

## ICE GLAZE DETERMINATION OF FROZEN SHELLFISH: COLLABORATIVE TRIAL

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*Nineteen laboratories collaboratively tested the MAFF Validated Method V13 (based on a Codex Alimentarius Commission procedure) and a West European Fish Technologists Association (WEFTA) method for the determination of ice glaze of some fish products. Samples of cooked prawns and cooked shrimps containing controlled amounts of glaze were analysed. Even under the near ideal conditions used in the trial, the methods for estimating the amount of glaze exhibited considerable variation with respect to accuracy and precision. The MAFF Validated Method performed marginally worse than the WEFTA method in terms of accuracy but considerably better in terms of precision. The recommendation that the V13 method should be used as the method of choice for the determination of ice glaze on frozen shellfish is, therefore, maintained.*

### INTRODUCTION

Temperature fluctuations during frozen storage can result in frozen fish and fishery products suffering surface dehydration due to the sublimation of water. As well as causing a dry, chalky appearance to the product, this sublimation also results in significant weight loss. When defrosted, the product is dry and spongy due to protein denaturation. Most manufacturers overcome this potential deterioration of quality by covering the product with a layer of ice (glazing) which partially sublimates during storage thus not affecting the water within the product. Controlling the amount of glaze applied to a product can be difficult since the amount of glaze pick-up is dependent on the temperature of both the fish and the water, the size and shape of the product, and the dwell time of the glazing process itself.

Article 8 of the EC Food Labelling Directive<sup>(1)</sup> requires, *inter alia*, that a declaration of the net weight of product, i.e. the weight of product excluding the glaze, is made on certain products packed in a protective medium. While the UK

has yet to implement Article 8 into its national legislation the question of how to measure the weight net of glaze still must be considered to enable proper enforcement once this does come into force. The European Commission has not stipulated any official methodology, but it is understood that internationally accepted methods are preferred and will be recognised in European trade.

A collaborative trial<sup>(2)</sup> conducted by MAFF in 1988/89 investigated different methods to determine the amount of ice glaze on frozen fish fillets and shellfish. This trial compared two Codex methods, for fish fillets<sup>(3)</sup> and shrimps and prawns<sup>(4)</sup>, a method developed at Lancashire County Public Analyst Laboratory<sup>(2)</sup>, and a British Frozen Food Federation (BFFF) method<sup>(2)</sup>. The principle of the methods used for the molluscs and shellfish are given in Appendix I. The study reported a wide variation with respect to precision and accuracy but concluded that the Codex methods were more accurate in determining the glaze, and the Lancashire method was more precise. The principal difference between these two approaches is that in the Codex methodology the frozen fish is immersed in a water bath at 27°C until the glaze can be felt to have been removed, whereas in the Lancashire method all samples are immersed in the water bath for fixed time (2 minutes), regardless of size. A similar principle was used by the BFFF method, albeit with different times. None of the methods was considered to be satisfactory for molluscan shellfish.

The Codex method for frozen crustaceans<sup>(4)</sup> is the most widely used in international trade. However, recently Germany has adopted a method developed by the West European Fish Technologists Association (WEFTA). This is a modification of Codex methodology in that a lower water bath temperature of 15° to 20°C is used, but the deglazed prawn is dried with a paper towel rather than drained for a fixed time. Although the lower temperature is reputed to improve the precision of the method by making it easier to judge when the ice glaze has been removed, no published collaborative evidence exists to support this view.

The trial reported in this paper has been designed to check the precision of the WEFTA method, to compare it with the Codex method, which has also been published as the MAFF Validated Method V13<sup>(5)</sup>, and also to establish whether with the WEFTA method it is possible to determine the level of glaze on molluscs, notably scallops.

## ORGANISATION OF THE TRIAL

### Participants

The trial was organised by the Ministry of Agriculture, Fisheries and Food, Food Labelling and Standards Division, in conjunction with Ross Foods, Grimsby, who supplied, glazed and despatched the frozen samples. Nineteen laboratories (15 UK public analysts and four industry laboratories) participated in the trial. Two of the industry laboratories were from the same establishment; that establishment received two sets of sample which were analysed independently by two different analysts. The results are therefore being treated as originating from two separate laboratories.

### Samples

#### *Samples and glazing*

Frozen raw king scallops, cooked prawns and cooked shrimps, all supplied unglazed, were glazed at Ross Foods, Grimsby on a standard production line equipped with two series of water spray nozzles across the width of the belt. The volume of water in the sprays was adjusted to achieve the nominal levels of glaze given in Table I. The target glaze levels are also given in the Table as are the sample code numbers used in the trial. The glaze was applied in a single passage through the line, with the higher levels of glaze requiring both sets of sprays. The level of applied glaze was checked after passage through the fluidised-bed freezer by the Codex method (V13), and by weighing the starting and finished product.

Participants received 10 individual samples which consisted of five "blind" duplicates. The sample code numbers used are given in Table I.

### Methods of Analysis

The methods of analysis used by the participants in the trial are described in Appendices II and III.

### Results

Participants were asked to report glaze content on the sample as received.

The results obtained by participants for the glaze contents (as g/100g on the sample as received) and are given in Tables II-VI and summarised in Table VII.

**Table I**  
**SAMPLES USED IN ICE GLAZE CONTENT COLLABORATIVE TRIAL**

Sample	Target glaze level	Mass balance	Ice Glaze Added (g/100 g of Original Product)	Sample Code Numbers
Scallops	ca. 20%	-†	7 to 7.5*	6, 9
Small cooked shrimps	ca. 17%	14.6	15.8	1, 5
Small cooked shrimps	ca. 28%	26.9	28.9	3, 8
Large cooked prawns	ca. 15%	14.1	13.5	2, 4
Large cooked prawns	ca. 25%	24.4	25.0	7, 10

\* The size of the king scallops was particularly large and therefore it was not possible to achieve as high a glaze level as initially desired.

† A fault in the initial weighing of the scallops resulted in a 'negative' mass balance, i.e. the final weight was less than the initial weight, and thus had to be disregarded.

The total weight of sample material for each code number (1 to 10) was ca. 400g (100g for the WEFTA method and 300g for the Codex method). Each laboratory received blind duplicates of each of the shellfish types and glaze levels. Two complete sets of reference samples were retained by Ross Foods.



**Table II**  
**ICE GLAZE CONTENT OF SCALLOP SAMPLE (g/100 g of Original Product): Ice Glaze Added ~ 7.5%**

LABORATORY	WEFTA METHOD		V13 METHOD	
	Sample 6	Sample 9	Sample 6	Sample 9
1	4.78	5.56	5.80	4.83
2	6.31	6.08	0.44	- 1.90
3	9.10	9.77	6.06	6.66
4	7.2	7.6	5.3	4.3
5	6.6	6.1	5.5	5.0
6	13.0	11.5	7.1	7.2
7	11.3	9.2	1.8	1.0
8	7.7	8.9	4.3	4.3
9	5.7	8.6	3.6	6.6
10	5.9	5.8	2.9	3.8
11	9.51	7.72	4.47	3.80
12	9.4	9.0	5.6	8.4
13	10.3	9.8	3.3	4.4
14	6.3	6.8	5.0	8.1
15	5.9	6.8	7.1	6.4
16a	7.1	11.5	4.0	6.5
16b	11.28	13.4	7.3	7.8
17	5.9	6.3	3.7	4.3
18	9.1	9.1	6.6	6.4
MEAN		8.21		5.05
r		3.10		3.79
S <sub>r</sub>		1.11		1.36
RSD <sub>r</sub>		13.51		26.85
R		6.31		6.36
S <sub>R</sub>		2.25		2.27
RSD <sub>R</sub>		27.45		45.03

For key, see Table VIII

**Table III**  
**ICE GLAZE CONTENT OF SMALL COOKED SHRIMPS SAMPLE**  
**(g/100 g of Original Product): Ice Glaze Added 15.8%**

LABORATORY	WEFTA METHOD		V13 METHOD	
	Sample 1	Sample 5	Sample 1	Sample 5
1	12.58	11.56	12.55	12.64
2	15.89	17.02	8.23	6.84
3	20.53	16.92	15.0	15.08
4	22.0	21.6	15.8	15.4
5	14.7	14.4	14.2	14.0
6	22.2	22.0	17.9	16.1
7	13.4	14.6	8.5	8.8
8	12.0	12.9	14.0	14.5
9	12.3	10.1	12.0	11.6
10	16.4	16.1	12.9	12.1
11	17.97	21.86	12.75	13.01
12	15.8	20.4	11.9	14.6
13	17.8	16.6	12.5	12.6
14	11.7	9.6	12.6	12.2
15	18.0	19.2	15.4	14.2
16	16.2	19.0	11.7	10.9
17	15.2	13.8	12.3	11.1
18	13.3	15.9	13.4	15.7
19	16.9	20.0	13.6	13.5
MEAN		16.27		12.95
r		4.37		2.17
S <sub>r</sub>		1.56		0.78
RSD <sub>r</sub>		9.59		5.99
R		9.91		6.50
S <sub>R</sub>		3.54		2.32
RSD <sub>R</sub>		21.75		17.91

For key, see Table VIII

Table IV

**ICE GLAZE CONTENT OF SMALL COOKED SHRIMPS SAMPLE  
(g/100 g of Original Product): Ice Glaze Added 28.9%**

LABORATORY	WEFTA METHOD		V13 METHOD	
	Sample 3	Sample 8	Sample 3	Sample 8
1	26.62	27.85	27.84	28.28
2	31.68	31.03	19.07 <sup>(b)</sup>	18.74 <sup>(b)</sup>
3	33.15	34.20	29.28	28.2
4	34.3	37.6	29.2	30.2
5	28.3	29.1	26.5	28.8
6	34.3	38.8	31.5	31.5
7	31.2	33.2	22.4	23.8
8	25.6	28.2	26.3	29.1
9	25.7	27.6	26.4	26.3
10	28.9	29.2	25.7	27.7
11	33.91	36.83	26.18	29.25
12	32.1	36.2	28.1	29.6
13	31.8	33.9	25.7	28.2
14	26.1	24.5	26.5	26.8
15	30.1	31.8	25.2	28.9
16a	30.6	35.2	24.6	25.6
16b	29.5	33.9	25.9	29.4
17	29.3	27.4	26.7	27.6
18	29.7	28.9	27.6	28.6
MEAN		31.01		27.48
r		5.16		3.85
S <sub>r</sub>		1.84		1.38
RSD <sub>r</sub>		5.95		5.01
R		10.07		5.66
S <sub>R</sub>		3.59		2.02
RSD <sub>R</sub>		11.59		7.36

