on those results remaining after removal of outliers. The results are given in Table II.

Comments on the Results Obtained

The results obtained by laboratory 9 were not included in the statistical

TABLE II
SURVIVAL OF *E. COLI* FROM RIVER WATER IN NATURAL MINERAL WATER WHEN HELD AT 20°C (EXPERIMENT 1) AND 2°C (EXPERIMENT 2)

	Concentration of *E. coli* (*cfu/100 ml*)							
	In river water	In natural mineral water						
Time *h*	0	0	4	24	48	144	168	192
Experiment 1								
Sample 1	13	11	14	7	2	2	—	—
2	65	44	42	22	22	14	—	—
3	130	uc	73	54	31	20	—	—
Experiment 2								
Sample 1	145	132	78	67	57	75	65	58
2	290	200	134	107	110	116	120	

uc, uncountable

analysis of the results due to incubator failure during the initial isolation procedure.

Neither laboratory 7 nor 8 isolated any *E. coli* from the natural mineral water, although laboratory 8 did isolate coliforms. It is possible that the high temperature, 14°C, of the sample on arrival at laboratory 7 adversely influenced the survival, and thus the isolation, of *E. coli.* However, this seems unlikely since the temperature of the sample at laboratory 6 was 17·5°C and *E. coli* was isolated, although these results were subsequently eliminated from analysis by Cochran's test at the 5 per cent. level. At laboratory 8 the sample temperature was 7°C, and thus it is unlikely to have influenced the poor isolation of *E. coli.* Since both laboratories 7 and 8 used the same isolation medium, Lactose Agar with Tergitol 7, but different confirmatory media, it would seem likely that the isolation medium used was the cause of the poor recovery of *E. coli* from the samples. These results were not included in the analysis of the trial results.

The majority of laboratories used Membrane Lauryl Sulphate Broth or Agar, or Membrane Enriched Teepol Broth as the isolation medium followed by Lactose Peptone Water and Tryptone Water for confirmation of *E. coli.* Statistical analysis of these results demonstrated that this method was capable of yielding reproducible and repeatable results when approximately 60 *E. coli* colony forming units (cfu) were present per 250 ml sample. However, it should be noted that no indication of the sensitivity of the methods was attempted. The Community Directive[1] requires the absence of *E. coli* in 250 ml. Thus a suitable method should be able to detect 1 cfu per 250 ml. Preparation of samples containing only 1 cfu would be extremely difficult.

Conclusions

E. coli were not isolated from natural mineral water containing approximately 60 cfu/ml when Lactose Agar with Tergitol 7 was used. These results suggest that this medium is not a suitable isolation medium. However, this study demonstrates that isolation using Membrane Lauryl Sulphate Broth, or Agar, or

Membrane Enriched Teepol Broth followed by confirmation using Lactose Peptone Water and Tryptone Water is a suitable method for detecting *E. coli* in natural mineral water containing approximately 60 cfu per 250 ml.

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Appendix I: Sample Preparation

The following experimental work was conducted to enable spiked samples containing approximately 60 *E. coli* cfu per/250 ml of natural mineral water, at the time of the trial, to be prepared. In all the experiments *E. coli* were enumerated by placing membrane filters, through which 100 ml aliquots of sample had been filtered, on sterile pads pre-soaked in Membrane Enriched Teepol Broth and incubating them for 4 h at 30°C followed by 18 h at 44°C. Initial experiments demonstrated that a laboratory strain of *E. coli* and a freeze-dried strain were unsuitable for use as the test organism as neither strain survived the transfer to natural mineral water. River water was eventually chosen as the source of *E. coli.*

EXPERIMENT 1

A sample of river water containing 1300 *E. coli* cfu per/100 ml was used to inoculate three 1 litre bottles of natural mineral water with 10, 50 and 100 ml of river water respectively. The decline of *E. coli* at 20°C in the natural mineral water was monitored for seven days.

EXPERIMENT 2

Experiment 1 was repeated using 2 × 1 litre bottles and storage at 2°C.

