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Evaluation of Microbiological Methods of Analysis for Natural Mineral Waters

Part 4

Detection of Faecal Streptococci

Collaborative Trial

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The results of a collaborative trial of two methods for the detection of faecal streptococci in natural mineral water are reported. Twenty-seven UK laboratories participated in the trial to validate liquid enrichment and membrane filtration procedures prescribed by the International Standards Organisation for use with potable waters. The results from this trial indicate that both procedures are suitable for the detection of faecal streptococci in natural mineral water. The membrane filtration procedure is recommended for enumeration of faecal streptococci in natural mineral water when present in numbers exceeding 100 cfu/ml.

The European Council of Ministers has adopted a Directive (80/777/EEC) on the Approximation of the Laws Relating to the Exploitation and Marketing of Natural Mineral Waters (NMW)[1]. This Directive was subsequently translated into legislation for England, Wales and Scotland[2] and also for Northern Ireland[3].

The Directive 80/777/EEC prescribes microbiological standards for NMW at source and specifies:

- the absence of coliforms and *Escherichia coli* in 250 ml at 30°C and 44.4°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
- the determination of total revivable colony count at 22°C and 37°C

Under consideration are microbiological methods for the examination of NMW and in particular the microbiological methods for potable waters published by

the International Organisation for Standardisation (ISO). Methods being discussed in the EU are assessed within the UK by carrying out collaborative trials. Three trials of microbiological methods for NMW have already been carried out by MAFF, for the detection of *Escherichia coli* (Part I)[4], enumeration of total revivable colony count (Part II)[5] and detection of *Pseudomonas aeruginosa* (Part III)[6]. This report describes the fourth trial organised by MAFF to validate microbiological methods of analysis for NMW and in particular for the demonstration of the absence of faecal streptococci as required by the Directive. The aims and objectives of these trials have been given previously[7].

Development of the Method of Analysis Under Test

The following ISO methods were investigated:

- ISO 7899-1:1984 Water Quality, Detection and enumeration of faecal streptococci by enrichment in liquid medium. [8]

- ISO 7899-2:1984 Water Quality, Detection and enumeration of faecal streptococci by membrane filtration. [9]

EC Directive 80/777/EEC, requires demonstration of the absence of faecal streptococci in 250 ml of NMW. The ISO liquid enrichment procedure (Part 1 of ISO 7899) was used for the detection of faecal streptococci and not for their enumeration.

The ISO membrane filtration procedure (Part 2 of ISO 7899) was used for the detection and enumeration of faecal streptococci in those test samples which had "significant" numbers of the organism present.

Definition

For the purpose of this trial the following definition applies: Faecal streptococci - micro-organisms capable of growth in dextrose azide broth or on KF Streptococcus or m-enterococcus agars incubated at 37°C and which also produce characteristic colonies when grown on bile aesculin azide agar incubated at 44°C.

Collaborative Trial Organisation

Participants

A total of 27 UK laboratories participated in the trial, comprising 14 Public Analyst laboratories, 11 Public Health laboratories, one commercial laboratory and the MAFF Food Science Laboratory, Norwich.

Preparation and Distribution of Samples

Full details of sample preparation procedures are given in Appendix I. Protocols were distributed to participants several weeks in advance of the trial to allow for familiarisation with the methods.

NMW samples were prepared and cooled to *ca* 6°C on the morning of the day of distribution. They were transported in cool boxes (<10°C) and delivered to participating laboratories by overnight carrier to arrive the following morning. Participants were instructed to store samples at 4°C for 3 days prior to testing. Each laboratory received 5 × 250 ml samples of NMW for examination using the liquid enrichment technique and 5 × 500 ml samples of NMW for examination using the membrane filtration procedure. Participants were asked to determine the presence/absence of faecal streptococci in each of the 10 NMW samples.

Each batch of 10 samples of NMW was identical and comprised 3 sample types; samples were dispatched to participants as blind duplicates randomly coded. The sample types were as follows:

> Sample type A: 2 × 250ml and 2 × 500ml samples containing approximately 50 cfu *Enterococcus faecalis* NCDO 581/250ml NMW and 50 cfu *Staphylococcus aureus* NCDO 1499 /250 ml (positive sample, high level of contamination).
>
> Sample type B: 2 × 250ml and 2 × 500ml samples containing approximately 10 cfu *Enterococcus faecalis* NCDO 581/250ml NMW and 50 cfu *Staphylococcus aureus* NCDO 1499 /250 ml NMW (positive sample, low level of contamination)
>
> Sample type C: 1 × 250ml and 1 × 500ml sample containing approximately 50 cfu *Staphylococcus aureus* NCDO 1499/250 ml NMW (negative sample)

Methods

The methods participants were required to use are given in Appendices II and III.

Results

The results (expressed as presence/absence) obtained by each laboratory using the liquid enrichment technique are given in Table 1. The results for the membrane filtration technique were reported as both presence/absence and as colony counts; they are given in Tables 2 and 3. As counts were reported for the membrane filtration method, precision characteristics (repeatability and reproducibility) for the method were calculated using the procedures outlined in the International Protocol on the conduct and evaluation of collaborative study data [10]; these are given in Table 4.

Discussion of results

Liquid enrichment method

All samples containing *Enterococcus faecalis* NCDO 581 at a level of *ca* 50 cfu/250 ml NMW (sample type A), were correctly identified by participants using the liquid enrichment technique. However, one laboratory reported a false negative result from one of the samples containing *Enterococcus faecalis* NCDO 581 at the lower level of contamination. The remaining samples inoculated at this lower level were correctly identified. One laboratory also reported a false positive result from a sample that had not been inoculated with *Enterococcus faecalis* (sample type C).

Participants reported that the liquid enrichment method was easy to use but a number of laboratories commented that detection of the colour change, resulting from production of acid in the dextrose azide broth, was difficult. The single false positive result for the uninoculated sample was reported as producing only a weak colour change in the dextrose azide broth. Some participants suggested an alternative formulation of dextrose azide broth which did not contain bromocresol purple and relied on production of turbidity in the broth as an indication of the presence of presumptive faecal streptococci. This modification would overcome the difficulty in interpretation of the colour change but would presumably result in an increase in the number of presumptive positive dextrose azide broths requiring confirmation on bile aesculin azide agar, as a number of bacterial species are capable of producing turbid growth in dextrose azide broth.

Reported results for confirmation of positive dextrose azide broths revealed that 25 of the 27 laboratories used the prescribed bile aesculin azide agar (BAAA) as the confirmation medium. Of these, 19 laboratories reported typical growth on BAAA after 6 hours incubation at 44°C and 4 laboratories reported that they were unable to detect typical growth on BAAA until 24 hours. Of the remaining 2 laboratories using BAAA, one did not report results for examination of plates at 6 hours and the other laboratory reported typical growth from all samples containing *Enterococcus faecalis* after 6 hours incubation but interestingly, the confirmation of the isolate from the uninoculated sample (false positive) required 24 hours incubation before typical growth was detected.

Two laboratories did not use BAAA for confirmation of faecal streptococci but used either kanamycin aesculin azide agar, which required 24 hour incubation for development of typical growth, or used bile aesculin agar; no indication of growth after 6 hours was reported but typical growth developed after 24 hours incubation. Three laboratories expressed the opinion that the ±0.25°C tolerance for incubation of the BAAA was not practical.

In summary, use of the liquid enrichment procedure enabled the correct identification of 268 of 270 natural mineral water samples (99.3%) with a very low risk (0.7%) of obtaining either a false positive or false negative result. The method is simple to carry out but the requirement for large volumes of double strength dextrose azide broth is a problem which is exacerbated when large numbers of samples are examined. Although the ISO method indicates that the procedure can be used for enumeration as a most probable number (MPN) technique, the media, materials and time required to process samples would probably make this procedure impractical for processing large numbers of samples.

The liquid enrichment method is recommended for samples where low numbers of faecal streptococci are anticipated, e.g. bottled natural mineral water.