For key, see Table VIII

**Table V****ICE GLAZE CONTENT OF LARGE COOKED PRAWNS SAMPLE  
(g/100 g of Original Product): Ice Glaze Added ~ 13.5%**

LABORATORY	WEFTA METHOD		V13 METHOD	
	Sample 2	Sample 4	Sample 2	Sample 4
1	11.18	10.41	11.50	10.95
2	13.25	13.45	6.38 <sup>(b)</sup>	5.33 <sup>(b)</sup>
3	16.65	17.28	13.64	12.48
4	18.0	19.6	12.3	12.4
5	11.8	12.7	11.4	11.4
6	17.5	17.6	15.2	12.0
7	13.3 <sup>(a)</sup>	8.7 <sup>(a)</sup>	6.1 <sup>(b)</sup>	3.1 <sup>(b)</sup>
8	11.2	10.9	15.7	11.0
9	11.3	10.0	10.6	9.1
10	13.6	12.5	10.6	9.3
11	16.63	17.09	11.11	11.26
12	17.0	18.4	10.5	10.0
13	16.0	14.8	11.1	10.5
14	10.8	10.9	11.1	11.1
15	14.4	15.2	11.8	11.4
16a	14.1	14.9	8.0	8.5
16b	16.4	15.5	12.6	13.5
17	12.9	11.9	11.6	11.3
18	11.7	12.0	11.3	12.9
MEAN		14.15		11.45
r		1.76		3.13
S <sub>r</sub>		0.63		1.12
RSD <sub>r</sub>		4.44		9.76
R		7.67		4.52
S <sub>R</sub>		2.74		1.62
RSD <sub>R</sub>		19.35		14.42

For key, see Table VIII

Table VI

**ICE GLAZE CONTENT OF LARGE COOKED PRAWNS SAMPLE  
(g/100 g of Original Product): Ice Glaze Added ~ 25%**

LABORATORY	WEFTA METHOD		V13 METHOD	
	Sample 7	Sample 10	Sample 7	Sample 10
1	20.19	22.56	22.11	21.85
2	25.59	25.19	15.17	13.19
3	24.74	25.90	22.40	22.6
4	27.6	28.1	23.0	23.1
5	22.9	22.8	20.9	19.1
6	26.8	27.1	24.0	26.4
7	23.0	24.0	17.0	15.6
8	22.4	22.0	21.2	22.2
9	20.6	21.0	17.9	21.0
10	22.8	23.3	21.0	18.9
11	27.20	26.95	21.24	22.29
12	26.1 <sup>(a)</sup>	31.2 <sup>(a)</sup>	25.4	21.6
13	24.7	26.5	20.1	20.6
14	18.6	19.1	22.4	19.9
15	26.6	26.8	20.3	22.5
16a	26.0	23.9	17.3	21.3
16b	25.4	26.5	20.6	21.2
17	22.9	20.7	20.5	20.2
18	21.7	20.9	21.3	23.4
MEAN		23.97		20.81
r		2.27		4.01
S <sub>r</sub>		0.81		1.43
RSD <sub>r</sub>		3.39		6.87
R		7.39		7.49
S <sub>R</sub>		2.64		2.68
RSD <sub>R</sub>		11.00		12.86

For key, see Table VIII

**Table VII**  
**SUMMARY OF RESULTS OBTAINED TRIAL**

Samples	Ice glaze g/100g				
	Scallop	Small Cooked Shrimps	Small Cooked Shrimps	Large Cooked Prawns	Large Cooked Prawns
Added glaze	~7.5	15.8	28.9	13.5	25
WEFTA Method					
$\bar{x}$	8.21	16.27	31.01	14.15	23.97
r	3.10	4.37	5.16	1.76	2.27
R	6.31	9.91	10.07	7.67	7.39
Recovery (%)	109	103	107	105	96
n	19	19	19	18	18
Codex Method (V13)					
$\bar{x}$	5.05	12.95	27.48	11.45	20.81
r	3.79	2.17	3.85	3.13	4.01
R	6.36	6.50	5.66	4.52	7.49
Recovery (%)	67	82	95	85	83
n	19	19	18	17	19

**Table VIII**  
**Key to Tables II to VII**

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(a)	An outlying result by Cochran's Test at $P < 0.01$ level, not used in calculation of mean, repeatability or reproducibility.
(b)	An outlying result by Grubb's Test at $P < 0.01$ level, not used in calculation of the mean, repeatability or reproducibility.
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 material under the same conditions may be expected to lie with 95% probability.
$S_r$	The standard deviation of the repeatability.
$RSD_r$	The relative standard deviation of the repeatability ( $S_r \times 100/\text{mean}$ )
R	Reproducibility (between-lab variation). The value below which the absolute difference between two single test results obtained with the same method on the identical test material under different conditions may be expected to lie with 95% probability.
$S_R$	The standard deviation of the reproducibility.
$RSD_R$	The relative standard deviation of the reproducibility ( $S_R \times 100/\text{MEAN}$ )

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### Statistical Analysis of the Results

The data were examined for evidence of individual aberrant systematic error ( $p < 0.01$ ) using Cochran's and Grubbs' Tests progressively, following procedures described in the internationally agreed Protocol for the Design, Conduct and Interpretation of Collaborative Studies<sup>(5)</sup>. Mean values and precision parameters were calculated, and are given in Tables II to VI, which also identify rejected outliers.

The repeatability (r) and the reproducibility (R) as defined by that Protocol<sup>(6)</sup> were calculated after the removal of outliers. Corresponding values for standard deviation (S) and relative standard deviation (RSD, expressed as a percentage; i.e. "Coefficient of Variation") were also computed.

### **Summary of Results from Previous Trial**

The results obtained from the previous trial are summarised in Table IX for comparison purposes.

### **DISCUSSION OF RESULTS**

If a given method for the determination of the ice-glaze is accurate then the amount of glaze determined by the method should be equivalent to the amount of glaze added to the sample. In this trial the results for added and recovered glaze are summarised in Table VII.

Assuming that the added glaze has been accurately determined during sample preparation, then the percentage of recovered glaze is significantly better when participants used the WEFTA method than when they used the Codex method. The principle differences between the two methods are the water bath temperature and the drying procedures used on the deglazed sample. Both methods used the same subjective end-point determination for assessing when the sample is no longer covered in glaze. The WEFTA method probably allows more time in which to assess the end-point as the water bath is cooler, and hence may give slightly better accuracy. In addition, it may be that there is a greater removal of water by drying with a paper towel (the WEFTA method) than by draining for a set time.

However, the converse consideration applies when considering the precision of the methods. In this case both the repeatability and reproducibility precision values are better for the Codex method. This is probably indicative of the effectiveness of the drying procedure - in effect, more reproducible but not as quantitative.

Overall, in view of the greater precision being achieved with the Codex method with some loss of accuracy (as measured by the "recovery") as compared to the WEFTA method, it is recommended that the Codex method is retained as the method of choice.

The Codex method exhibited similar precision characteristics as in the previous MAFF trial thus indicating that any refinements to the procedure or experience with it since that trial have not improved the performance of the participants.

In addition, participants made similar comments on the methods as assessed in this trial as were made and given in the report of the previous trial<sup>(2)</sup>.



**Table IX**  
**SUMMARY OF RESULTS OBTAINED IN PREVIOUS**  
**COLLABORATIVE TRIAL**

Samples	Ice glaze g/100g						
	SCW - prawns	Scampi	Cockles	SCW - prawns	LWW - prawns	SWW - prawns	Scallops
Added glaze	20.7	7.8	10.7	13.3	16.9	22.9	5.7
Codex Method							
$\bar{x}$	25.1	5.1	5.0	20.6	16.2	22.6	1.9
r	3.1	3.1	3.1	3.3	3.3	3.1	1.7
R	5.1	5.0	4.6	5.2	6.3	5.7	4.3
n	12	10	10	12	12	12	10
Lancs. Method							
$\bar{x}$	26.9	6.5	6.3	22.8	18.7	25.2	3.3
r	3.4	3.1	2.8	2.2	1.6	3.8	1.9
R	3.3	3.6	3.5	2.9	2.5	4.2	2.7
n	12	11	12	10	11	11	12
BFFF Method							
$\bar{x}$	25.7	6.0	6.9	20.9	15.8	22.3	3.3
r	2.5	2.3	2.2	4.9	2.4	3.2	0.9
R	4.6	4.1	4.1	6.3	2.8	3.4	1.5
n	12	11	12	12	10	10	10

SCW: Cold water prawns - cooked and small

LWW: Warm water prawns

SWW: Warm water prawns - cooked and small

## CONCLUSIONS

Since there were relatively small amounts of frozen material passing through the glazing line, it should be appreciated that the preparation of samples for this trial is inherently variable, despite the precautions taken to glaze under strictly controlled conditions, and to ensure homogeneity of the samples as best possible. This could account for unacceptable accuracy and precision exhibited by the methods being assessed in this trial, and why the performance of the Codex method is slightly worse than the previous trial. Commercial samples would be expected to show less variability in glaze content.

Nevertheless, because the methods being assessed are highly empirical and subjective in nature, they must be expected to exhibit more variability than would normally be anticipated in a method of analysis. These results confirm that expectation.

From the results reported in this trial it is possible to make the following recommendation:

The recommendations made in the previous Report regarding the determination of ice glaze on frozen shellfish are to be maintained and the Codex procedure (MAFF Validated Method V13) is to be preferred to the WEFTA method.

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## APPENDIX I

### **SUMMARY OF METHODS USED IN PREVIOUS TRIAL CODEX ALIMENTARIUS COMMISSION - DETERMINATION OF NET CONTENTS OF QUICK FROZEN SHRIMPS AND PRAWNS**

#### *Scope And Field Of Application*

The method is designed to determine the net contents of raw and cooked quick frozen shrimps and prawns covered by glaze.

#### *Principle*

The sample is thawed by immersion into a container into which running water is introduced in the case of raw product, or immersed in water maintained at 27°C until thawing is deemed, by sight or feel, to be completed. The weight loss is assumed to be loss of ice glaze.

### **LANCASHIRE COUNTY COUNCIL - DETERMINATION OF ICE GLAZE FOR IQF COOKED AND PEELED PRAWNS**

#### *Scope And Field Of Application*

The method is designed to determine the ice-glaze content of IQF cooked and peeled prawns.