The results of experiments 1 and 2 are shown in Table II.

After a reduction in numbers of approximately 50 per cent. in the first few hours the *E. coli* levels remained relatively stable for the next week when held at 2°C.

These two experiments demonstrated that river water could be used as a source of *E. coli* with which to spike natural mineral water and, after an initial reduction of the *E. coli* present, their numbers could be held steady at a low temperature long enough to transport the samples and conduct the trial.

EXPERIMENT 3

To confirm this conclusion the transport conditions and testing of a spiked sample were investigated.

The results were as shown in Table III.

TABLE III
SURVIVAL OF *E. COLI* IN NATURAL MINERAL WATER UNDER TRANSPORT CONDITIONS FOR TRIAL

	← Time *h*		
	0	24	48
E. coli (cfu/100 ml)	59	NT	38
Temperature (°C)	5	8·5	15

NT, Not tested

EXPERIMENT 4

The variability in the number of *E. coli* in individual samples prepared at the same time was assessed by determining their number in nine 100-ml aliquots of a spiked natural mineral water sample.

The results were as follows: Counts, 47 46 47 50 40 55 52 51 44; Mean, 48; Standard deviation, 4·5.

Appendix II: Method of Analysis for *Escherichia coli*

1. DEFINITION[a]

For the purpose of this method, the following definition applies:

1.1 *Presumptive* Escherichia coli (E. coli):

Organisms capable of forming colonies aerobically at 44 ± 0·5°C on a selective and differential lactose culture medium with the production of acid within 24 h, and which also produce gas from lactose and indole from tryptophan at 44 ± 0.5°C.

2. PRINCIPLE

The detection of *E. coli* in 250 ml of natural mineral water is based on the filtration of the sample through a membrane filter with a pore size which will

(a) See section 8 for further information on these organisms.

retain bacteria. The membrane is placed on either a selective lactose agar culture medium or on an absorbent pad saturated with a liquid medium containing lactose and incubated for 4 h at 30°C followed by 18 ± 2 h at 44°C and examined for the presence of E. *coli*. Characteristic colonies formed on the membrane are subcultured and subjected to confirmatory tests for gas and indole production.

3. APPARATUS AND GLASSWARE

The apparatus required is normal microbiological laboratory equipment, including:

3.1 *Incubators or Water Baths*

Capable of being maintained at 30 ± 1°C and 44 ± 0·5°C.

3.2 *Membrane Filtration Apparatus*

Consisting of

(a) sterile filtration units with vacuum flask tubing, a moisture trap flask and a vacuum source;
(b) sterile forceps;
(c) sterile membrane filters, approximately 47 mm diameter, with a pore size equal to or less than 0·45 μm.

4. CULTURE MEDIA, DILUENT AND REAGENTS

It is recommended that media be prepared from dehydrated basic materials or commercially available dehydrated media. The media shall be prepared according to the manufacturer's instructions and selective agents added, as supplements, at the given concentrations. Media may be stored in the dark at between 0 and 5°C for no longer than 1 month, unless otherwise stated. Care must be taken to avoid any changes during storage.

All chemicals used in the preparation of culture media and diluents shall be of analytical quality unless otherwise stated.

4.1 *Isolation Media*

4.1.1 *Lactose TTC Agar with Tergitol 7*

4.1.1.1 *Basal Medium*

Lactose	20 g
Peptone	10 g
Yeast extract	6 g
Meat extract	5 g
Bromothymol blue	0·05 g
Agar	10–20 g[a]
Distilled water	1000 ml

Preparation: Dissolve the ingredients in the water by heating. If necessary, adjust the pH so that after sterilisation it is 7·2 ± 0·1 at 20°C. Dispense the

[a] Depending on the gelling power of the agar.

medium in bottles in volumes of 100 ml and sterilise in the autoclave at 121 ± 1°C for 15 min.