Membrane filtration method

Faecal streptococci were detected using membrane filtration, in conjunction with KF-streptococcus and m-enterococcus agars, in all samples of NMW containing *Enterococcus faecalis* at levels of 10 and 50 cfu/250 ml (Sample types A and B). However, one laboratory reported a false positive result, when using KF-streptococcus agar, for a sample that had not been inoculated with *Enterococcus faecalis* (sample type C) but correctly identified the sample using m-enterococcus agar. This laboratory also reported the false positive result using the liquid enrichment technique.

Statistical analysis of the counts from replicate samples revealed that there was no significant difference ($p<0.05$) in counts obtained between the KF-streptococcus and m-enterococcus media used in the membrane filtration technique. Four laboratories reported growth on filters incubated on KF-streptococcus agar from samples which had not been inoculated with *Enterococcus faecalis* but no growth was obtained from the corresponding filter incubated on m-enterococcus agar. Pre-trial studies did not indicate that KF-streptococcus agar was less selective than m-enterococcus agar, when *Staphylococcus aureus* was investigated as a potential competitor to *Enterococcus faecalis*. Therefore, it seems likely that the growth on the KF-streptococcus agar was autochthonous flora from the NMW, rather than the inocula added for the purposes of the trial.

Twenty-five laboratories used BAAA to confirm presumptive isolates obtained on the membrane filters as faecal streptococci. These laboratories reported typical growth after 6 hours incubation from 88% of faecal streptococci isolated using m-enterococcus agar and 90% of isolates cultured on KF-streptococcus agar. The BAAA plates from 4% of isolates were only examined after 24 hours incubation and the isolates which were incorrectly confirmed as faecal streptococci (false positives) required incubation for 24 hours before typical growth was produced.

Of the 2 laboratories that did not use BAAA for confirmation, one used kanamycin aesculin azide agar, which required 6 hours incubation for development of typical growth and the other laboratory used bile aesculin agar and gave no indication of growth after 6 hours incubation but reported typical growth after 24 hours incubation.

A higher proportion of isolates were confirmed after 6 hours incubation with the membrane filtration procedure, than with the liquid enrichment technique. This is presumably due to the higher inoculum applied to the BAAA plate when subculturing from a colony on a solid medium, as opposed to a broth culture.

In summary, faecal streptococci were detected in >99% of NMW inoculated with *Enterococcus faecalis* using either m-enterococcus agar or KF-streptococcus agar using the membrane filtration technique. The principle advantages of the membrane filtration technique are that it gives an indication of the level of contamination with faecal streptococci and is suitable for rapid processing of large numbers of samples, with no requirement for large quantities of isolation media. The membrane filtration technique is recommended for water samples where higher numbers of target organism are expected, e.g. >100 cfu/ml.

The precision of the membrane filtration method, determined by statistical analysis of the colony counts, appeared to be better for sample type A which

had a higher mean colony count than for sample type B, this was not unexpected However, repeatability values were poor when compared to the reproducibility of the method. There was little difference in precision between the two media used for membrane filtration.

Conclusion

The results of this trial indicate that the liquid enrichment and membrane filtration methods are suitable for detection of faecal streptococci in NMW. As there was no significant difference between the counts obtained on KF-streptococcus and m-enterococcus agars or an effect on precision, the value of using both media (as indicated by ISO) for examination of NMW is questionable. From the limited data available from this trial, m-enterococcus agar is the medium of choice for the membrane filtration procedure, as no false positive results were reported and fewer presumptive isolates which did not confirm as faecal streptococci were obtained with this medium. The membrane filtration technique is recommended for enumeration of faecal streptococci, provided >100 cfu/ml are likely to be present in the sample.

The incubation period for presumptive isolates of faecal streptococci to develop typical growth on BAAA incubated at 44°C should be at least 24 hours, particularly when using the liquid enrichment procedure. However there is value in examining BAAA plates after 6 hours incubation, as a large proportion of faecal streptococci can be confirmed after this incubation period.

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TABLE 1

Faecal Streptococci in Natural Mineral Water Collaborative Trial Results For Liquid Enrichment Technique

	SAMPLE TYPE		
Laboratory Reference Number	**A**	**B**	**C**
1	+;+	+;+	-
2	+;+	+;+	-
3	+;+	+;+	-
4	+;+	+;+	-
5	+;+	+;+	-
6	+;+	+;+	-
7	+;+	+;+	-
8	+;+	+;+	-
9	+;+	+;+	-
10	+;+	+;+	-
11	+;+	+;+	-
12	+;+	+;+	-
13	+;+	+;+	-
14	+;+	+;+	-
15	+;+	+;+	-
16	+;+	+;-	-
17	+;+	+;+	-
18	+;+	+;+	-
19	+;+	+;+	+
20	+;+	+;+	-
21	+;+	+;+	-
22	+;+	+;+	-
23	+;+	+;+	-
24	+;+	+;+	-
25	+;+	+;+	-
26	+;+	+;+	-
27	+;+	+;+	-
Intended Results	+;+	+;+	-

Sample Type A: 50 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type B: 10 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type C: 50 cfu *Staphylococcus aureus*/250ml
+ Faecal streptococci detected
- Faecal streptococci not detected

TABLE 2
Collaborative Trial Results for Membrane Filtration Technique Using KF-Streptococcus Agar

	SAMPLE TYPE		
Laboratory Reference Number	**A**	**B**	**C**
1	+(47) ; +(39)	+(9) ; +(5)	-(0)
2	+(61) ; +(47)	+(15) ; +(5)	-(0)
3	+(52) ; +(41)	+(11) ; +(11)	-(0)
4	+(49) ; +(49)	+(13) ; +(14)	-(0)
5	+(43) ; +(54)	+(3) ; +(7)	-(0)
6	+(49) ; +(70)	+(3) ; +(5)	-(0)
7	+(67) ; +(50)	+(21) ; +(23)	-(0)
8	+(37) ; +(51)	+(10) ; +(11)	-(0)
9	+(40) ; +(50)	+(10) ; +(9)	-(0)
10	+(47) ; +(51)	+(7) ; +(7)	-(0)
11	+(56) ; +(53)	+(7) ; +(12)	-(0)
12	+(53) ; +(61)	+(9) ; +(17)	-(0)
13	+(34) ; +(53)	+(10) ; +(15)	-(0)
14	+(51) ; +(36)	+(9) ; +(8)	-(0)
15	+(59) ; +(39)	+(9) ; +(14)	-(0)
16	+(58) ; +(52)	+(12) ; +(11)	-(0)
17	+(53) ; +(70)	+(8) ; +(10)	-(0)
18	+(49) ; +(34)	+(3) ; +(10)	-(0)
19	+(56) ; +(60)	+(16) ; +(11)	+(16)
20	+(46) ; +(40)	+(10) ; +(10)	-(0)
21	+(54) ; +(43)	+(12) ; +(17)	-(0)
22	+(42) ; +(40)	+(6) ; +(6)	-(0)
23	+(16) ; +(47)	+(13) ; +(16)	-(0)
24	+(37) ; +(45)	+(12) ; +(9)	-(0)
25	+(44) ; +(57)	+(11) ; +(13)	-(0)
26	+(58) ; +(43)	+(10) ; +(5)	-(0)
27	+(44) ; +(49)	+(14) ; +(10)	-(0)
Intended Results	**+(50) ; +(50)**	**+(10) ; +(10)**	**-(0)**

Sample Type A: 50 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type B: 10 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type C: 50 cfu *Staphylococcus aureus*/250ml
+ Faecal streptococci detected
- Faecal streptococci not detected
Number in brackets is reported cfu on membrane from 250 ml volume.