#### *Principle*

The sample is immersed in water at 27°C for a set time of two minutes during which it is gently agitated. The sample is then drained in a sieve and weighed. The weight loss is assumed to be the loss of ice-glaze.

### **BRITISH FROZEN FOOD FEDERATION - DETERMINATION OF ICE- GLAZE FOR IQF COOKED AND PEELED PRAWNS**

#### *Scope And Field Of Application*

The method is designed to determine the ice-glaze content of IQF cooked and peeled prawns.

#### *Principle*

The sample is immersed in water maintained at 20°C for a set time of 30 seconds during which it is continuously agitated. The sample is then drained in a sieve and weighed. The weight loss is assumed to be the loss of ice-glaze.

## APPENDIX II

### MAFF VALIDATED METHOD V13: CODEX ALIMENTARIUS COMMISSION

#### 1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the net contents of shellfish including quick-frozen raw and cooked prawns (shrimps) covered by ice glaze.

#### 2. DEFINITION

Content of ice glaze: the percentage weight of ice glaze as determined by the method specified.

#### 3. PRINCIPLE

The sample is thawed by immersion into a container into which running tap-water is introduced (in the case of the raw product), or immersed in tap-water maintained at 27°C until thawing is deemed to be completed (in the case of the cooked product). The weight loss is assumed to be loss of ice glaze.

#### 4. REAGENTS

None.

#### 5. APPARATUS

5.1 Analytical balance.

5.2 Sieve: Clean and dry, with woven wire cloth of nominal square aperture size 2.8mm and conforming to the requirements of ISO R565, or of aperture size 2.38mm and conforming to the requirements of US No. 8 Standard Screen. Sieves are to be of diameter 200mm or 300mm.

5.3 Container with inlet/overflow into which fresh tap-water at room temperature can be introduced at the bottom of the container at the rate of approximately 25 l/min.

5.4 Water bath: A vessel containing tap-water at  $27 \pm 1^\circ\text{C}$  equal in weight to 8 times the weight of sample taken (6.1).

#### 6. PROCEDURE

6.1 Place the sample in a freezer of temperature  $-18 \pm 2^\circ\text{C}$  and allow to equilibrate. For analysis, remove the sample from low temperature storage, open immediately, accurately in g to one decimal place ( $m_0$ ).

- 6.2 Weigh a clean dry sieve (5.2), using a 200mm diameter sieve if the sample weight is 500g or less, or 300mm if greater than 500g. Let the weight in g of the sieve, to one decimal place, be  $m_1$ .
- 6.3 Transfer the weighed portion to the sieve. Deglaze by one of the following methods:
  - 6.3.1 Frozen raw products: immerse the sieve and test sample in the container with running water (5.3).
  - 6.3.2 Frozen cooked product: immerse the sieve and test sample in the water bath containing the specified quantity of tap-water (5.4).
- 6.4 Leave the product immersed until all the ice is melted. After all the glaze that can be seen or felt has been removed (i.e. when the external surface of the sample becomes soft) and the shrimps or prawns separate easily, remove the sieve and test sample, incline the sieve at an angle of about 20° and drain for two minutes.
- 6.5 Weigh the sieve containing the drained product. Let the final weight in g, to one decimal place, be  $m_2$ .

## 7. EXPRESSION OF RESULTS

The ice glaze content of the original sample, expressed as a percentage by weight, is given by:

$$\% \text{ ice glaze content} = 100 \times (m_0 + m_1 - m_2) / m_0$$

where:

$m_0$  is the initial frozen weight taken (6.1);

$m_1$  is the initial weight of sieve alone (6.2);

$m_2$  is the observed deglazed weight with sieve (6.5).

## APPENDIX III

### WEST EUROPEAN FISH TECHNOLOGISTS ASSOCIATION (WEFTA) METHOD

#### 1. SCOPE AND FIELD OF APPLICATION

The method is designed to determine the net contents of shellfish including quick-frozen raw and cooked prawns (shrimps) covered by ice glaze.

#### 2. DEFINITION

Content of ice glaze: the percentage weight of ice glaze as determined by the method specified.

#### 3. PRINCIPLE

The pre-weighed glazed sample is immersed into a water bath until all the glaze is removed. This is determined by feeling the sample and, when the surface becomes rough, the still frozen sample is removed from the water bath and dried on a paper towel before estimating the net product content by repeated weighing. By this procedure thaw drip losses and/or re-freezing of adhering moisture can be avoided.

#### 4. REAGENTS

None.

#### 5. APPARATUS

- 5.1 Analytical balance.
- 5.2 Water bath, preferably with adjustable temperature.
- 5.3 Circular sieve with a diameter of 200mm and 1 to 3mm mesh apertures (ISO R565).
- 5.4 Paper or cloth towels with smooth surface.

#### 6. PROCEDURE

##### 6.1 Sample Preparation

- 6.1.1 The product temperature should be adjusted to -18 to -20°C to achieve standard deglazing conditions.
- 6.1.2 The water bath shall contain an amount of fresh potable water equal to about ten times the declared weight of the product. The temperature should be adjusted to between 15° and 20°C.

**6.2** Determination of Gross Weight, 'A'.

After removal of the packing the weight of the glazed product is estimated:

- (i) Large shrimps/prawns or single fillets - single weights are recorded  
( $A_1 - A_n$ );
- (ii) Small sized shrimps - the unit should be divided into sub-samples of *ca.* 100g. Sub-samples to be stored in a cold box until required.

**6.3** Removal Of Glaze

The pre-weighed samples/sub-samples are placed in a monolayer on the sieve and transferred into the water bath where they are kept immersed by hand. The product may be carefully agitated until no more glaze can be felt by the fingertips on the surface of the product (texture changes from slippery to rough). The time required will be dependent on the size, shape and glaze content and will typically be in the range 10 to 60 seconds.

**6.4** Determination of net weight, 'B'.

The deglazed sample/sub-sample, after removal of adhering water by use of a towel (no pressure applied) is immediately weighed.

**6.5** Determination Of Glaze Weight, 'C'.

Glaze weight, C = Gross weight, A - Net weight, B

**6.6** Calculation Of Percentage Proportions

% net contents of the product, F =  $(B/A) \times 100$

% glaze related to the gross weight of product, G =  $(C/A) \times 100$

% glaze related to the net weight of product, H =  $(C/B) \times 100$



**MAFF VALIDATED METHODS FOR THE ANALYSIS OF  
FOODSTUFFS**

**METHOD FOR THE ENUMERATION OF *LISTERIA*  
*MONOCYTOGENES* IN MEAT AND MEAT PRODUCTS**

**V38**

Correspondence on this method may be sent to Sue Scotter, CSL Food Science Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UQ.

Correspondence on the MAFF Validated Methods Series may be sent to Roger Wood, Food Labelling and Standards Division, CSL Food Science Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UQ.

**COSHH AND SAFETY CONSIDERATIONS**

*Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Codes of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.*

*Each laboratory should follow its own safety rules and national regulations, particularly COSHH, with respect to the sample preparation.*

The procedures specified in this method shall only be carried out in laboratories with suitable facilities and under control of a qualified microbiologist.

These procedures shall not be performed in quality control laboratories, or in food manufacturing or processing premises, where there is a risk of contamination of the environment.

Full bacteriological precautions shall be taken at all times whilst carrying out the procedure specified in this method. Particular attention shall be given to the sterilisation of used equipment and media after testing suspect samples prior to disposal or reuse.

Note - For further and more detailed safety precautions reference is made to ISO 7218, Methods for microbiological examination of food and animal feedingstuffs, Part O, 1996, General laboratory practices (10.3).

**1. SCOPE AND FIELD OF APPLICATION**

This method specifies procedures recommended for the enumeration of *Listeria monocytogenes* in meat and meat products, and is based on the

USDA method for the detection of *L. monocytogenes* in meat and meat products (10.1).

## 2. DEFINITIONS

For the purpose of this recommended method the following definitions apply:

### 2.1 *Listeria monocytogenes*:

Pathogenic bacteria that form typical colonies on the specified solid selective medium and which display the morphological, physiological and biochemical characteristics described, when tests are carried out in accordance with this method.

### 2.2 Enumeration of *Listeria monocytogenes*:

Determination of the number of viable and confirmed *L. monocytogenes* bacteria per gram of product when the examination is carried out in accordance with this method.

## 3. PRINCIPLE

In general, the enumeration of *L. monocytogenes* necessitates three successive stages as in 3.1 to 3.3. See also the diagram of procedure in Appendix I.

### 3.1 Preparation of the test sample

The test sample is homogenised in suspension medium and decimal dilutions are prepared as necessary.

### 3.2 Enumeration and presumptive identification

The selective agar is inoculated from the initial suspension (3.1) and dilutions thereof, incubated at 30°C and examined after 48 h to check for the presence of colonies which, from their appearance, are considered to be presumptive *Listeria* spp..

### 3.3 Confirmation of identity

Colonies of presumptive *Listeria* spp. (3.2) are sub-cultured onto a non-selective solid medium for confirmation of identity by means of appropriate morphological, physiological and biochemical tests.

## 4. CULTURE MEDIA AND REAGENTS

### 4.1 Basic materials

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

The chemical products used for the preparation of the culture media and reagents shall be of recognised analytical quality.

The water used shall be distilled or deionised water, free from substances that might inhibit the growth of microorganisms under the test conditions.

When agar is specified, the amount used should be varied according to the manufacturer's instructions to give media of suitable firmness.

Measurements of pH shall be made using a pH meter, measurements being referred to a temperature of 25°C. Adjustments, if necessary, are made by adding either 1 M hydrochloric acid or 1 M sodium hydroxide solution.

If the prepared culture media and reagents are not used immediately, they shall, unless otherwise stated, be stored in the dark at a temperature between 2 and 5°C for no longer than 1 month, conditions which do not produce any change in their composition.

### 4.2 Diluent

	g/L
Peptone	1.0
Sodium chloride	8.5

Preparation: Dissolve the components in 1000 mL of water by gently heating. Dispense into final containers such that after autoclaving each tube or bottle contains  $9.0 \pm 0.2$  mL. Adjust the pH so that after autoclaving it is  $7.0 \pm 0.2$  at 25°C. Autoclave at 121°C for 15 min.

### 4.3 Culture media

#### 4.3.1 Sample suspension medium (UVM 1 formulation)

##### 4.3.1.1 Base

	g/L
Protease peptone	5.0
Tryptone	5.0
Meat extract	5.0
Yeast extract	5.0
Sodium chloride	20.0
Di-sodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by boiling. Autoclave at 121°C for 15 min. Cool to 50°C.