4.1.1.2 *TTC Solution*

2, 3, 5-triphenyl-tetrazolium chloride (TTC)	0·05 g
Distilled water	100 ml

Preparation: Dissolve the TTC in some of the water and make up to 100 ml. Sterilise by membrane filtration.

4.1.1.3 *Tergitol 7 Solution*

Tergitol 7	0·2 g
Distilled water	100 ml

Preparation: Dissolve the Tergitol 7 in some of the water and make up to 100 ml. Sterilise in the autoclave at 121 ± 1°C for 15 min.

4.1.1.4 *Complete Medium*

Basal medium (4.1.1.1)	100 ml
TTC solution (4.1.1.2)	5 ml
Tergitol 7 solution (4.1.1.3)	5 ml

Preparation: Melt the basal medium (4.1.1.1) and cool to 44 ± 2°C. Add the TTC (4.1.1.2) and Tergitol (4.1.1.3) solutions aseptically, mixing thoroughly but avoiding the formation of bubbles after each addition. Dispense into Petri dishes to a depth of about 5 mm. If not for immediate use, store at 4°C in the dark for not longer than 10 days.

4.1.2 *Lactose Agar with Tergitol 7*

Peptone	5 g
Yeast extract	3 g
Lactose	10 g
Tergitol 7	0·1 ml
Bromothymol blue	0·025 g
Agar	10–20 g[a]
Distilled water	1000 ml

Preparation: Dissolve the ingredients in the water by heating. Dispense volumes of 100 ml into individual bottles and autoclave at 121 ± 1°C for 15 min. For use, melt and dispense into Petri dishes to a depth of about 5 mm; store at 4 ± 1°C in the dark for not longer than 10 days.

4.1.3 *Membrane Enriched Teepol Broth*

Peptone	40 g
Yeast extract	6 g
Lactose	30 g
Phenol red (0·4% m/v aqueous solution)	50 ml
Teepol 610	4 ml
Distilled water	1000 ml

Preparation: Add the peptone and yeast extract to the water and steam to dissolve; add the lactose, phenol red and Teepol afterwards and mix gently to avoid froth. The final pH of the sterile medium should be 7·4 to 7·5 and it may be

[a] Depending on the gelling power of the agar.

necessary to adjust the pH to about 7·6 before sterilisation in order to achieve this. Distribute into screw-capped bottles and autoclave at 115 ± 1°C for 10 min.

4.1.4 *Membrane Lauryl Sulphate Broth*

Peptone	40 g
Yeast extract	6 g
Lactose	30 g
Phenol red (0·4% m/v aqueous solution)	50 ml
Sodium lauryl sulphate—specially pure	1 g
Distilled water	1000 ml

Preparation: Add the ingredients to the water and mix gently to avoid froth. The final pH of the sterile medium should be 7·4 to 7·5 and it may be necessary to adjust the pH to about 7·6 before sterilisation to achieve this. Distribute into screw-capped bottles and autoclave at 115 ± 1°C for 10 min. If Membrane Lauryl Sulphate Agar is required 10–20 g of agar[a] should be added and dissolved by heating prior to sterilisation.

4.1.5 *mFC Medium*

Tryptose or biosate	10·0 g
Proteose peptone	5·0 g
Yeast extract	3·0 g
Sodium chloride	5·0 g
Lactose	12·5 g
Bile salts mixture	1·5 g
Aniline blue	0·1 g
Distilled water	1000 ml

Preparation: Dissolve the ingredients in the distilled water to which has been added 10 ml of 1 per cent. rosolic acid in 0·2 N NaOH. Heat the medium to the boiling point, promptly remove from heat and cool to below 45°C. Do not sterilise by autoclaving. The final pH should be 7·4. The finished media should be stored at 2 to 10°C and any unused medium discarded after 96 h.

Notes: 1. This medium may be solidified by the addition of 1·2–1·5 per cent. (w/w) agar before boiling.

2. Rosolic acid reagent will decompose if sterilised by autoclaving. The stock solution should be stored in the dark at 2 to 10°C and be discarded after 2 weeks, or sooner if its colour changes from dark red to muddy brown.