TABLE 3

Collaborative Trial Results for Membrane Filtration Technique Using m-Enterococcus Agar

Laboratory Reference Number	SAMPLE TYPE A	B	C
1	+(46) ; +(38)	+(10) ; +(6)	-(0)
2	+(50) ; +(39)	+(15) ; +(4)	-(0)
3	+(49) ; +(48)	+(7) ; +(11)	-(0)
4	+(48) ; +(52)	+(12) ; +(7)	-(0)
5	+(55) ; +(40)	+(4) ; +(15)	-(0)
6	+(59) ; +(60)	+(19) ; +(6)	-(0)
7	+(46) ; +(49)	+(10) ; +(15)	-(0)
8	+(50) ; +(59)	+(17) ; +(8)	-(0)
9	+(40) ; +(47)	+(7) ; +(14)	-(0)
10	+(50) ; +(57)	+(8) ; +(13)	-(0)
11	+(49) ; +(53)	+(9) ; +(10)	-(0)
12	+(42) ; +(58)	+(6) ; +(12)	-(0)
13	+(51) ; +(57)	+(8) ; +(17)	-(0)
14	+(45) ; +(55)	+(15) ; +(10)	-(0)
15	+(48) ; +(51)	+(17) ; +(15)	-(0)
16	+(57) ; +(44)	+(8) ; +(8)	-(0)
17	+(54) ; +(60)	+(14) ; +(13)	-(0)
18	+(60) ; +(60)	+(9) ; +(7)	-(0)
19	+(42) ; +(41)	+(8) ; +(8)	-(0)
20	+(50) ; +(49)	+(6) ; +(12)	-(0)
21	+(60) ; +(36)	+(10) ; +(9)	-(0)
22	+(40) ; +(40)	+(9) ; +(21)	-(0)
23	+(33) ; +(42)	+(7) ; +(4)	-(0)
24	+(46) ; +(49)	+(9) ; +(11)	-(0)
25	+(48) ; +(42)	+(9) ; +(9)	-(0)
26	+(60) ; +(31)	+(11) ; +(4)	-(0)
27	+(51) ; +(53)	+(6) ; +(11)	-(0)
Intended Results	**+(50) ; +(50)**	**+(10) ; +(10)**	**-(0)**

Sample Type A: 50 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type B: 10 cfu *Enterococcus faecalis*/250ml and 50 cfu *Staphylococcus aureus*/250ml
Sample Type C: 50 cfu *Staphylococcus aureus*/250ml
+ Faecal streptococci detected
- Faecal streptococci not detected
Number in brackets is reported cfu on membrane from 250 ml volume.

TABLE 4

Log_{10} performance characteristics for membrane filtration method

Sample type	Performance characteristics	m-Enterococcus agar	KF Streptococcus agar
A	Mean (cfu/250ml)	1.68	1.68
	SD	0.05	0.06
	SD_r	0.07	0.08
	RSD_r%	4.10	4.6
	r	0.19	0.22
	SD_R	0.07	0.08
	RSD_R%	4.24	4.61
	R	0.20	0.22
B	Mean (cfu/250ml)	0.97	0.98
	SD	0.10	0.16
	SD_r	0.20	0.15
	RSD_r%	20.50	14.98
	r	0.56	0.41
	SD_R	0.18	0.19
	RSD_R%	18.02	19.9
	R	0.56	0.55

Key to performance characteristics

SD	Overall standard deviation of the mean.
r	repeatability (within laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on identical test materia under the same conditions may be expected to lie within a 95% probability
SD_r	the standard deviation of the repeatability
RSD_r%	The relative standard deviation of the repeatability $SD_r \times 100/\times$
R	reproducibility (between laboratory variation). The value below which the absolute difference between two single test results obtained with the same method on identical test materia under different conditions may be expected to lie within a 95% probability
SD_R	the relative standard deviation of the reproducibility
RDS_R%	The relative standard deviation of the reproducibility $(SD_R \times 100)/\times$

Appendix I

Sample preparation

Natural mineral water for artificial inoculation

Samples of commercially available bottled NMW were purchased on several occasions prior to the trial from a retail outlet and examined for the presence of faecal streptococci using the liquid enrichment technique. Autochthonous flora were present in the source but no faecal streptococci were detected on any occasion so for the purposes of the trial, this source was considered to be suitable for artificial inoculation.

Selection of organisms for artificial inoculation of samples

Ideally, a range of samples were required containing not only typical strains of faecal streptococci but additionally, organisms which give similar reactions to faecal streptococci but are not members of this group. A number of isolates from culture collections were examined for their reactions using the methods prescribed for the trial, these included *Escherichia coli*, 5 *Bacillus* species, 2 *Micrococcus* species, 4 *Staphylococcus* species, 2 *Streptococcus* species and *Aerococcus viridans*. None of these bacterial species produced similar reactions to faecal streptococci in all media, under the trial conditions. However *Staphylococcus aureus* NCDO 1499 produced acid in dextrose azide broth to a limited extent.

Pre-trial investigations also demonstrated that both *Enterococcus faecalis* NCDO 581 and *Staphylococcus aureus* NCDO 1499 were stable in NMW for at least a week, therefore bulk samples of the following sample types were prepared:

Sample Type A - NMW containing approximately 50 cells/250 ml of *Enterococcus faecalis* NCDO 581 and 50 cells/250 ml of *Staphylococcus aureus* NCDO 1499 (positive sample, high level of contamination).

Sample Type B - NMW containing approximately 10 cells/250 ml of *Enterococcus faecalis* NCDO 581 and 50 cells/250 ml of *Staphylococcus aureus* NCDO 1499 (positive sample, low level of contamination).

Sample Type C - NMW containing approximately 50 cells/250 ml of *Staphylococcus aureus* NCDO 1499 (negative sample).

After thorough mixing, appropriate volumes were aseptically dispensed into sterile plastic screw top containers, to provide sufficient samples for each participating laboratory and to allow a further 10 samples of each sample type to be examined by both the liquid enrichment and membrane filtration procedures for homogeneity testing at the Food Science Laboratory.

Appendix II

METHOD I
Detection by Enrichment in a Liquid Medium

1. Scope and Field of Application

Faecal streptococci normally inhabit the intestines of man and warm-blooded animals and can provide an indication of faecal pollution. This protocol specifies a method for the detection of faecal streptococci (those possessing the Lancefield Group D antigen) in natural mineral water by enrichment in a liquid medium.

2. Definition

2.1 Presumptive Faecal Streptococci: Organisms capable of aerobic growth at 37 ± 0.5°C in a liquid culture medium containing glucose and sodium azide with the production of acid in 48h.

2.2 Faecal Streptococci: Presumptive faecal streptococci as defined above which can also grow at 44 ± 0.25°C in the presence of bile salts and sodium azide and hydrolyse aesculin.

3. Principle

3.1 Enrichment culture

The detection of faecal streptococci in a 250 ml volume of natural mineral water is based on inoculation of the sample into a selective liquid medium, azide glucose broth, followed by incubation for 24 and 48h at 37°C. Faecal streptococci grow in this medium and ferment glucose with the formation of acid, which causes a change in the colour of the pH indicator from purple to yellow.

3.2 Confirmation

All enrichment cultures showing positive reactions after 24 or 48 h are subcultured to a confirmatory medium to eliminate false positive reactions such as those by other Gram-positive cocci or rods. The confirmatory medium, bile-aesculin-azide agar (BAAA), is then incubated at 44°C for up to 48 h. Faecal streptococci grow on this medium and hydrolyse aesculin; the end-product, 6,7-dihydroxycoumarin, combines with iron (III) ions to give a tan-coloured to black compound which diffuses into the medium. Colonies which

give a positive aesculin reaction on BAAA may be regarded as faecal streptococci.

4. Culture Media and Reagents

WARNING - All selective media described in this method contain sodium azide. As this substance is highly toxic and mutagenic, precautions should be taken to avoid contact with it, especially by the inhalation of fine dust during the preparation of commercially available dehydrated complete media. Azide-containing media should not be mixed with strong inorganic acids, as toxic hydrogen azide (HN_3) may be produced. Solutions containing azide can also form explosive compounds when in contact with metal pipework, for example from sinks.

4.1 Basic materials

In order to improve the reproducibility of results, it is recommended that for the preparation of diluents and culture media, dehydrated basic components or complete dehydrated media be used. Commercially prepared reagents may also be used. The manufacturer's instructions shall be rigorously followed.

All chemicals and reagents shall be of recognised analytical quality.

Water used shall be distilled or deionised and free from substances which may inhibit the growth of micro-organisms under the test conditions.

Measurements of pH shall be made using a pH meter, measurements being referred to a temperature of 25°C.