##### 4.3.1.2 Supplement per Litre of medium

	mg
Nalidixic acid	20.0
Acriflavine HCL	12.0

Preparation: Dissolve the components in 4 mL of water. Sterilise by filtration through a filter of pore size 0.22 µm (5.1.13).

##### 4.3.1.3 Preparation of the complete medium

Aseptically add the supplement to the basal medium. Invert gently to dissolve. Adjust the pH so that, after sterilisation and the addition of supplement, it is  $7.4 \pm 0.2$  at 25°C. Aseptically distribute the complete medium into 225 mL volumes. The complete medium may be stored for up to one week at 2 - 5°C before use.

#### 4.3.2 Listeria selective agar Oxford formulation

##### 4.3.2.1 Base

	g/L
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0
Agar	10.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by boiling. Autoclave at 121°C for 15 min. Cool to 50°C.

#### 4.3.2.2 Supplement per Litre of medium

	mg
Cycloheximide	400.0
Colistin sulphate	20.0
Acriflavin	5.0
Cefotetan	2.0
Fosfomicin	10.0

Preparation: Dissolve the components in 10 mL of a 1:1 solution of ethanol:water. Sterilise by filtration through a 0.22 µm pore size filter (5.1.13).

#### 4.3.2.3 Preparation of the complete medium

Aseptically add the supplement to the basal medium and mix thoroughly. Adjust the pH so that, after sterilisation and addition of supplement, it is  $7.0 \pm 0.2$  at 25°C. Transfer the complete medium in quantities of about 15 mL to sterile Petri dishes and allow to solidify on a level surface. The complete medium may be stored for up to one week at 2 - 5°C before use.

#### 4.3.3 Tryptone Soya Yeast Extract Agar (TSYEA)

	g/L
Tryptone soy broth	30.0
Yeast extract	16.0
Agar	12.0 to 18.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by boiling. Adjust the pH so that after sterilisation it is  $7.3 \pm 0.1$  at 25°C. Autoclave at 121°C for 15 min and allow to cool to 50°C. Transfer quantities of about 15 mL to sterile Petri dishes and allow to solidify on a level surface (5.1.14).

#### 4.3.4 Tryptone Soya Yeast Extract Broth (TSYEB)

	g/L
Tryptone soy broth	30.0
Yeast extract	16.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by heating gently. Adjust the pH so that after sterilisation it is  $7.3 \pm 0.1$  at 25°C. Transfer the TSYEB in quantities of about 10 mL to tubes or bottles. Sterilise for 15 min at 121°C.

**4.3.5 Blood agar (not required if microwell haemolysis test used)****4.3.5.1 Base**

	g/L
Protease peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by boiling. Sterilise the blood agar base for 15 min at 121°C. Cool the medium to  $47 \pm 1^\circ\text{C}$ .

**4.3.5.2 Supplement per Litre of medium**

	mL
Washed sheep red blood cells	70.0

Preparation: Centrifuge defibrinated sheep blood at  $900 \times g$  for 30 min, aseptically removing the supernatant liquid and re-suspend the pellet in sterile 0.85% saline solution to the original volume. If the centrifuged suspension has haemolysed, a fresh suspension must be prepared.

**4.3.5.3 Preparation of the complete medium**

Add the washed sheep red blood cells to the sterilised agar base and mix well. Adjust the pH so that, after sterilisation and addition of supplement, it is  $7.0 \pm 0.1$  at  $25^\circ\text{C}$ . Transfer the medium in quantities of about 15 mL to sterile Petri dishes and allow to solidify on a level surface.

**4.3.6 Brain heart infusion broth**

	g/L
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Protease peptone	10.0
Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate	2.0

Preparation: Dissolve the dehydrated components in 1000 mL water by heating gently. Adjust the pH so that after autoclaving it is  $7.4 \pm 0.2$  at  $25^\circ\text{C}$ . Distribute in 10 mL volumes in screw-capped containers and autoclave at  $121^\circ\text{C}$  for 15 min.

**4.3.7 Phosphate Buffered Saline (PBS)**

	g/L
Sodium chloride	8.0
Potassium chloride	0.2
Disodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2

Preparation: Dissolve the dehydrated components in 1000 mL water by heating gently. Adjust the pH so that after autoclaving it is  $7.3 \pm 0.1$  at 25°C. Dispense in 10 mL volumes in screw-capped containers and autoclave at 115°C for 10 min.

**4.3.8 Carbohydrate utilisation broth****4.3.8.1 Base**

	g/L
Protease peptone	10.0
Beef extract	1.0
Sodium chloride	5.0
Bromocresol purple	0.02

Preparation: Dissolve the dehydrated components in 1000 mL water by gentle heating. Distribute into tubes or bottles in quantities of 10 mL. Sterilise at 121°C for 15 min. Adjust the pH so that after sterilising it is  $6.8 \pm 0.2$  at 25°C.

**4.3.8.2 Carbohydrates**

	g/L
Rhamnose	50.0
Xylose	50.0

Preparation: Dissolve each carbohydrate separately in 1000 mL water, do not heat to dissolve. Sterilise by filtration through a 0.22 µm pore size filter (5.1.13).

**4.3.8.3 Preparation of the complete medium**

For each carbohydrate, aseptically add 1 mL carbohydrate solution (4.3.8.2) to each tube or bottle of basal medium (4.3.8.1).

**4.3.9 Motility medium**

	g/L
Casein peptone	20.0
Meat peptone	6.1
Agar	3.5

Preparation: Dissolve the dehydrated components in 1000 mL water by boiling. Adjust the pH so that after sterilisation it is  $7.3 \pm 0.2$  at 25°C. Dispense in tubes or bottles in quantities of about 10 mL. Sterilise for 15 min at 121°C.

#### 4.3.10 CAMP (Christie/Atkins/Munch-Peterson) test agar

Very thin-layered sheep blood agar plates are required for this test.

##### 4.3.10.1 Base

	g/L
Protease peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

Preparation: Dissolve the dehydrated components in 1000 mL of water by boiling. Adjust the pH so that after sterilisation it is  $7.0 \pm 0.1$  at 25°C. Sterilise the blood agar base for 15 min at 121°C. Cool the medium to 50°C and transfer quantities of about 15 mL to sterile Petri dishes and allow to solidify on a level surface.

##### 4.3.10.2 Sheep blood medium

	mL
Basal medium (4.3.10.1)	100.0
Washed sheep red blood cells (see 4.3.5.2)	7.0

Preparation: Add the washed cell suspension to the sterilised, molten base cooled to  $47 \pm 1^\circ\text{C}$ .

##### 4.3.10.3 Preparation of the complete medium

Pour a very thin layer of sheep blood medium (4.3.10.2) over the basal medium (4.3.10.1) using no greater than 3 mL per plate. Allow to solidify in an even layer. If the blood is added to dishes containing the basal medium which have been prepared in advance, it may be necessary to warm the dishes by placing them in an incubator at 37°C for 20 min before pouring the blood layer. Dry plates before use.

##### 4.3.10.4 CAMP reaction cultures

A weakly  $\beta$ -haemolytic strain of *Staphylococcus aureus* (eg NCTC 1803) and a strain of *Rhodococcus equi* (eg NCTC 1621) are required to



undertake the CAMP test. Not all strains of *Staphylococcus aureus* are suitable for the CAMP test.

Maintain stock cultures of *S. aureus*, *R. equi*, *L. monocytogenes*, *L. innocua* and *L. ivanovii* by inoculating TSYEA plates (4.3.3), incubating at 37°C for 24 - 48h, or until growth has occurred and storing at 4°C.

## 5. APPARATUS & GLASSWARE

Usual microbiological laboratory equipment, and in particular:

### 5.1 Apparatus

#### 5.1.1 Apparatus for dry sterilisation (oven) or wet sterilisation (autoclave)

Apparatus that will enter into contact with the culture media, the dilution fluid or the sample, except for apparatus that is supplied sterile (particularly plastic apparatus), shall be sterilised either by:

being kept at 170 to 175°C for not less than 1 h in an oven or

by being kept in contact with saturated steam at 121°C for not less than 15 min in an autoclave.

An autoclave is also necessary for the sterilisation of culture media and reagents. It shall be capable of being maintained at 121°C.

**5.1.2 Incubator:** capable of being maintained at 30°C ± 1°C.

**5.1.3 Incubator:** capable of being maintained at 37°C ± 1°C.

**5.1.4 Incubator:** capable of being maintained at 25°C ± 1°C.

**5.1.5 Waterbath:** capable of being maintained at 47°C ± 1°C.

#### 5.1.6 Blending equipment

One of the following shall be used:

a) a rotary blender, operating at a rotational frequency between 8000 and 45000 min<sup>-1</sup>, with glass or metal bowls fitted with lids, resistant to the conditions of sterilisation.

b) a peristaltic type blender (Stomacher Model 400), with sterile plastic bags.

Note - The bowls or plastic bags should have sufficient capacity to allow the sample to be properly mixed with the appropriate amount of diluent. In general, the volume of the container should be equal to about twice the volume of the sample plus diluent.

**5.1.7 Loops:** of platinum-iridium, nickel-chromium or plastic of diameter approximately 3 mm.

- 5.1.8 Inoculating needle:** of platinum-iridium, nickel-chromium or plastic.
- 5.1.9 pH-meter:** (for measuring the pH of prepared media and reagents), having an accuracy of calibration of 0.1 pH unit at 25°C.
- 5.1.10 Refrigerator:** (for storage of prepared media and reagents), capable of being maintained at 2 to 5°C.
- 5.1.11 Sterile round-bottom microtitre plates,** for microwell haemolysis.
- 5.1.12 Automatic pipette,** capable of dispensing 100 µL volumes.
- 5.1.13 Filters,** of 0.22 µm pore size suitable for the filtration of aqueous solutions and organic solvents.
- 5.2 Glassware:** The glassware shall be resistant to repeated sterilisation.
  - 5.2.1 Culture bottles or flasks,** for sterilisation and storage of culture media and incubation of liquid media.
  - 5.2.2 Test tubes,** of dimensions approximately 16 mm x 125 mm fitted with lids.
  - 5.2.3 Screw-capped bottles** of approximately 25 mL capacity.
  - 5.2.4 Flasks or bottles,** of capacity 250 mL.
  - 5.2.5 Measuring cylinders,** for preparation of the complete media.
  - 5.2.6 Graduated pipettes,** of nominal capacity 1 mL graduated in divisions of 0.1 mL.
  - 5.2.7 Sterile Petri dishes,** of glass or plastic of diameter 90 to 100 mm.
  - 5.2.8 Spreaders:** of glass or plastic.
  - 5.2.9 Microscope slides/coverslips**

## 6. PROCEDURE

See the diagram of procedure in Appendix I.