4.2 *Confirmatory Media*

4.2.1 *Lactose Peptone Water (for gas production)*

Peptone	10 g
Sodium chloride	5 g
Lactose	10 g
Phenol red (0·4 per cent. m/V aqueous solution)	2·5 ml
(or Andrade's indicator, prepared as below)	10 ml
Distilled water	1000 ml

Preparation: Dissolve the ingredients in the water and adjust to pH 7·5. Add the phenol red indicator and distribute in 5 ml volumes into test tubes containing

[a] Depending on the gelling power of the agar.

inverted Durham tubes. (Alternatively, adjust to pH 6·8–7·0, and add the Andrade's indicator.) Autoclave the plugged tubes at 110°C for 10 min. Test for sterility by incubation at 37 ± 1°C for 24 h.

Andrade's Indicator

This is prepared by dissolving 0·5 g of acid fuchsin in 100 ml of distilled water. Add 17 ml of sodium hydroxide solution (1 mol/litre) and leave at room temperature overnight. The solution should be straw coloured the following morning. If it is at all brownish, add a little more sodium hydroxide solution and allow to stand again. This solution is strongly alkaline, and consequently media to which it is added should be adjusted previously to a pH of about 6·8.

4.2.2 *Tryptone Water (for indole reaction)*

Certain peptones which give satisfactory results in tests at 37°C are not satisfactory for the indole test at 44°C. Tryptone has been found satisfactory and is recommended.

Tryptone	20 g
Sodium chloride	5 g
Distilled water	1000 ml

Preparation: Dissolve the ingredients in the water and adjust to pH 7·5. Distribute in 5 ml volumes into suitably sized plugged test tubes or universal bottles, and autoclave at 115 ± 1°C for 10 min.

4.2.3 *Lauryl Tryptose Mannitol Broth with Tryptophan*

Tryptose	10 g
Mannitol	5 g
Sodium chloride	5 g
Dipotassium hydrogen phosphate	2·75 g
Potassium dihydrogen phosphate	2·75 g
Sodium lauryl sulphate	0·1 g
L (−) Tryptophan	0·2 g
Distilled water	1000 ml

Preparation: Add the tryptose, mannitol, sodium chloride, phosphates and tryptophan to the water and heat to dissolve. Add the sodium lauryl sulphate and mix gently to avoid frothing. Adjust to pH 6·8 ± 0·2. Distribute in 5-ml volumes in tubes containing an inverted Durham tube. Plug the tubes and autoclave at 115 ± 1°C for 10 min.

4.2.4 *Schubert's Medium*

Tryptophan	0·2 g
Glutamic acid	0·2 g
Magnesium sulphate	0·7 g
Ammonium sulphate	0·4 g
Sodium citrate	0·5 g
Sodium chloride	2 g
Oxoid tryptone	10 g
Mannitol	7·5 g
Distilled water	500 ml
Phosphate buffer pH 7·6 (see 4.3.2)	500 ml

Preparation: Dissolve the solid ingredients in the water. Add the buffer solution. Distribute in 7 to 8 ml volumes in test tubes containing inverted Durham tubes. Plug the tubes and sterilize at 115 ± 1°C for 10 min.

4.3 *Reagents*

4.3.1 *Kovac's Reagent for Indole*

Paradimethylaminobenzaldehyde	5 g
Amyl Alcohol (free from organic bases)	75 ml
Hydrochloric acid (d = 1·18 g/ml)	25 ml

Preparation: Dissolve the aldehyde in the alcohol. Add the concentrated acid with care. Protect from light and store at 4 ± 1°C.

Note: The reagent should be light yellow to light brown in colour; some samples of amyl alcohol are unsatisfactory, and give a dark colour with aldehyde.