4.2 Isolation medium

Azide Glucose Broth (double-strength)

Beef extract	9.0 g
Tryptone	30.0 g
Glucose	15.0 g
Sodium chloride (NaCl)	15.0 g
Sodium azide (NaN_3)	0.4 g
Bromocresol purple (ethanolic solution 15 g/l)	2 ml
Water	up to 1000 ml

Preparation : Dissolve the ingredients in the water by boiling. Adjust the pH so that after sterilisation it will be 7.2 ± 0.1 at 25°C. Distribute in

250 ml volumes in screw-capped bottles of 500 ml capacity. Sterilise the medium for 15 min at 121°C.

4.3 Confirmation medium

Bile-aesculin-azide Agar	(Difco 0525-01-5 or equiv.)
Tryptone	17.0 g
Peptone	3.0 g
Yeast extract	5.0 g
Ox-bile, dehydrated	10.0 g
Sodium chloride (NaCl)	5.0 g
Aesculin	1.0 g
Ammonium iron (III) citrate	0.5 g
Sodium azide (NaN_3)	0.15 g
Agar	10 to 20 g [1]
Water	up to 1000 ml

[1] Depending on the gelling power of the agar

Preparation : Dissolve the ingredients in the water by heating. Adjust the pH so that after sterilisation it will be 7.2 ± 0.1 at 25°C. Distribute in screw-capped bottles of 500 ml capacity and sterilise for 15 min at 121° C. Cool to 50 to 60°C in a water bath and pour into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface. Plates may be stored between 2 to 8°C for up to 1 week in sealed containers to prevent drying.

5. Apparatus

Usual microbiological laboratory equipment including:

5.1 Incubators capable of being maintained at 37 ± 0.5°C

5.2 Incubators capable of being maintained at 44 ± 0.25°C.

5.3 Water bath capable of being maintained at 50 ± 1°C

6. Procedure

6.1 Enrichment

Add 250 ml of sample to 250 ml of azide glucose broth (**4.2**) and incubate at 37 ± 0.5°C for 22 ± 2 h. In addition, add 250 ml of sterile distilled water to 250 ml of azide glucose broth (**4.2**) and incubate at 37 ± 1°C for 22 ± 2 h (uninoculated control).

Consider all cultures showing a yellow colour (throughout the whole container or in the lower part of the container only) as giving a positive reaction. Reincubate negative samples for an additional 22 ± 2 h.

After this incubation even a faint colour change to reddish purple should be considered indicative of acid production. In order to improve the interpretation, the colour of the inoculated cultures should be compared with the colour of the uninoculated control medium. All containers showing a positive reaction should be considered to contain presumptive faecal streptococci.

6.2 Confirmation

Confirm each enrichment culture showing acid production by streaking a loopful of the resuspended enrichment broth on a plate of bile-aesculin-azide agar (**4.3**) and incubating at 44 ± 0.25°C for up to 48h. Blackening of the medium and/or colonies may occur within 6h. Participants are asked to record the time at which blackening was first visible. Regard all plates showing a tan to black colour in the colonies and/or in the surrounding medium as confirmation of the presence of faecal streptococci.

NOTE : Use of heavy inocula will increase the speed of reaction. More than one isolate may be streaked onto one plate of medium.

7. Expression of Results

Express the results as presence (+) or absence (-) of faecal streptococci in 250 ml natural mineral water.

8. Quality Control

The isolation and confirmation procedures should be assessed by the inclusion of control organisms.

Recommended controls: positive - *Enterococcus faecalis* NCTC 775
negative - *Escherichia coli* NCTC 9001

9. References

9.1 British Standard 1989 Water Quality, Part 4, Section 4.3. Detection and enumeration (most probable number) of faecal streptococci by the multiple tube technique. BS 6068 : 4.3.

9.2 International Standard 1984 Water Quality, Detection and enumeration of faecal streptococci - Part 1 : Method by enrichment in a liquid medium. ISO 7899/1.

Appendix III

Method II
Detection by Membrane Filtration

1. Scope and Field of Application

Faecal streptococci normally inhabit the intestines of man and warm-blooded animals and can provide an indication of faecal pollution. This protocol specifies a method for the detection of faecal streptococci (those possessing the Lancefield Group D antigen) in natural mineral water by membrane filtration.

2. Definitions

2.1 Presumptive Faecal Streptococci : Organisms capable of aerobic growth and of forming characteristic pink to maroon coloured colonies by reduction of 2,3,5 -triphenyltetrazolium chloride (TTC) to the red dye formazan, on a selective isolation medium containing sodium azide and glucose within 48h at 37 ± 0.5°C.

2.2 Faecal Streptococci : Presumptive faecal streptococci as defined in 2.1 which can also grow at 44 ± 0.25°C in the presence of bile salts and sodium azide and hydrolyse aesculin.

3. Principle

3.1 Filtration and Incubation

The detection of faecal streptococci in a 250 ml volume of natural mineral water is based on filtration of the sample through a membrane filter with a pore size which will retain the bacteria. The filter is placed on a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and 2,3,5-triphenyltetrazolium chloride, a colourless indicator, that is reduced to red formazan by faecal streptococci.

After a period of resuscitation and incubation, all raised colonies which show a red, maroon or pink colour, either in the centre of the colony or throughout, are counted as presumptive faecal streptococci.

3.2 Confirmation

Presumptive faecal streptococci are subcultured to the confirmatory medium, bile-aesculin-azide agar (BAAA), which is incubated at 44°C for up to 48 h. Faecal streptococci grow on this medium and hydrolyse aesculin; the end-product, 6,7-dihydroxycoumarin, combines with iron (III) ions to give a tan-coloured to black compound which diffuses into the medium.

Colonies which give a positive aesculin reaction are regarded as faecal streptococci.

4. Culture Media and Reagents

WARNING - All selective media described in this method contain sodium azide. As this substance is highly toxic and mutagenic, precautions should be taken to avoid contact with it, especially by the inhalation of fine dust during the preparation of commercially available dehydrated complete media. Azide-containing media should not be mixed with strong inorganic acids, as toxic hydrogen azide (HN_3) may be produced. Solutions containing azide can also form explosive compounds when in contact with metal pipework, for example from sinks.

4.1 Basic materials

In order to improve the reproducibility of results, it is recommended that for the preparation of diluents and culture media, dehydrated basic components or complete dehydrated media be used. Commercially prepared reagents may also be used. The manufacturer's instructions shall be rigorously followed.

All chemicals and reagents shall be of recognised analytical quality.

Water used shall be distilled or deionised and free from substances which may inhibit the growth of micro-organisms under the test conditions.

Measurements of pH shall be made using a pH meter, measurements being referred to a temperature of 25°C.

4.2 Isolation Media

Use both of the following isolation media as directed:

4.2.2 KF-streptococcus agar (Kenner)

4.2.2.1 Basal Medium

Proteose peptone	10.0 g
Yeast extract	10.0 g
Sodium chloride (NaCl)	5.0 g
Sodium glycerophosphate	10.0 g
Maltose	20.0 g
Lactose	1.0 g
Sodium azide (NaN_3)	0.4 g
Bromocresol purple (ethanolic solution 15 g/l)	1 ml
Agar	10 to 20 g[(1)]
Water	up to 1000 ml

[(1)] Depending on the gelling power of the agar

Preparation : Add the ingredients to the water and heat to boiling to completely dissolve. Heat for an additional 5 min. Allow to cool to 50 to 60°C.

4.2.2.2 TTC Solution

2, 3, 5-triphenyltetrazolium chloride	1 g
Water	100 ml

Preparation : Dissolve the dye in the water by stirring. Sterilise by filtration (0.22 μm). The solution should be protected from light.

4.2.2.3 Complete Medium

Basal medium	1000 ml
TTC solution	10 ml

Preparation : Add the TTC solution to the basal medium cooled to 50°C to 60°C. TTC is thermolabile, so that overheating must be avoided. Adjust the pH if necessary to 7.2 with a sterile solution of sodium carbonate (100 g/l). Pour the medium into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface. Poured plates may be stored in the dark for up to 30 days at 4 ± 2°C.