### 6.1 Preparation of test sample, initial suspension and dilutions

Add 225 mL sample suspension medium (4.3.1) to 25 g test sample in a Stomacher bag or blender bowl. Blend for 2 min. Prepare dilutions from the initial suspension as necessary in the diluent (4.2).

## 6.2 Inoculation

Transfer by means of a sterile pipette, 0.1 mL of the initial suspension ( $10^{-1}$  dilution) to each of two selective agar plates (4.3.2). Repeat the procedure for  $10^{-2}$  dilution and further dilutions as necessary. Carefully spread the inoculum as quickly as possible over the surface of the agar plate, trying not to touch the sides of the dish, using a glass or plastic spreader (5.2.8). Use a sterile spreader for each plate. Retain the plates at room temperature, on a level surface for about 15 min with the lids uppermost to allow the inoculum to soak into the agar.

## 6.3 Incubation

Invert the plates prepared according to 6.2 and incubate them at  $30 \pm 1^{\circ}\text{C}$  for 48 h.

## 6.4 Counting and selection of colonies

Select dishes at two consecutive dilutions containing less than 150 typical colonies, that is colonies surrounded by a dark brown or black halo. Count these suspect colonies.

## 6.5 Confirmation

### 6.5.1 Selection of colonies for confirmation

From each plate containing less than 150 typical colonies (6.4) select five typical or suspect colonies or, if there are fewer than five such colonies, select all for confirmation.

### 6.5.2 Subculturing

Streak the selected colonies onto the surface of TSYEA plates (4.3.3) in a manner which will allow well isolated colonies to develop. Incubate the plates at  $30^{\circ}\text{C}$  for 24 h or until growth is satisfactory.

## 6.6 Confirmation

### 6.6.1 Catalase reaction

From each TSYEA plate (6.5.2) pick a typical colony and place it on a coverslip (5.2.9). Add a drop of 3% hydrogen peroxide solution to a microscope slide (5.2.9). Invert the coverslip and place onto the slide. This technique is used to prevent aerosol formation. All *Listeria* spp. are catalase positive demonstrated by the formation of gas bubbles.

### 6.6.2 Morphology and staining properties

Test for Gram reaction. From each TSYEA plate (6.5.2) pick a typical colony and prepare a heat-fixed mount on a microscope slide (5.2.9).

Gram stain and examine under oil immersion on a light microscope. All *Listeria* spp. are Gram-positive short rods.

### 6.6.3 Motility at 25°C

From each TSYEA plate (6.5.2) select a well isolated typical colony and remove by means of a sterile inoculating needle (5.1.8). For each isolate stab inoculate a tube of motility medium (4.3.9) and incubate at 25°C for 48 h. Examine for growth around the stab, if negative reincubate for a further 5 days. *Listeria* spp. are motile giving a typical umbrelliform growth pattern.

### 6.6.4 Haemolysis (see also 6.6.5)

If the morphological and physiological characteristics, and catalase reaction indicate the possibility of *Listeria* spp., inoculate blood agar plates (4.3.5) to determine the haemolytic reaction.

Dry the agar surface well before use. Select a typical colony from each TSYEA plate (6.5.2) and streak the colony onto the blood agar by means of a loop (5.1.7). Simultaneously inoculate blood agar plates (4.3.5) with positive and negative control cultures (*L. monocytogenes*, *L. ivanovii* and *L. innocua*).

After 48h incubation at 37°C, examine the test strains and controls. *L. monocytogenes* shows narrow, slight zones of clearing ( $\beta$ -haemolysis): *L. innocua* should show no clear zone. *L. ivanovii* usually shows wide, clearly delineated zones of  $\beta$ -haemolysis. Remove the colony to examine the haemolysis underneath the colony. Hold plates up to a bright light to compare test cultures with controls.

### 6.6.5 Haemolysis using microwell technique

As an alternative to the preparation of blood agar plates for the determination of haemolytic activity, a microwell method may be used.

From each TSYEA plate (6.5.2) select a well isolated typical colony and remove by means of a sterile loop (5.1.7). For each isolate inoculate a Brain Heart Infusion Broth (4.3.6) and incubate at 37°C for 48 h.

Prepare a 2% sheep erythrocyte suspension by washing i.e. centrifuging and resuspending, sterile defibrinated sheep blood three times in PBS (4.3.7). From this suspension pipette 100  $\mu$ L in duplicate into wells of a round bottom microtitre plate (5.1.11).

To the erythrocyte suspension add 100  $\mu$ L of Brain Heart Infusion broth culture. Incubate the microtitre plate for 45 min at 37°C followed by incubation for 2 h at 4°C. The presence of haemolysins are shown by a

homogeneous red liquid. A clear supernatant with a layer of red blood cells on the bottom of the well indicate no haemolytic activity. Reference strains of *L. monocytogenes*, *L. ivanovii* and *L. innocua* should be run concurrently with this test.

### 6.6.6 Further biochemical confirmation

For these assays a culture in TSYEB (4.3.4) corresponding to the typical colony used for the haemolysis reaction (6.6.4 or 6.6.5) is required. Pick a typical colony from each TSYEA plate (6.5.2) and suspend in a tube containing TSYEB (4.3.4). Incubate for 24 h at 37°C.

#### 6.6.6.1 Carbohydrate utilisation

Inoculate the carbohydrate fermentation broths (4.3.8) each with one loopful of the TSYEB culture (6.6.6). Incubate for up to 7 days at 37°C, although positive reactions (acid formation indicated by a yellow colour) occur mostly within 24 - 48 h. Reference strains of *L. monocytogenes*, *L. ivanovii* and *L. innocua* should be run concurrently with this test.

#### 6.6.6.2 CAMP test

Streak the *S. aureus* and *R. equi* cultures in single lines across the blood agar plate (4.3.10) so that the two cultures are parallel and diametrically opposite. A thin, even inoculum is required. This can be obtained by using an inoculating needle (5.1.8) or a loop (5.1.7) held at right angles to the agar. Streak the test strain in a similar fashion at right angles to these cultures so that the test culture and reaction cultures do not touch but at their closest are about 1 - 2 mm apart. Several test strains may be streaked on the same plate.

Simultaneously, streak control cultures of *L. monocytogenes*, *L. innocua* and *L. ivanovii*. Incubate the plates at 37°C for 18 - 24 h.

Positive reactions are indicated by an enhanced zone of  $\beta$ -haemolysis at the intersection of the test strain with either the *S. aureus* or *R. equi* culture. However, the appearance of positive results varies with the reaction culture. A positive reaction with *R. equi* is seen as a wide (5 - 10 mm) 'arrow-head' of haemolysis. Small (about 1 mm) zones of weak haemolysis around the intersection of the test and *R. equi* cultures are negative reactions. A positive reaction with *S. aureus* is seen as a small rounded zone of enhanced haemolysis extending only about 2 mm from the test strain and within the weakly haemolytic zone due to growth of the *S. aureus* culture. Large zones of haemolysis around the *S. aureus* culture do not occur.

*L. monocytogenes* and *L. seeligeri* show a positive CAMP reaction with *S. aureus* but not *R. equi*. *L. ivanovii* reacts with *R. equi* but not with *S. aureus*. The other *Listeria* spp. show negative CAMP reactions with both *S. aureus* and *R. equi*.

## 6.7 Interpretation of morphological and physiological properties and biochemical reactions

All *Listeria* spp. are small Gram-positive rods (only with 24 h old cultures) that demonstrate an umbrelliform growth pattern in the motility medium. They are catalase positive. *L. monocytogenes* utilises rhamnose but not xylose.

*L. monocytogenes*, *L. ivanovii* and *L. seeligeri* (weak) produce  $\beta$ -haemolysis on blood agar plates and positive reactions in the microwell haemolysis test. Of the three haemolytic *Listeria* spp. only *L. monocytogenes* fails to utilise xylose and is positive for rhamnose utilisation.

*L. monocytogenes* and *L. seeligeri* show a positive CAMP reaction with *S. aureus* but not with *R. equi*. *L. ivanovii* reacts with *R. equi* but not with *S. aureus*. The other *Listeria* spp. show negative CAMP reactions with both reaction cultures.

## 7. CONTROL CULTURES

Control cultures of *L. monocytogenes*, *L. ivanovii* and *L. innocua* should be run concurrently with all confirmatory tests.

## 8. EXPRESSION OF RESULTS

### 8.1 General

If all of the selected typical colonies (6.4) confirm as *L. monocytogenes*, the number of organisms present will be the same as that given by the count in 6.4. In all other cases the number shall be calculated from the percentage of isolates confirmed positive in relation to the total number of selected colonies (6.4).

Round the result to a whole number of colonies.

## 8.2 Calculation of the weighted mean

Calculate the number,  $N$ , of *L. monocytogenes* per gram of product using the following equation:

$$N = \frac{\sum c}{(n_1 + 0.1n_2)0.1d}$$

$\sum c$  = the sum of confirmed colonies on all dishes retained

$n_1$  = the number of dishes retained at the first dilution

$n_2$  = the number of dishes retained at the second dilution

$d$  = the dilution factor corresponding to the first dilution

Round the result calculated to two significant figures.

Take as the result the number of micro organisms per gram of product, expressed as a number between 1.0 and 9.9 multiplied by  $10^x$ , where  $x$  is the appropriate power of 10.

## 8.3 Estimation of small numbers

If the two dishes corresponding to the initial suspension contain less than 15 colonies, calculate the arithmetic mean  $m$  of the colonies counted on both dishes.

Report the result as follows:

estimated number  $N_E$  of *L. monocytogenes* per gram:

$N_E = m \times d^{-1}$  where  $d$  is the dilution factor of the initial suspension.

## 8.4 No characteristic colonies

If the two dishes corresponding to the initial suspension contain no characteristic colonies report the result as follows:

less than  $1 \times d^{-1}$  *L. monocytogenes* per gram, where  $d$  is the dilution factor of the initial suspension.