4.3.2 *Phosphate Buffer (pH 7·6)*

Aqueous solution of Na_2HPO_4 anhydrous M/15 (9·47 g/litre): 83 parts
Aqueous solution of KH_2PO_4 anhydrous M/15 (9·073 g/litre): 13 parts

4.4 *Diluents*

4.4.1 *Peptone Diluent (0·1 per cent.)*

Peptone	1·0 g
Distilled water	1000 ml

Preparation: Dissolve the peptone in about 950 ml of the water. Adjust the pH with sodium hydroxide solution (40 g/litre) or hydrochloric acid (36·5 g/litre) so that after sterilisation it will be 7·0 ± 0·1. Make up to 1000 ml with water and autoclave at 121 ± 1°C for 15 min.

4.4.2 *Peptone Saline Solution*

Peptone	1·0 g
Sodium chloride	8·5 g
Distilled water	1000 ml

Preparation: Dissolve the ingredients in about 950 ml of the water by boiling. Adjust the pH with sodium hydroxide solution (40 g/litre) or hydrochloric acid (36·5 g/litre) so that after sterilisation it will be 7·0 ± 1. Make up to 1000 ml with water, dispense in convenient volumes and autoclave at 121 ± 1°C for 15 min.

4.4.3 *Quarter Strength Ringer's Solution*

Sodium chloride	2·25 g
Potassium chloride	0·105 g
Calcium chloride, anhydrous	0·12 g
Sodium bicarbonate	0·05 g
Distilled water	1000 ml

Preparation: Dissolve the ingredients and dispense in convenient volumes. Autoclave at 123 ± 1°C for 15 min.

5. PROCEDURE

5.1 *Filtration and Incubation*

Filter two 250 ml volumes of natural mineral water in the following manner: With sterile forceps, aseptically remove the membrane filter. Centre the membrane grid side up or face-up on the filter holder base. Place a filter funnel on to the assembly and secure it.

With vacuum on, add 20–30 ml of one of the sterile diluents (4.4). Release vacuum. Aseptically transfer the specified volume from a well-mixed sample of natural mineral water to the funnel. Apply the vacuum and filter the entire contents. Rinse the funnel with 20–30 ml of the same sterile diluent twice, applying the vacuum continuously. Turn off the vacuum immediately after the last rinse has passed through the filter. Remove the filter funnel and with sterile forceps remove the membrane filter from the base.

If a solid isolation medium is chosen roll the membrane filter onto the agar surface in the Petri dish, making sure that air is not trapped between the membrane and the agar surface. If an air bubble is observed, the membrane should be raised and again rolled onto the agar to eliminate the air.

Alternatively, if a liquid isolation medium is chosen, roll the membrane filter in a similar manner onto a sterile pad soaked with this medium. The same precaution to avoid air bubbles should be taken. Incubate each membrane for 4 h at 30 ± 1°C followed by 10–24 h at 44 ± 0·5°C.

5.2 *Examination of Membranes*

After incubation examine the membranes and count as presumptive *E. coli* organisms all colonies, irrespective of size, which show the following characteristics:

(a) On Lactose TIC Agar with Tergitol 7 (4.1.1): a yellow, orange or brick red coloration with a yellow central halo in the medium under the membrane.
(b) On Lactose Agar with Tergitol 7 (4.1.2): a yellow central halo in the medium under the membrane.
(c) On Membrane Enriched Teepol Broth (4.1.3): a yellow colour extending to the membrane.
(d) On Membrane Lauryl Sulphate Broth or Agar (4.1.4): a yellow colour extending to the membrane.
(e) On mFC Medium (4.1.5): a blue colour.

5.3 *Confirmatory Tests*

It must be noted that the colonies on membranes are only presumptive *E. coli.* Since gas production is not detected, it is important to carry out confirmatory tests.