4.2.3 m-Enterococcus Agar (Slanetz and Bartley)

4.2.3.1 Basal Medium

Tryptose	20.0 g
Yeast extract	5.0 g
Glucose	2.0 g
Dipotassium hydrogen orthophosphate (K_2HPO_4)	4.0 g
Sodium azide (NaN_3)	0.4 g
Agar	10 - 20 g [(1)]
Water	to 1000ml

[(1)] Depending on the gelling power of the agar

Preparation : Add the ingredients to the water and heat to boiling to completely dissolve. Heat for an additional 5 min. Cool to 50 to 60°C.

4.2.3.2 TTC Solution

2, 3, 5-triphenyltetrazolium chloride	1 g
Water	100 ml

Preparation : Dissolve the dye in the water by stirring. Sterilise by filtration (0.22 µm). The solution should be protected from light.

4.2.3.3 Complete Medium

Basal medium	1000 ml
TTC solution	10 ml

Preparation : Add the TTC solution to the basal medium cooled to 50°C to 60°C. TTC is thermolabile, so that overheating must be avoided. Adjust the pH if necessary to 7.2 with a sterile solution of sodium carbonate (100 g/l). Pour the medium into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface. Poured plates may be stored in the dark for up to 30 days at 4 ± 2°C.

4.3 Confirmation Medium

4.3.1 Bile-aesculin-azide Agar (Difco 0525-01-5 or equiv.)

Tryptone	17.0 g
Peptone	3.0 g
Yeast extract	5.0 g
Ox-bile, dehydrated	10.0 g
Sodium chloride (NaCl)	5.0 g
Aesculin	1.0 g
Ammonium iron (III) citrate	0.5 g
Sodium azide (NaN_3)	0.15 g
Agar	10 to 20 g [1]
Water	up to 1000 ml

[1] Depending on the gelling power of the agar

Preparation : Dissolve the ingredients in the water by heating. Adjust the pH so that after sterilisation it will be 7.1 ± 0.1 at 25°C. Distribute in volumes of 250 ml in screw-capped bottles of 500 ml capacity. Sterilise for 15 min at 121°C. Cool to 50 to 60°C and pour into Petri dishes to a depth of at least 3 mm and allow to set on a cool, horizontal surface. Poured plates may be stored between 2 to 8°C for up to 1 week.

5. Apparatus

Usual microbiological equipment including:

5.1 Incubators, capable of being maintained at 37 ± 0.5°C and 44 ±0.25°C.

5.2 Membrane filtration apparatus, of a sufficient capacity to contain 250 ml of sample, with vacuum flask tubing, a moisture trap flask and a vacuum source.

5.3 Sterile forceps

5.4 Sterile membrane filters, with a pore size of 0.22 μm and approximately 47 mm in diameter.

6. Procedure

6.1 Filtration and Incubation

After thorough mixing by shaking, from a 500 ml sample, filter a 250 ml volume of water. With sterile forceps, place the membrane filter face upwards onto the KF-streptococcus agar (**4.2.2.3**) without trapping air bubbles under the membrane. Without rinsing the filter apparatus, filter the second 250 ml volume and place the membrane onto m-enterococcus agar (**4.2.3.3**). Incubate the plates at 37 ± 0.5°C for 44 ± 4 h.

NOTE - Funnels should be disinfected by immersion in boiling water for at least 1 minute and then cooled between different samples.

After incubation, consider all raised colonies which show a red, maroon or pink colour, either in the centre or throughout the colony as presumptive faecal streptococci.

NOTE - Occasionally bacteria other than group D streptococci may produce this type of colony.

6.2 Confirmation

Subculture a representative sample of typical colonies from both isolation media to plates of bile-aesculin-azide agar. Incubate at 44 ± 0.25°C for up to 48 h. Blackening of the medium and/or colonies may occur within 6h. Participants are asked to record the time at which blackening was first visible. Regard all plates showing a tan to black colour in the colonies and/or in the surrounding medium as confirmed faecal streptococci.

7. Expression of Results

Express the results as the number of presumptive faecal streptococci and confirmed faecal streptococci per 250 ml of sample.

8. Quality Control

The isolation and confirmation procedures should be assessed by the inclusion of control organisms.

Recommended controls: positive - *Enterococcus faecalis* NCTC 755
negative - *Escherichia coli* NCTC 9001

9. References

9.1 British Standard 1989 Water Quality, Part 4, Section 4.4. Detection and enumeration of faecal streptococci by the membrane filtration technique. BS 6068 : 4.4.

9.2 International Standard 1984 Water Quality, Detection and enumeration of faecal streptococci - Part 2 : Method by membrane filtration. ISO 7899/2.

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Annual Report of the Council of the Association of Public Analysts for 1994

presented at 42nd Annual General Meeting of the Association at
the Stakis Hotel, Northampton on 29th April 1995
by the Honorary Secretary, Dr. Peter Clare

This report is concerned with the activities of the Association of Public Analysts (APA) for the year ending December 31st 1994 and discusses events that influence the development of the Association and its members. The Council of the Association met on five occasions during 1994. Its roles are to co-ordinate the activities of the Association and to provide for the representation of the members of the APA to outside organisations. The objectives of the Association were set out in 1953 in the Articles and Memorandum of Association of the APA, the first objective being stated as "to assist in upholding and maintaining the character and position of public analysts and official agricultural analysts and of the profession of analytical chemistry and to protect and advance the interests of public analysts and to promote co-operation between them."

Public Analysts' Laboratories

Thirty two Public Analysts Laboratories are situated throughout mainland Britain a further four in the Channel Islands, Isle of Man and Northern Ireland. Overseas members are placed in Eire and Australia. Around 700 persons are employed in these laboratories and the scientific disciplines to be found within them include matters associated with food and food technology, agriculture, waters and the environment, occupational hygiene, toxicology, and consumer goods such as cosmetics, and toys. It is a feature common to all members laboratories that the results of the scientific investigations that are conducted may be the subject of scrutiny by the courts, industry, other experts, and accreditation bodies.

A growth in the activities of bodies organising performance assessment and accreditation schemes has occurred in recent years. These schemes all have a potential for feedback of useful information concerning the scientific work carried out in the laboratory and the associated administration although because of the varied and investigatory nature of much of the scientific work in a PA laboratory it is not unreasonable to expect that generic rather than specific schemes will be both more financially viable and professionally workable.

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Annual conference and Exhibition

The Annual Conference in 1994 was held in April at the Old Ship Hotel in Brighton and took the theme of "Essential Science for Local Government". It had the objective of exploring the science based nature of food law enforcement and other local government issues such as health and safety, waste disposal and hazards associated with the use of industrial chemicals. An exhibition of leading suppliers of scientific equipment was held for the duration of the conference.

Mrs. Jill Moore of the National Federation of Consumer Groups and Councillor Bill Mackay, of the Local Authorities Co-ordinating Committee on Food and Trading Standards (LACOTS) chaired the two sessions. Key items in the programme were delivered by Mr. Charles Cockbill of the Ministry of Agriculture, Fisheries and Food (MAFF) and Mr. John Wood of the Food and Drink Federation (FDF). Mr. Cockbill discussed the anticipated demands that will be placed upon Public Analysts in the light of the requirements of both the Food Safety Act 1990 and legislation evolving from Brussels. The basic requirements of an official laboratory he described as being efficient, being able to generate reliable results and capable of responding to developing situations. Mr. Woods discussed the enforcement role of the Public Analyst drawing attention to the necessary interaction with food manufacturers and the foodstuffs produced.

Other issues confronting members of the APA were highlighted in deliveries from Professor J.G.Firth, former Director of the Occupational Medicine and Hygiene Laboratories of the Health and Safety Executive and Mr. Mel Billington, Public Analyst, Birmingham City Council. Professor Firth discussed the practical implications of the science of Health and Safety and its effective application within local authorities and industry, and Mr. Billington explained the need for scientific services in local authorities as applied to waste disposal, industrially contaminated land and incidents such as spillages and fires where industrial chemicals are present.

At the Annual Dinner of the Association, an occasion which was characterised by an excellent rack of lamb and after dinner entertainment from Mr. Peter McDonald, consultant surgeon at Northwick District Hospital, the certificates of those who have been successful (1993) in becoming Masters of Chemical Analysis (M.Chem.A.), Mrs. Patricia Urwin and Mr. Christopher Hunt, were presented.