## 9. VALIDATION

The procedure as described in this protocol has been used in an on-going proficiency test exercise organised by the Ministry of Agriculture, Fisheries and Food, CSL Food Science Laboratory, Norwich (10.5). A summary of results obtained is given in Appendix II. Test materials were distributed on four occasions. These comprised freeze-dried minced beef test materials, artificially inoculated with the target organism and a simulated autochthonous flora; in order to simulate, as closely as possible,

a natural foodstuff. On each occasion analysts received duplicate blind test materials and were asked to use the method prescribed in this protocol.

All test materials used in the proficiency testing exercise were assessed for homogeneity (Appendix II) using the recommended procedures described in the ISO/IUPAC/AOAC International Harmonised Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (10.6). Homogeneity was assessed immediately following preparation of the test materials (day 0) and again on the date of examination by laboratories (test day). For distribution rounds 1 to 3, test day was 12 days after preparation of the test materials. For round 4, test day was 19 days after preparation.

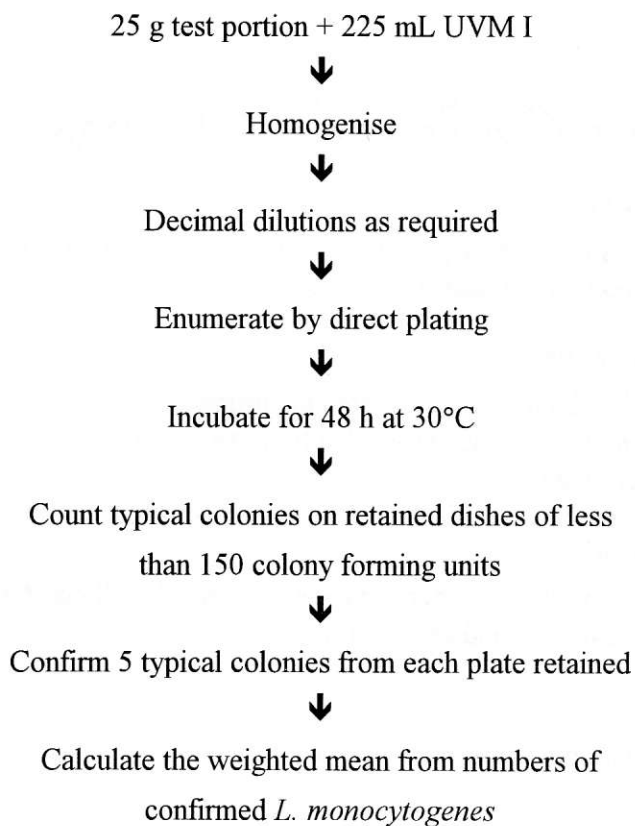
Statistical analyses of the results for any one test material are as described in the ISO/IUPAC/AOAC International Protocol for the Design, Conduct and Interpretation of Collaborative Studies (10.4).

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**APPENDIX I**  
**METHOD FOR THE ENUMERATION OF *L. MONOCYTOGENES***



## APPENDIX II

### STATISTICAL ANALYSIS OF THE RESULTS FROM AN ON-GOING PROFICIENCY TESTING SCHEME

#### A.1 Matrix

All test materials were prepared on a minced beef matrix

#### A.2 Laboratories

Data points from participating laboratories were used in statistical analyses after the removal of aberrant results

#### A.3 Statistical outliers

Entries among tables derived from the original test results that deviate so much from comparable entries that they are considered to be irreconcilable with other data (10.4).

#### A.4 Assigned value

The robust mean calculated from data returned by all participants who carried out the method as prescribed

#### A.5 $S_r$

the standard deviation of the repeatability

#### A.6 $r$

repeatability (within laboratory variation) - the value below which the absolute difference between two single test results obtained with the same method on an identical test material under the same conditions may be expected to lie within a 95% probability

#### A.7 $S_R$

the standard deviation of the reproducibility

#### A.8 $R$

reproducibility (between laboratory variation) - the value below which the absolute difference between two single test results obtained with the same

method on an identical test material under different conditions may be expected to lie within a 95% probability

**Table 1**  
**Precision characteristics ( $\text{Log}_{10}$  colony forming units per gram) of the method derived from the results of an on-going proficiency testing scheme**

Date of Testing	No. of laboratories	Homogeneity	No. of statistical outliers	Assigned value	$S_r$	$r$	$S_R$	$R$
May 1994	12	satisfactory	0	4.70	0.08	0.22	0.15	0.42
November 1994	15	satisfactory	0	3.87	0.08	0.22	0.15	0.42
November 1995	18	satisfactory	2	5.19	0.09	0.24	0.16	0.44
November 1996	20	Satisfactory	1	4.46	0.09	0.24	0.23	0.64
January 1997	20	satisfactory	1	3.53	0.07	0.19	0.11	0.31

THE HISTORY OF THE  
CITY OF BOSTON

From the first settlement in 1630 to the present time.

By  
JOHN B. BOSTON

NEW YORK

## **Annual Report of the Council of the Association of Public Analysts for 1996**

*Presented at the 44th Annual General Meeting of the Association held at the Royal York Hotel, York on 19th April 1997 by the Honorary Secretary, Dr. Peter Clare.*

### **Introduction**

This report reviews the activities of the Association of Public Analysts (APA) during the year ending December 31st 1996 and discusses external events that influence the development of the Association and its members. 1996 has been without precedent in public awareness for the need for food that is safe and wholesome, without disease, that is nutritious, and unadulterated, and free from substitution.

Bovine Spongiform Encephalopathy (BSE) and the contamination of food by *Escherichia coli* 0157 have had serious repercussion on the confidence in the safety and composition of food bought and consumed and in consequence debate has been renewed concerning the need for a food safety body whose principle role would be addressing public confidence in the food. The role of the Ministry of Agriculture, Fisheries and Food (MAFF) is perceived as representing both the consumer and food producer interests, and consequently a food safety body which is able to demonstrate both authority and independence may well be able to address this lack of consumer confidence.

### **Public Analysts' Laboratories**

Public Analysts' Laboratories are situated throughout mainland Britain and are also to be found in the Channel Islands, Isle of Man and Northern Ireland. Overseas Public Analysts Laboratories are found in many countries including Australia, Nigeria and the Bahamas. 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 subject to scrutiny by the courts, industry, other experts and accreditation bodies.

The work of a Public Analyst centres on his laboratory but is not confined to it. The provision of a laboratory with adequate facilities and equipment and quality standards, together with the staff required, to carry out the broad range of work required and likely to be expected is a major undertaking. Over the years mergers, take-overs and closures of laboratories have occurred, in some cases for good business reasons. However the trend in numbers has been progressively downward with access to PAs and their staff becoming ever more

geographically remote. Records show that the number of PA laboratories in 1985 was 42, by 1993 it had dropped to 33 and currently is 31.

### **Food Safety and Composition**

A regular feature of the work of Public Analysts is the analysis and examination of foods pursuant to their statutory responsibilities. This work addresses the safety, composition, and labelling of foods. During the year Public Analysts have reported on:

- a) Unfit and out of date food.
- b) Poultry meats which have been previously frozen and nevertheless sold as fresh.
- c) Minced meats of specified descriptions contaminated with other species for example minced lamb contaminated with beef, minced beef contaminated with pork.
- d) The substitution of branded whiskies by those of a lower malt content and the manufacture of spirit drinks from entirely bogus and dangerous sources including the use of methylated spirit containing the toxic alcohol methanol.
- e) The sale of ham made reformed meat and yet described as "made from selected cuts of pork leg.
- f) The composition of children's confectionery, with special reference to the presence and amount of synthetic colouring agents.
- g) Medicinal claims made on herbal remedies and food.
- h) A high proportion of meat products being deficient in meat.
- i) Added water present in meats and intoxicating drinks.
- j) The substitution of cress, a pungent salad seedling by rape, a seedling of bland taste.
- k) The composition of animal feedingstuffs.

### **Minced Meat**

At the close of 1995 the Association published a review of the fat content of minced beef in the Journal of the Association of Public Analysts (JAPA). This

review preceded the publication of the Minced Meat and Meat Preparations (Hygiene) Regulations 1995, which enacted Council Directive 94/65/EC and laid down a number of compositional and hygiene criteria including compositional standards for the fat content of a variety of named minced meats. These names as described in the regulations are quite specific and so for example minced pure beef and lean minced beef are specific designations for which the compositional and hygiene standards of these regulations apply. Without these specific names the products sold, for example as 100% minced beef, are not considered (in guidance notes to these regulations and published by MAFF) as subject to these compositional standards.

The Association has subsequently recommended in a further article that the fat content of beef minces should be generally limited by a guide level of 20% and an absolute maximum of 25%. Similarly the term lean has in recent years produced a confusion of opinion. Lean meat contains not more than 10% of fat, this being intramuscular and not visible. It is now accepted that the criteria for fat content of all lean minced meats should meet this criterion. The description lean as applied to mince effectively means that lean mince should be made from lean meat, that is meat which has been trimmed free of visible fat - an unsurprising conclusion.

### **Food Labelling**

Food legislation continues to change in line with the implementation of EU directives and regulations, and Government initiatives on deregulation and reregulation and the subject of food labelling has been a part of this development. New Food Labelling Regulations were made in 1996, which continue to implement Directive 79/112/EEC and these have updated and consolidated the previous regulations. Additionally the labelling of foods can be subject to the requirements of other regulations, for example the Meat Products etc. Regulations 1984. These latter are due for review and at issue will be a definition of meat, and the application of popular names, such as Pork Sausage, to products which may well be allowed a lower meat content than is now both statutory and customary.

Foods are appearing on the market which have been subjected to specific treatments. Both the UK Food Labelling Regulations and corresponding EC directive address this aspect of food labelling. For example the directive states the name under which a food is sold shall include particulars as to the physical condition of the foodstuff or specific treatment which it has undergone, where omission of such information could create confusion" (Art. 5 para. 3 of the directive).

However, recent reports that genetically modified soya beans cannot be so labelled because of the manner of harvesting and the deliberate selling of poultry, described as fresh and yet which has been previously frozen, provide examples of food labelling whereby the consumer is denied information with which to make an informed choice. This latter example regularly results in prosecution of this offence.