5.3.1 *Gas and Indole Production*

To confirm the membrane results, subculture ten typical colonies or 20 per cent. of all typical colonies (whichever is the greater number) (5·2) to separate tubes of lactose peptone water (4.2.1) and to separate tubes of tryptone water (4.2.2) and incubate at 44 ± 0·5°C for 24 h. Alternatively each colony of

presumptive *E. coli* may be subcultured to separate tubes of one of the prescribed single step media (4.2.3 or 4.2.4) and incubated at 44 ± 0·5°C for 24 h. Look for:

(a) production of gas;
(b) development of a red colour at the surface of the culture after the addition of 0·2–0·3 ml of Kovac's reagent (4.3.1).

These reactions confirm the presence of presumptive *E. coli.*

Notes: 1. If Schubert's medium is used only Lactose TTC Agar with Tergitol 7 (4.1.1) or Membrane Lauryl Sulphate Broth (or Agar) (4.1.4) should be used as the isolation medium.

2. Typical colonies may be subcultured onto nutrient agar and incubated at 30°C for up to three days before continuing with confirmation steps as described above.

6. CALCULATION OF RESULTS

Calculate the proportion of all typical colonies subcultured in 5.3.1 which were confirmed as presumptive *E. coli.* Using this number calculate the number of presumptive *E. coli* obtained in 5.2.

Example: assuming nine out of ten colonies are confirmed as being *E. coli,* the proportion of all typical colonies subcultured in 5.3.1 which can be regarded as presumptive *E. coli* is thus 90%. Therefore the number of presumptive *E. coli* forming colonies in 5.2 may be calculated as = 0·9 × the total number of typical colonies counted.

7. TEST REPORT

The test report should include:

(a) A statement of the number of *E. coli* found in each 250 ml sample of natural mineral water tested.
(b) The isolation medium used.
(c) The confirmatory medium/media used.
(d) Any operation(s) not specified in the method or considered optional which may have influenced the results.
(e) Any particular occurrence(s) observed during the course of the analysis, in particular any which may have influenced the final choice of method for the EC Directive detailing microbiological methods of analysis for natural mineral waters.
(f) The temperature of the sample at the time of testing.

8. FURTHER MICROBIOLOGICAL INFORMATION RELEVANT TO THE COLIFORM GROUP OF ORGANISMS

Presumptive *E. coli* are Gram-negative, non-sporing, oxidase-negative, rod-shaped bacteria, which are capable of aerobic and facultatively anaerobic growth in the presence of bile-salts (or other surface-active agents with similar growth-inhibiting properties), and which are also able to ferment lactose with the production of acid (or aldehyde), and gas within 48 h when incubated at a temperature of 44°C.

Presumptive *E. coli* which also give a positive result in the methyl red test and can decarboxylate 1-glutamic acid, but which are not able to produce acetyl methyl carbinol, utilise citrate as the sole source of carbon or to grow in potassium cyanide broth, may be regarded as *E. coli.*

J. Assoc. Publ. Analysts, 1988, **26**, 49–50

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J. Assoc. Publ. Analysts, 1987, **25**, 113–128

The Distribution of Heavy Metals in Soil and Metal Uptake into Vegetation, at Beaumont Leys Sewage Farm, Leicester

Part I: Analytical Methodology and Metal Distribution in Soil

S. D. MUSGROVE

Several mistakes occurred in Table VII of the above paper. The correct Table is shown overleaf

TABLE VII

HEAVY METALS IN SOIL SAMPLES (BEAUMONT LEYS—200 FT GRID POINTS). PERCENTAGE METAL EXTRACTED WITH VARIOUS EXTRACTANTS

		Antimony	Arsenic	Barium	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
Mean total metal content mg element/kg ds.		—	16·1	92	3·0	281	76	168	0·67	72	350
Metal available in 0·5 M acetic acid	Mean concn.	—	—	—	1·36	—	5·5	—	—	17·7	87
	per cent. of total	—	—	—	45·3	—	7·2			24·6	24·9
Metals extracted by Toys (Safety) Regs. Method	Mean concn.	0·11	1·22	66	2·86	6·98	—	21·7	0·015	—	—
	per cent. of total	—	7·6	71·7	95·3	2·5		12·9	2·2		