Training and Meetings

The Association has a full programme of events which are the initiative of its training committee and the APA educational trust which was established in 1988 to advance training

and education in the field of scientific analysis and examination. During 1994 a series of one day meetings was held on the topics of food authenticity, the microbiological examination of foods and waters, and on gas chromatographic techniques in food analysis. In addition the APA annual residential training course was held at the University of Reading, Berkshire during March. These annual training courses are designed so that in a three year cycle both the scientific and administrative activities to be encountered in a "typical" Public Analysts laboratory are covered.

A training initiative has been discussed with the Food and Drink Federation and this has resulted in Public Analysts being invited to address meetings which were organised by the FDF on the topic of the quantitative determination of colouring matter in food, this being of particular relevance in view of the European Council Directive on this matter.

European Issues

In 1993 Council Directive 93/99/EC on the subject of additional measures concerning the official control of foodstuffs was published. This sets out the operational criteria for the official control of foodstuffs. The Association has commented on two draft papers circulated by the MAFF which concerned this directive, the first on the qualifications for food control officials and the second on the exchange of information between member states on food control matters. The final form of these papers has yet to be determined but will most probably do so as codes of practice issued in accordance with the provisions of section 40 of the Food Safety Act 1990.

The forum of Food Law Enforcement Practitioners (FLEP) has met twice this year, the meetings being held in London and in Holland and the Association being represented at these seminars by Mr. Anthony Harrison, former President of the Association. At these meetings the subject of the community inspection service was discussed in addition to the other aspects of the food control directives. Officials from the Community Inspection Service will be charged with the duty of monitoring and evaluating the equivalence and effectiveness of the official food control systems in operation in the various member states. (A visit to the UK by the commission food inspectorate which took place in February 1995 and was addressed by the President of the Association, Mr. Malvern Barnett, on the official scientific service in the UK.)

Scientific Affairs

The exercise "Guide Levels for Food Quality" has continued and at issue have been the concepts of standards for food quality being led either by the market or by a standard based on a more fundamental quality such as the description of the food. The matter of

breaded fish fillets was discussed in May and a standard of at least 60% of fish in these products was agreed although a market based standard of 50% was apparent. It is noteworthy that the least amount of fish reported in this survey of over 400 samples was 28%, a product that could be described as containing a slither of fish in an abundance of coating, and that the highest amount was over 95%, a product with just a dusting of breadcrumbs.

The data already gathered concerning minced meats will raise similar points concerning the quality of food for retail sale. In recent years many Public Analysts have, for example, found samples of minced meat described as lean to contain more fat than those sold without such a description implying a low fat content. The progress of this exercise will be timely in the light of Council Directive 94/65/EC which lays down the requirements for the production and marketing of certain minced meats and meat products.

The Validated Enforcement Methods Service (VEMS) has now, through the offices of the Association's science writer, produced over 50 in a series of documented analytical procedures which are set out in a style which has been approved by a lead assessor of the National Measurement Accreditation Service (NAMAS).

Towards the end of the year the Drinking Water Inspectorate of the Department of the Environment issued an updated "Report 71 on the Microbiology of Water". This report contains the accepted laboratory procedures for the examination of drinking waters for microbiological criteria. A significant change incorporated into this revised document is the criterion for the evaluation of Coliform organisms, the principle indicators of faecal pollution.

LACOTS has established three sub panels to the Quality Standards Panel. These are in the fields of Labelling, Fertilisers and Feeding Stuffs, and Sampling and Analysis. The sub panels are to be chaired by Public Analysts Mr.R.A. Stevens (Labelling) and Mr.J.Fulstow (Sampling and Analysis), and by Mr.N.Edwards, of the Institute of Trading Standards Administration, (Fertilisers and Feedingstuffs). A prime purpose of the panel dealing with the sampling and analysis of foodstuffs will be to advise on aspects of the Community co-ordinated sampling programme and expertise from within the membership of the Scientific Affairs Committee of the Association will be channelled appropriately.

Honorary Vice Presidents

At the initiative of Mr.E.B.Reynolds, the Co-ordinator of Scientific Affairs, the Articles of Association of the APA were amended in order to allow for the appointment of Honorary Vice Presidents. Lord Dainton FRS, former President of the Royal Society of Chemistry and Councillor. W.Mackay former Chairman LACOTS and Chairman of Mendip District Council have accepted the first of these positions. In anticipation of these appointments the Articles of Association of the APA were specifically amended so that "persons of note or distinction" could become Honorary Vice Presidents of the APA.

Policy Matters

Under the leadership of the President of the Association, the policy committee of the Association have prepared a number of documents which have been presented to Council. These documents deal with matters that reflect both on internal relationships between members of the Association and on the enhancement of the status of Public Analysts.

a) A Code of Ethical Conduct - the revision of this code, which was previously reviewed in 1987 and which deals with the professional relationship between members of the Association, has been seen as necessary in the light of changes in the employment status of many Public Analysts.

b) Arrangements for the Conduct of Council Meetings these arrangements include instructions for the presentation of papers to Council for discussion and action has become a prime factor in the increased efficiency of Council meetings

c) Non official testing - the statistics that are concerned with food enforcement activity in the UK and must be forwarded to Brussels under the obligations of community law have in recent years included the results of analysis of foods that have been described as "screen testing". This briefing paper on non official testing sets out to explain the terms official, non official and screen-testing as they are applied to samples taken during food law enforcement activities.

d) A briefing document concerning the role of Public Analysts - this paper is designed to be an aide memoire for Members of Parliament and for additional use by Public Analysts in the light of a further round of local government reorganisation.

A determined programme of publicity by Council which has been headed by Mr.P.Lenartowicz, Public Analyst to Mid Glamorgan, has resulted in the production of two brochures entitled "Protecting the Quality of Life" and "Qualified to Protect". The first set out in some detail the role of the Public Analyst in all areas of consumer

protection and the second included an explanation of the breadth and depth of the scientific and legal content of the syllabus to M.Chem.A. examination.

Food Authenticity

The analysis of food for authentication has long been a central role of Public Analysts and most recently the speciation of red meats and fish have been the subject of investigations. Recent reports in the national press have focused on "competition" on the high seas for a reduced fish stocks. It is not therefore surprising to record that substitution for named popular fish such as cod and haddock by other species is commonly encountered during the analysis of sampled fish.

Laboratory analysis continues to demonstrate the presence of mixtures of pork and lamb in products sold as minced beef and most recently a sample of halal minced lamb was found to consist a mixture of beef and pork.

Similarly, substitution of brand leader whiskys by others containing a lower proportion of malt whisky is a regular finding in Public Analyst's laboratories.

A regular and festive event at Christmas time in many laboratories is the enzymatic analysis of poultry in order to establish whether these poultry are fresh or have been subject to a storage by freezing; subsequent presentation of the birds for sale being achieved after thawing. Such a retail sale is a direct contravention of an aspect of the Food Labelling Regulations 1984 which was designed to protect both the consumer against a product which is potentially microbiologically unsafe and the purchase of food incorrectly described and the trader against an unfair trade practice.

Conclusions

The Ministry (MAFF) have indicated that the provisions of the food control directives relating to official laboratories will have to implemented by 1st November 1998. Public Analysts laboratories are participating in accreditation and performance assessment schemes. However the scientific and technical criteria for accreditation and performance assessment yet needs to be resolved and published before the laboratories of the Association can clearly identify the detail of these exercises and most cost effectively take on this mantle. It is quite apparent that the introduction to the 1989 Annual Report of Council has stood the test of time - our members continue to "live in interesting times".

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ANNUAL STATISTICS FOR THE PUBLIC ANALYST SCIENTIFIC SERVICE 1994

Introduction

The following report presents data related to samples submitted to Public Analysts' laboratories by local authorities during the calendar year 1994.

The statistics presented are purely in terms of numbers of samples and are not a direct measure of the total amount of the actual work undertaken by laboratories. Some samples may only require minimal analysis and interpretation whilst others can result in a research project in order to certify with confidence that they are or are not satisfactory - a difference that is rarely evident when merely viewing the sample as a mere commodity.