### **Enforcement of Food Law in the United Kingdom**

The establishment of food law in the United Kingdom is a responsibility divided between several Central Government Ministries, the Ministry of Agriculture Fisheries and Food (MAFF) and the Department of Health (DoH) in England and Wales and the Scottish and Northern Irish Offices as appropriate. The last major revision of food law occurred in the late 1980s and stemmed largely from food contamination incidents, both deliberate and accidental and involved the finding in food and the subsequent media reporting of foreign bodies such as glass, and micro-organisms such as *Listeria* and *Salmonella*. Codes of practice were introduced within the terms of the Food Safety Act 1990 which were concerned with food sampling, administration of the Act, and inspections of manufacturing and food handling premises. These codes addressed food compositional and hygiene standards. In addition two directives from the EU, (89/397/EEC and 93/99/EEC), both concerned with food control, set the framework and mechanism for the official control of food in member states. The status of official food control laboratories, qualifications of scientists and inspectors and exchange of information are included in these specifications for food control. In the United Kingdom local government authorities have a responsibility of enforcing food law. The officials responsible for the sampling and enforcement are placed within trading standards and environmental health departments. The enforcement scientists are identified in food legislation as Public Analysts, Food Analysts and Food Examiners. The appropriate qualification for Public Analysts and Food Analysts is the Mastership in Chemical Analysis, a postgraduate qualification awarded by the Royal Society of Chemistry. When the enforcement procedures which are detailed within the Food Safety Act 1990 are followed it is entirely open for a local authority to prosecute should an infringement of food law occur. The scientific results of Public Analysts and Food Examiners, and their corresponding interpretations of these results are therefore paramount in any judicial procedure.

Veterinary inspection is responsible for admitting animal flesh into the food chain and the licensing of pesticides is controlled by MAFF.



## **Independent Food Safety Body**

The Association has produced a discussion paper on the merits and role of an independent food safety body (see introduction). Interest in the concept of such a body has developed because of the recent incidents of BSE in cattle, contamination of cooked meats by *E. coli* 0157 and contamination of minced meats by other species. In the view of the Association the key roles for this body are:

- a) the inspection of statutory enforcement systems
- b) the duty of explanation of issues of food safety and composition
- c) providing an overview of food and agriculture research carried out in the UK and appropriate advice

The paper recognises that the food law enforcement system in the UK is already independent of central government and the food producing industry including farming and fisheries. Most recently the Government has announced that a Food Safety Council, headed by a Food Safety Advisor can fulfil such a function, this council reporting directly to government ministers. Other authorities have indicated that only a food agency, independent of the Ministries can propagate the necessary confidence in food safety.

## **Meetings and Training**

Brian Taylor, the training officer of the Association, in his first year in office has continued with the programme of meetings and training so essential to the continued development of the members of the APA and indeed the Association itself. Meetings have been held on the subjects of the detection of irradiation foods, and generic accreditation of testing methods. In addition a factory visit to HJ. Heinz Co. Ltd. at Wigan, Lancashire, was arranged. A particular emphasis on canning technology formed a focus for this visit. The European Food Law Association (EFLA) has held two meetings during the year. One on the topic of Single Market- Benefit or Burden? and the other was on the topical theme of 'An Independent Food Agency'.

During the year the training committee produced a third edition of the training guide on legislation. This guide contains details of UK and EU legislation on food, agriculture, water, environment, consumer protection, Medicines and other subjects of interest to PA laboratories. The annual training school of the Association was once again held at the Food Science Department at Reading University. This week long training session is primarily intended for those studying for the MChemA examination. It does however offer considerable

value for all analytical scientists in the content and quality of the subject matter covered.

### **Scientific Affairs**

A series of discussion meetings whereby scientists from the Laboratory of the Government Chemist meet with those from the Association has been initiated. These meetings will provide a valuable forum for the exchange of experience concerning food analysis, food technology, and report and certificate writing. The subjects of beer analysis and the incorporation of water into fish formed the first subjects at this forum.

The anticipated implementation of legislation requiring the 'quantitative declaration of ingredients' of foods has prompted fundamental debate concerning the quantification of fish in fish products such as fish fingers and spreads. Central to the discussion is the amount of water likely to be taken up by fish flesh after the fish has been harvested and before it has been landed at the dock side and subsequently processed. The Ministry (MAFF) has established a working party, drawing membership from representatives of the trade, and both central and local government with the aim of producing a code of practice on the determination of the fish content of manufactured fish products and their labelling.

The Scientific Affairs Committee of the Association, under the leadership of Brian Dredge, has drawn up a first protocol for a generic testing regime. A protocol for high pressure liquid chromatography, HPLC, has been completed and forwarded to the United Kingdom Accreditation Service, UKAS, for comment. The purpose of this generic approach is to enable Public Analysts to continue to cope with the necessary development of analytical science, necessary because so many of the problems presented to laboratories are both novel and unexpected.

In September the Council of the Association agreed the final wording of a policy statement on "Reliability of Analytical Measurement". Norman Michie, the Chairman of the quality assurance subcommittee has embarked on the next stage which will take the form of a detailed guidance note to assist in the calculation and presentation of reliability parameters. It is anticipated that the results of this exercise will lead to an enhanced understanding of analytical measurements and their interpretation.

### **Representation on Outside Bodies**

The Association through the voluntary activity of its members continues to provide extensive representation to a wide range of outside bodies which include LACOTS panels, MAFF Food Authenticity Groups and Food Analysis

Performance Assessment Scheme (FAPAS) Steering Group, The Royal Society of Chemistry, Food Law Enforcement Practitioners (FLEP), Council of European Standards (CEN), the Department of the Environment and the British Standards Institute (BSI).

The Local Government Association (LGA) will during 1997 become the new voice for local government in England and Wales "to promote vigorous, responsive and democratic local leadership", Sir Jeremy Beecham, its chairman has stated. It will be the single voice of more than 400 local authorities and so replace the three local authority Associations. A key objective of this new authority will be to increase the power and responsibilities of local government. The supply of scientific advice to the LGA will be a responsibility of the APA.

### **Annual Conference and Exhibition 1996**

The annual conference and exhibition was held during the year at the Strand Palace Hotel, London. The last occasion that the Association held its annual conference in London was 1981 and it was therefore timely that a London was again the venue. The conference took the theme 'the Interests of the Consumer' Jim Humble OBE, the chief executive of LACOTS explained the necessary quality of local government enforcement and the need for consistency to protect the consumer and fair trader. The corresponding views of the food industry was discussed by Joanna Scott, Head of External Relations at the Food And Drink Federation. These included an outline of the efforts being made by the FDF in the area of consumer education. Chris Leftwich, Chief Inspector at the Fishmongers Company explained the effects of EC fisheries policy and hygiene requirements in processing, especially those associated with farmed fish. John Kneale of the Medicines Control Agency (MCA) presented a paper which dealt with the licensing of medicinal products and herbal remedies. Of particular interest was a discussion on medicinal claims associated with foods and guidelines used by the MCA in order to assess whether a claim was indeed a medicinal claim. Consumer based organisations were represented by Linda Reddy of the Consumers Association and Dr. Helen Wallis of Greenpeace. Linda Reddy discussed the operation procedures of the consumers association and placed the subject of a "Food Agency" before the meeting. Dr. Wallis highlighted the need for a proactive assessment of risks and the public perception of them. The migration of phthalate plasticizers used in polyvinylchloride plastics used as food packaging and the potential for alleged hormone disruption effects were used to illustrate these aspects. The Ministry (MAFF) was represented by Colin Perry of the Veterinary Medicines Directorate and Grant Meekings of the Food Labelling and Standards Division

Professor Eddie Abel, President of the Royal Society of Chemistry was guest of Honour at the Annual Dinner the main dish being based on loin of lamb. At

these occasions it is customary to present the certificates of Mastership in Chemical Analysis to those candidates who have recently been successful in this statutory examination. Mr. John Waller, of the Leicestershire Public Analyst's Laboratory, was the recipient in 1996.

### **The Royal Society of Chemistry**

The Royal Society of Chemistry is the examining body for Public Analysts and all Public Analysts are members of the RSC. Two developments of particular significance that originate from the RSC are in need of recording.

Five years ago discussions were entered into with the National Council for Vocational Qualifications (NCVQ) and the Department of Employment with a view to the RSC running a project to develop a National Vocational Qualification (NVQ) at level 5 for analytical chemistry. This project, which was lead by Peter Cobb of the RSC, took evidence from professional analytical chemists in the fields of industrial chemistry, forensic science, water chemistry, pharmaceutical chemistry and public analysis and resulted in four key roles for analytical chemists being identified, namely interfacing with clients and other professions, sampling, analysis, and management. These were expressed as units of competence and have been published by the RSC. The report recognises that at level five for example a professional analytical scientist must not only be able to select an analytical technique and method but should be also able to extend or develop an analytical method.

The other significant development was the move of the Laboratory of the Government Chemist into the private sector as an independent non profit distributing company. The RSC has taken an equity stake in this new company. During the discussions on this privatisation it was agreed that the independence and integrity of the LGC should be preserved and the involvement of the RSC, through its stake, would provide a positive role in ensuring these, the prime interest being the maintenance of a source of scientific excellence to the benefit all members of the RSC.

### **Environmental Matters**

In last year's annual report, the role and development of the Standing Committee of Analysts which was set up by the Department of the Environment was described. Peter Holroyd, of Glasgow City Council, has been recently appointed as the representative of both the RSC and the APA on the main committee.

Proposals for the revision of Council Directive 80/777/EEC which relates to Natural Mineral Waters have included one which would allow ozone rich air to be used for the removal of iron, manganese, sulphur compounds and arsenic

“provided that this treatment does not alter the essential constituents”. What this means and how it will be enforced is not dear although conditions are to be laid down in this respect and the treatment will have to be stated on the label. Additionally “Spring Water” which previously has not been considered as a natural mineral water for the purpose of compositional and hygiene standards will be recognised in this revision as a water which will need to meet the standards of the above directive.

### **Conclusion**

This annual report for 1996 highlights some of the science based activities of Public Analysts and the need to keep abreast of developments analytical science and food technology and the law associated with these. It has a bias this year towards food. topics. In common with other years Public Analysts have continued to be active in the other fields of consumer protection (e.g. toys, cosmetic products), occupational hygiene, and environmental matters.

In all these activities the Public Analyst offers advice that is independent and without conflict of interest.

## **Annual statistics for the Public Analyst service 1996**

*Presented at the AGM on 19th April 1997 by Paul Lenartowicz, Public Relations Officer*

### **Introduction**

The following report presents data related to samples submitted to Public Analysts' laboratories by local authorities during the calendar year 1996.

As usual 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 result in a virtual research project in order to certify with confidence that they are or are not satisfactory - a difference that is rarely evident if the sample is viewed as a simple commodity. The recognition of need and matching of analytical response and interpretation with attendant quality assurance, always to a standard acceptable to a Court of Law, is a primary aspect of the work of Public Analysts.