Data

Responses were received from all but two of the Public Analysts' Laboratories in the United Kingdom, one not returning figures as a consequence of political policy, the other merely declining to submit data. The submitted data has been calculated for the full population of the UK by interpolation. Detailed figures broken down by sample type appear in the Appendix.

This year the figures for Central and South Wales (covering the bulk of the population) have been separated from the English statistics in order to give an indication of the relative position in Wales. However, as the northernmost counties of Wales submit their samples to a laboratory that also serves a number of English authorities, complete separation of Wales has not been possible and the following summaries must be viewed accordingly.

Data received from one of the Channel Islands' laboratories appears for comparative purposes on the charts, but has not been included in calculations of United Kingdom statistics

Food Work

There was about a 3% fall in sampling levels for food in 1994 compared with 1993, although levels remained well above those of the preceding three years. It was postulated last year that the increase recorded for 1993 may have been a consequence of pressure on local authorities to achieve the World Health Organisation's recommended minimum sampling rate of 2.5 samples per 1000 population. Although it is not known if this was indeed the reason, it is nevertheless concerning to note a

decline this year, particularly when levels generally are still far short of the target level.

The mean total number of food samples per 1000 population in the UK in 1994 was 1.74, against 1.81 in 1993 but only 1.18 in 1992. Breaking this down on a regional basis reveals a wide spread in sampling: the Scottish authorities again have sampled consistently above the recommended minimum of 2.5, achieving an average of 4.1, with Northern Ireland, Wales (Central & South), and England (including North Wales) all falling below 2.5 at 2.21, 1.57 and 1.48 respectively.

Only five laboratories' areas in England and Wales sampled above the 2.5 per 1000 target minimum, while the lowest rate recorded by an individual area was only 0.72. It is interesting to note that the laboratory that had recorded the lowest level of 0.86 last year received a significantly higher rate of 1.22 in 1994.

Chart 1 illustrates the spread of samples on a laboratory-by-laboratory basis.

In addition to official samples, being those which have been taken under the Food Safety Act 1990 and upon which official action may be taken, the above totals include "informal" samples. Informal samples are those that have not been taken in the prescribed manner but which have been submitted to the official Public Analyst's laboratory. The formal sampling of food is a time consuming and highly skilled operation unlike the mass purchase of informal samples, and is therefore often used by local authorities for routine survey work as it saves substantially on the costs of sampling.

It is understood, however, that only official should be counted for the purposes of statistical returns to the European Community. In the collected statistics these equate to formal and complaint foods samples only. When informal food samples are excluded from the figures the results appear very different, with a total of only 0.67 official samples per 1000 population for the UK as a whole. No areas achieved the 2.5 level. The respective figures for the individual regions were as follows: Northern Ireland: 1.18, Wales (Central & South): 0.89, Scotland: 0.86 and England (& North Wales): 0.62.

Chart 2 illustrates the spread of official samples on a laboratory-by-laboratory basis, indicating also the proportions of formal and complaint samples.

Unsatisfactory Food Samples

Two thirds of laboratories responded to a request for information relating to unsatisfactory samples. indicating that 21% of routine food samples and 56% of complaint samples had been found to be in some way unsatisfactory.

Food Factory Inspection

A total of 1262 days were spent on food factory inspection during the year, slightly up on the previous yearns 1105. From individual laboratories' returns it is again dear that the use of the Public Analyst in factory inspections has only been adopted to any significant degree in a very small number of authorities. One laboratory accounts for well over three quarters of the time spent on factory inspection, with over half of the Association's laboratories having had no involvement at all.

Non-food Work

The major areas other than food in which Public Analysts' laboratories served local authorities during 1994 are as follows, in decreasing order of numbers of samples:

Environmental investigations (soil, water, tip leachates, atmospheric samples etc.)
Workplace monitoring and analysis
Water analysis (other than environmental)
Consumer Safety and Trade Descriptions
Radiation monitoring
Agricultural samples (Fertilisers and Animal Feeds)
Miscellaneous (including coroners/toxicology samples, building materials, drugs, etc.)

552 days were spent assisting with the inspection of non-food factories, providing scientific expertise to interpret and assess the scientific aspects of manufacturing control.

There ware 280 call-outs to emergency incidents involving chemicals (ranging from fires to chemical spillages), utilising the 24-hour-a-day. 365-day-per-year back-up service provided to the emergency services to advise and/or provide analytical facilities to help deal with any incident that may be a threat to the public or the environment. On average this equates to one call for assistance somewhere in the UK every 31 hours.

Accreditation and Proficiency testing schemes

The position with regard to accreditation is a little sketchy as only two thirds of laboratories responded to this part of the questionnaire. It would appear that the general position has changed little since a year ago, when 55% of member laboratories ware recorded as being accredited by NAMAS for aspects of food analysis, most also holding accreditation for one or more other aspects of their work. It does appear, however, that preparations in the remaining laboratories are more advanced than last year with many anticipating accreditation to be gained in 1995 or 1996.
The burden of participation in proficiency testing schemes is ever increasing as the number and scope of such schemes continue to increase - an aspect of work that is not reflected in the sample statistics. For example, full participation in the Ministry of Agriculture, Fisheries and Food's Food Analysis Performance Assessment Scheme (participation in which is to become a mandatory requirement for Official Laboratories under the Additional Measures Directive) currently involves thirty two circulations of samples annually - averaging at two different circulations in any three week period.

These samples all require full set-up. calibration and quality assurance procedures to be instigated, procedures that can be extremely time consuming for some circulations. If, as may often be the case, the circulations cannot be tied in with 'rear' samples for similar analysis these procedures still have to be carded out in full, imposing a substantial burden of work for no return.

This is true also of the many other proficiency testing schemes necessary or desirable in certain areas of work. for example the Regular Interlaboratory Counting Exchange (RICE) for asbestos, Workplace Analysis Scheme for Proficiency (WASP) for atmospheric analysis, *AQUACHECK' for water, etc.

Observations

With respect to food samples it is worrying to note a slight decrease in the number of samples submitted during 1994 compared with the improvement that had been observed in 1993, while the lack of any substantial increase in utilisation of Public Analysts' services in food factory inspection despite recognition of their value in statutory Codes of Practice continues to be a matter for concern.

Whilst the number of food complaints found to be unsatisfactory is perhaps not surprising, the high proportion of routine food samples also found to be unsatisfactory (21%) raises serious concerns about the current level of compliance with food safety, quality and labelling requirements.

This is of particular concern at this current time of local authority reorganisation which may lead to smaller weaker authorities with lesser resources to pursue errant manufacturers and without a proven commitment to food sampling. This is also just at the time when there are substantial proposals to reduce the degree of regulatory control in favour of self regulation by industry. With a 21% 'failure' rate when backed up by the potential for legal enforcement it will be interesting to observe the effect if controls are lifted, though of course with fewer statutory requirements there will presumably be fewer 'failures' in law - though perhaps consumers would view things rather differently.

The high rate of unsatisfactory samples detected also raises questions about continued testing of food by unofficial (non Public Analyst) laboratories. sometimes referred to as 'screening' laboratories, set up within some local authority departments. Samples tested in these laboratories are not only missing from the above statistics but also are not subject to the capability and quality control of the official laboratories and thus may miss detection of some faults. Furthermore, those that are identified as possibly unsatisfactory are resampled for submission to the Public Analyst, involving at least a duplication of effort, with also a disproportionate requirement (and therefore cost) for checking and rechecking. In addition. samples tested by such laboratories are effectively excluded from basic databases of samples lasted by Public Analysis thereby potentially distorting the 'norm' on which a possible future enforcement

Modern analysis in general and of food in particular - is becoming ever more complex. This, combined with the increasing burdens of accreditation and proficiency testing, makes it important for maximum use to be made of the resources available to local authorities within their Public Analysts' laboratories in order to ensure the detection of faults and spread the standing costs of maintaining a comprehensive and effective service over as many samples as possible.

The wide use of the Public Analyst by local authorities generally for non food analysis is beneficial to the ability to maintain the necessary skills and technical backup for a comprehensive public protection service, though it is evident from individual returns that the degree of use by Trading Standards and Environmental Health departments in respect of such work varies tremendously.

It can only be hoped that with local authority reorganisation the value of Public Analysts' laboratories will be realised. and that in the consequential rationalisation of work proper support is given to the provision and maintenance of effective scientific support by maximising use of the Public Analyst.

Chart 1: Total Food Samples per 1000 Population

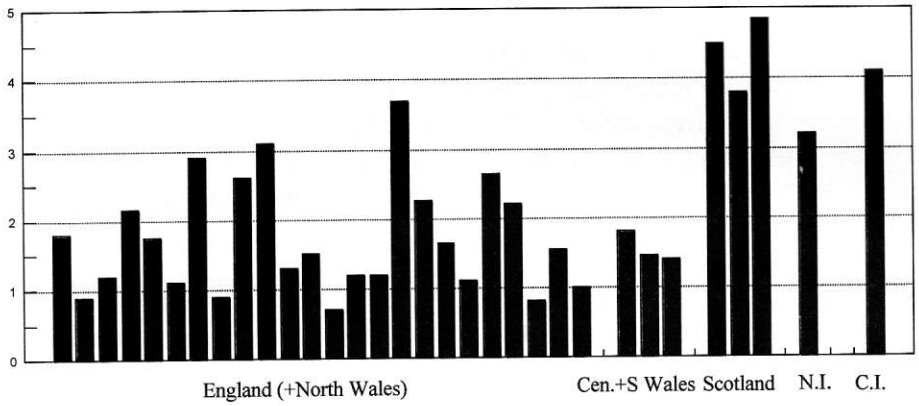
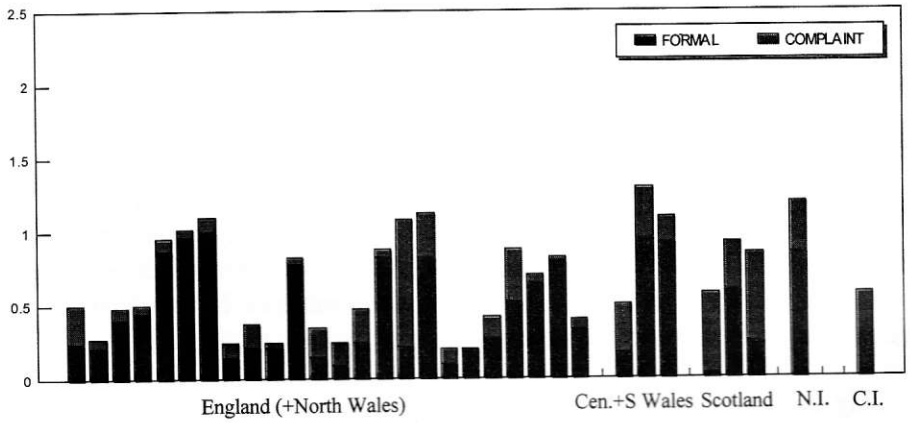


Chart 2: Official Food Samples per 1000 Population



APPENDIX

Data from returning laboratories interpolated to a total population of 56 million.

Foods - all formal	27011
Foods - all informal	62275
Foods - Home Authority (if identified)	2001
Foods - Port Health	1546
Foods - Complaints	8512
Foods - Bacteriological	4182
Milks (all)	6763
Mineral & other bottled waters	389
Drinking waters	21054
Swimming Pool Waters	4224
Other waters (incl. leachates, effluents etc.)	52667
Atmospheric samples	26018
Soils	2439
Workplace monitoring (excl. asbestos.)	4211
Asbestos (bulk & airborne)	22283
Feeding Stuffs - Agriculture Act	2543
Feeding Stuffs - Medicines Act	265
Fertilisers	738
Toys (Safety) Regulations	4717
Cosmetics (Safety) Regulations	934
Other Consumer Safety Act work	3194
Trade Descriptions samples	1965
Building Materials	1420
Coroners/toxicology	2871
Radiation monitoring	9084
Other Miscellaneous	12282
Total Foods for analysis	97798
Total foods bacteriological	4182
Total Waters (incl. environment.)	77945
(Total waters excl. environment.)	25278
Total Agriculture. Act + Medicines Act feeds	3546
Total Consumer Protection etc. (incl TDA)	10811
Total environmental samples	28457
Total workplace samples	23318
Total other samples	25657
TOTAL SAMPLES	296991
Emergency Callouts	280
Food factory inspection	1262
Other factory inspection	552

A statement from the Association of Public Analysts on official and non-official testing

OFFICIAL LABORATORIES

The Food Control Directive 83/397 and the Additional Food control Measures Directive 93/99, require each member state in the EU to provide a list of "official" laboratories whose analytical results can be relied upon to ensure the safety of the consumer within that member state and to reassure other member states that food tested in the official laboratory, and found to be satisfactory, is suitable for Inter-Union trade.

The Additional Food control Measures Directive sets out the requirements for official laboratories which include:-

(1) accreditation by a national body (in the UK - NAMAS)
(2) satisfactory performance in an external proficiency assessment scheme (in-the UK - FAPAS)
(3) the use of validated methods
(4) the employment of suitably qualified persons to carry out analyses.

Analytical data and conclusions reported from the Public Analyst's laboratory, the "official" laboratories are of the proper status to be sent to the Commission in Brussels, and thereby, fulfill the obligation of MAFF to provide valid annual returns.

QUALIFIED ANALYSTS

In the UK, the person suitably qualified to carry out analyses, or have them performed under his direction,. is the Public Analyst who is legally appointed under the Food safety Act 1990. The required qualification for the Public Analyst is the Mastership in Chemical Analysis a qualification awarded by the Royal Society of Chemistry after intensive examination in the chemistry, technology and legislation of food and related subjects.

SCREEN TESTING "NON-OFFICIAL" TESTING

Chemical testing carried out elsewhere in the local authority system, i.e. within Trading standards and Environmental Health departments is often called "screen testing". This term covers a wide range of testing activities from such simple tests as the determination of drained weights of canned fruits and the volumetric fat content

of milk to rather more sophisticated analyses carried out by persons who may have some knowledge or qualification in chemistry.

'Screen testing" in this context should more properly be described as 'NON-OFFICIAL TESTING". It is not carried out in official EU laboratories, it is not quality assured and it is not carried out under appropriate supervision as required by EU and UK law. Its results are not suitable to be provided in annual returns by MAFF to the EU Commission or used as a basis for InterUnion Trade.

'Screen testing" is a term which should not be used because it is likely to be confused with specific scientific activities officially or legally described as "screening". For example, the word 'screening' is used in UK legislation in the Erucic Acid in Food Regulations 1977 to describe complex analyses carried out in official laboratories.

LOCAL AUTHORITIES AND "NON-OFFICIAL TESTING"

The reasons given by local authorities for carrying out *"non-official testing'* vary widely but include -

economy - it is claimed that a large number of samples can be tested at a lesser cost than the Public Analyst might charge.

speed - it is claimed that simple tests can be carried out immediately the samples are taken back to the office.

targeting - where large numbers of samples of a similar type are tested, to determine whether a suspected problem actually exists.

accountability - full documentation need not be provided for each sample.

These apparent advantages are often illusory and disguise real disadvantages both to the local authority and to the laboratory as follows:-

- the official laboratory suffers by the diversion of resources with the result that the more sophisticated techniques essential in complex investigations carried out for local authorities assume a higher cost.
- reliable analytical results from surveillance or routine testing are not available as essential background data necessary to support court proceedings.
- resources put into non-official testing could be held to be a misuse of these resources by the local authority, since the capability for testing already exists in the legally appointed Public Analysts department.

SUMMARY

1. The EU requires a system of official laboratories for enforcement purposes. Every effort must be made to continue to provide and maintain a well structured and properly validated "official" service which it both efficient and cost effective.

2. With the greater sophistication in food production and the increasing complexity of analytical techniques it is illogical to divert resources of time and money away from the official laboratories, a course of action which denies the availability of background data for regulatory purposes.

3. Local authority inspection departments which currently divert resource into non-official testing should be required to identify and justify these costs in comparison with quotations supplied by their public Analyst for a properly accredited and administered service.

4. Data derived from non-official testing should neither be collected nor recognised by MAFF, with the exception of any which result from local agreement between the authority and the Public Analyst