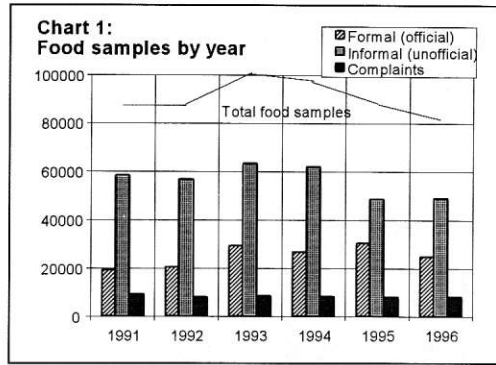
### **Data**

Data was returned by all but four member Laboratories in the United Kingdom, data having been calculated for the full population of the UK by interpolation. Detailed figures broken down by sample type appear in the Appendix.

The geographical split between Wales and England for the charted statistics has been complicated by the laboratory serving the northernmost Welsh counties also serving a number of English authorities. For this reason complete separation of statistics relating to Wales has not been possible and any references to England or Wales in the following discussion must be viewed accordingly. Data received from the Isle of Man and the two Channel Islands' laboratories has been recorded for comparative purposes, but has not been included in calculations of United Kingdom statistics.

### **Food Work**

Chart 1 shows the rates of submission of food samples for analysis in 1996 compared with the previous five years. It is clear from the chart that total food sampling levels have continued to drop steadily since their peak in 1993, with the total numbers in 1996 falling below any of the past five years. Most of the fall has been in formal samples, with informals and complaints remaining steady. The total decrease from 1995 to 1996 was 7%

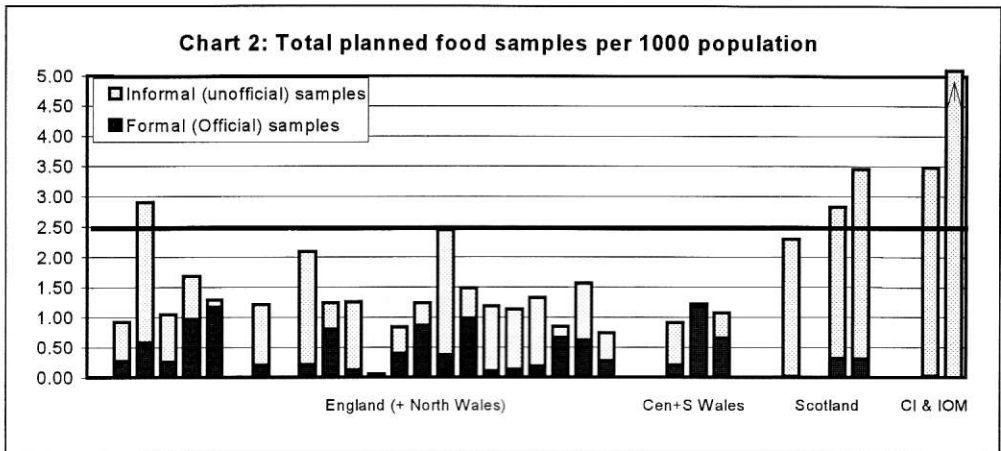


The mean number of planned food samples for analysis per 1000 population in the UK in 1996 was 1.31, against 1.47 in 1995, 1.59 in 1994 and 1.63 in 1993. (Total foods including complaints were 1.46, 1.62, 1.74 and 1.81 respectively for 1995, 1994 and 1993.) These averages, however, mask a great variation between individual authorities. Chart 2 illustrates the spread of planned samples on a laboratory-by-laboratory basis.

Some years ago the World Health Organisation recommended a minimum sampling level of 2.5 samples per 1000 population, a figure that has been endorsed by the Ministry of Agriculture Fisheries and Food and by the Local Authorities Co-ordinating body on Trading Standards (LACOTS). Taking the areas served by individual laboratories (which often include a number of local authorities), only three areas in the United Kingdom achieved levels in excess of this minimum in 1996 (compared with six areas in 1995).

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 for planned sampling include "informal" samples, which 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 notable that the areas sampling at the highest levels tend to be those with a relatively low proportion of formal samples. Chart 2 also indicates the relative proportions of official and unofficial samples.





### Unsatisfactory Food Samples

The rate of finding samples that contravene aspects of food legislation remain consistently high at an average of 20% of routine food samples. In addition 52% of food complaints were upheld. These figures are very similar to the previous year (21% and 55% respectively).

As in 1995 a little over half of the unsatisfactory samples (12%) failed to comply with labelling requirements. Despite the temptation sometimes to dismiss these as 'mere labelling errors', to do so would be a grave error of judgement as although a few may have been relatively minor infringements this category includes far more serious problems such as fraudulent claims, misleading or missing information (which in certain instances could prove hazardous to health, for example failure to declare ingredients to which a consumer might be allergic), failure to indicate the minimum durability (again with potentially dangerous results), in addition to failure to make correct statutory declarations and so on. The majority of labelling faults can only be detected by detailed analysis, and are not evident solely from a scrutiny of the label.

### Food Factory Inspection

The use of the Public Analyst in factory inspections continues to remain restricted to a few authorities despite his potential value as an expert to assess sampling and analytical records as well as other technical aspects of food production. One laboratory accounts for well over three quarters of the time spent on factory inspection and over half of the Association's laboratories had no involvement at all.

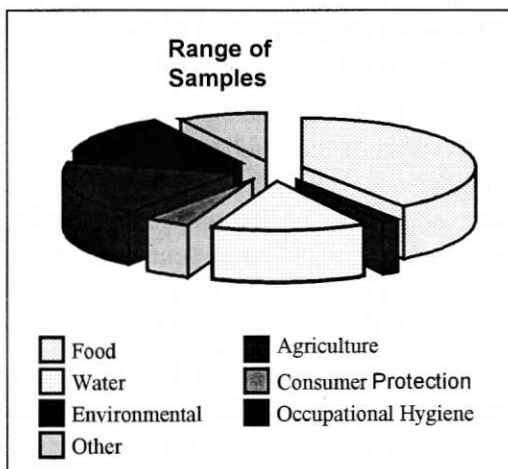


## Non-food Work

Many Public Analysts act as scientific advisers to the authorities they serve. This includes the provision of an emergency response service in case of incidents involving chemicals, whereby scientific expertise may be made available at any time to attend such incidents and provide advice as appropriate. In 1996 there were 227 such emergency call-outs, (ranging from fires to chemical spillages), on average equating to one call somewhere in the UK every 38 hours.

The major areas other than food in which Public Analysts' laboratories provided analysis to local authorities during 1996 are as follows, in decreasing order of numbers of samples:

- Water analysis (Mainly potable waters, but also swimming pools, etc.)
- Environmental investigations (soil, water, tip leachates, atmospheric samples etc.)
- Occupational Health (Asbestos identification and air testing, COSHH monitoring, etc.)
- Consumer Products (Toys, cosmetics, household chemicals, etc. for safety and to assess Trade Descriptions)
- Agricultural samples (Fertilisers and Animal Feeds)
- Other samples (Radiation monitoring, toxicology etc.)



## Proficiency testing schemes

All Public Analysts' laboratories participate in the Ministry of Agriculture, Fisheries and Food's *Food Analysis Performance Assessment Scheme*, FAPAS. Two laboratories participated in the full range of 14 different series offered in 1996, the average rate of participation being 9 series.

This scheme at full participation effectively involves up to one circulation of a foodstuff every seven working days. The 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 "real" samples for similar analysis these procedures still have to be carried out in full, imposing a substantial

burden of work for no return. The analytical cost for participation in the full range of FAPAS has been estimated to be up to £40,000 per annum.

The observations apply to 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., although none have the same vast scope applicable to food analysis.

### **Observations**

At a time of increasing public concern over food it is most worrying to record that rate of sampling food for analysis has decreased further since last year to an average of only 1.3 samples per 1000 head of population, just over half of the minimum rate of 2.5 recommended by the World Health Organisation. The reduction in sampling is of particular concern when set against the backdrop of a constant level of one fifth of foods analysed being found to be unsatisfactory.

The high rate of unsatisfactory samples detected continues to demonstrate the effectiveness of the Public Analyst service in applying quality analysis backed by solid science, skill, experience, development and professional co-operation. However, it must be recognised that the long term ability to maintain an effective service is dependant upon continued investment in both the seen and the unseen aspects of public protection science, which may be irreparably damaged by diversion or dilution of limited resources for short term gain.

The Public Analyst service is a fundamental part of the food law enforcement system and is entirely complementary to the other arms of that system. The proper provision of an efficient, competent, flexible and ever-current scientific capability requires adequate commitment of resources, both in terms of amount and stability.

The current trend in some quarters to view Public Analysts as mere providers of analysis on demand to be used or disused on a whim or as the sacrificial lamb when local authority budgets are constrained clearly indicates a misunderstanding of the whole basis of enforcement science and is detrimental not only to the current provision of the service but also to the long term ability to be effective. It is hoped that any review of the food law enforcement will address the issues raised in this and previous reports of the Association.

## APPENDIX

Data returned by laboratories for local authority work, 1996

	UK corrected for population	Isles
Population (millions)	56	0.2
Foods - all formal	24699	17
Foods - all informal	49009	1088
Foods - Complaints	8290	100
Foods - Bacteriological	5483	0
Potable waters (other than bottled)	27046	5312
Swimming Pool Waters	4962	59
Pollution water, effluents, tip leachates	9892	5023
Soils	2234	125
Workplace monitoring (excl. asbestos)	1747	0
Asbestos (bulk & airborne)	26053	184
Atmospheric samples	28531	3290
Feeding Stuffs	2888	0
Fertilisers	466	0
Toys (Safety) Regulations	4260	9
Cosmetics (Safety) Regulations	1176	2
Other Consumer Protection Act work	2870	0
Trade Descriptions samples	898	10
Radiation Monitoring	4854	525
Coroners/Toxicology	4269	57
Building Materials	350	1628
All other	8578	15362
<b>TOTAL SAMPLES</b>	<b>218555</b>	<b>32791</b>
Total food samples for analysis	81998	1205
Total foods bacteriological	5483	0
Total waters excl. environmental	32008	5371
Total Agriculture Act	3354	0
Total Consumer Protection etc (incl. TDA)	9204	21
Total environmental samples	40657	8438
Total workplace samples	27800	184
Total other samples	18051	17572