REPORT NO. 497

Pathogens In Food Proficiency Testing Program - Round 13

January 2006

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided by Ms Anne Gerry, Mrs Ingrid Flemming, IFM Quality Services Pty Ltd, who supplied and distributed the samples and Mrs Glenda Arnold, NATA Biological Accreditation Advisory Committee member.

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APPENDICES

1. FOREWORD

This report summarises the results of round thirteen of a series of proficiency testing programs involving the analysis of different food types for the detection of a range of pathogens.

NATA's Proficiency Testing Group conducted the exercise in October/November 2005. Note that from 1 January 2006 the delivery of proficiency testing services was transferred from NATA to a new, wholly owned subsidiary called Proficiency Testing Australia (PTA). The aim of the program was to assess laboratories' ability to competently perform the nominated tests.

2. FEATURES OF THE PROGRAM

(a) A total of 51 laboratories received samples, 50 of which returned results.

(b) The results reported by participants are presented in Appendix A.

(c) Laboratories were provided with five samples of flavoured pudding mix, "Instructions to Participants" and a "Results Sheet" (see Appendix D).

(d) NATA accredited laboratories were requested to perform the tests according to their routine methods, with the Australian Standard method being preferred.

(e) Each laboratory was randomly allocated a unique code number for the program to ensure confidentiality of results. Reference to each laboratory in this report is by its code number.

Please note that some laboratories reported more than one set of results and therefore one code number (with letter) could appear several times in the same data set.

3. FORMAT OF THE APPENDICES

APPENDIX A
Appendix A contains the results reported by participating laboratories for each of the five samples.

APPENDIX B
Appendix B contains a summary of the methods used by each laboratory.

APPENDIX C
Appendix C contains the results of the homogeneity and stability testing.

APPENDIX D
Appendix D contains the "Instructions to Participants" and pro-forma "Results Sheet".

4. DESIGN OF THE PROGRAM

Participants were asked to determine the presence of *Salmonella* and *Listeria* in five samples of flavoured pudding mix.

Each laboratory was provided with five samples labelled A, B, C, D and E and was requested to test 5 gram of each sample for each analysis.

- Samples A and D were duplicates. They contained *Salmonella* Adelaide and *Listeria innocua*, together with the natural flora in the samples. The levels of *Salmonella* Adelaide and *Listeria innocua* in the samples were approximately 20-30 cfu/g each.

- Sample B contained *Salmonella* Bredeney plus the natural flora in the sample. The level of *Salmonella* Bredeney in the sample was approximately 50-100 cfu/g.

- Samples C and E were duplicates. They contained *Listeria monocytogenes* and *Citrobacter freundii*, plus the natural flora of the samples. The levels of *Listeria monocytogenes* and *Citrobacter freundii* in the samples were approximately 50-100 cfu/g each.

5. HOMOGENEITY AND STABILITY TESTING

Prior to sample distribution, ten randomly selected samples from each matrix (A/D, B and C/E) were analysed for homogeneity by IFM Quality Services Pty Ltd. Based on the results of this testing, the homogeneity of samples B, C and E was established. Samples A and D were found to be homogeneous for *Salmonella*, but homogeneity could not be shown for samples A and D for *Listeria*.

Stability testing was performed on the samples by IFM Quality Services Pty Ltd seven days after sample distribution. The results showed that samples B, C and E were sufficiently stable for testing during this interval. Samples A and D were found to be stable for *Salmonella*, but stability could not be established for these samples for *Listeria*.

As a result of the homogeneity and stability testing, analysis of the results submitted by participants for samples A and D for *Listeria* was not performed for this round of the program. For more information on the homogeneity and stability testing, see Appendix C.

6. FALSE RESULTS

Testing methods were pooled and results examined for laboratories reporting false positives and false negatives. The false positive and false negative results for this round of the program are summarised in the following table.

TABLE A: FALSE RESULTS
(by laboratory code number)

Presence / Absence of *Salmonella* in Flavoured Pudding Mix		
Sample	False Positives	False Negatives
A		-
B		-
C	14, 35, 44, 48	
D		35
E	35, 36	
Presence / Absence of *Listeria* in Flavoured Pudding Mix *		
Sample	False Positives	False Negatives
A		
B	-	
C		-
D		
E		-

* Due to concerns about homogeneity, the determination of false results submitted by participants for samples A and D for *Listeria* was not conducted.

7. TECHNICAL COMMENTS

Response Rate

Of the 51 laboratories that participated in the program, 50 (98%) submitted results for inclusion in the final report. Forty nine of the 51 participants (96%) submitted results for *Salmonella*, while 44 of the 51 participants (86%) returned results for *Listeria*. Two laboratories (7 and 23) submitted more than one set of results.

Failure Rate

Of the 49 participants that submitted results for *Salmonella*, five submitted incorrect results. There were six false positive results for samples C and E, which were duplicate samples. These samples did not contain *Salmonella* but did contain *Citrobacter freundii* at a level of approximately 50-100 cfu/gram. Laboratories 14, 44 and 48 obtained false positive results for sample C only. Laboratory 36 obtained a false positive result for sample E only. Laboratory 35 obtained false positive results for both sample C and sample E. Participants reporting false positive results should review their procedures, in particular confirmation procedures.

Laboratory 35 also reported a false negative result for sample D, which contained *Salmonella* Adelaide at a level of approximately 20-30 cfu/gram.

The failure rates for the five different samples for *Salmonella* are calculated in Appendix A. The overall failure rate for *Salmonella* is also calculated in Appendix A. Here it was found that sample C had the highest failure rate of all the samples for *Salmonella* (7.8%), followed by sample E (3.9%) and sample D (2.0%). There were no false results reported for samples A and B. The overall failure rate for *Salmonella* was found to be 2.7%.

Of the 44 participants that submitted results for *Listeria*, there were no incorrect results submitted for samples B, C and E. Samples B, C and E were the only samples where failure rates were calculated for *Listeria*, due to doubts about the homogeneity of samples A and D (see Appendix C). Indeed, only 29 of the 46 (63%) *Listeria* results submitted for sample A indicated that *Listeria* was detected in the sample, while only 30 of the 46 (65%) *Listeria* results submitted for sample D indicated that *Listeria* was detected in the sample. The failure rates for the three samples analysed for *Listeria* (B, C and E) are calculated in Appendix A. The overall failure rate for *Listeria* is also calculated in Appendix A.

Method Commentary

For *Salmonella,* the majority of participants reported using cultural methods. Of these 30 participants used the Australian Standard method AS1766 for *Salmonella* isolation, however almost half of these used a modification. Alternative cultural methods included M-broth for selective enrichment and plating using Hektoen, desoxycholate agar, brilliant green and chromogenic agars. Nine participants used the AS5013 method. Ten participants used immunoassay methods. Three laboratories used PCR. It should be noted that some participants used more than one method for isolation. A considerable range of confirmatory tests was reported by participants in this round. From the results reported, there were no noticeable differences between the methods used for *Salmonella.*

Detailed method information for *Salmonella* is provided in Appendix B1.

For *Listeria* testing the majority of participants used the cultural methods of either the food or dairy Australian Standard methods. Enrichment media used include *Listeria* enrichment broth, buffered *Listeria* enrichment broth, half Fraser broth, UVM and full strength Fraser broth. Most participants used Oxford agar for plating, with the majority of these using Palcam agar in addition. Fourteen participants used enzyme immunoassay methods. Six participants reported using PCR. Again some labs have used more than one method for detection. From the results reported, there were no noticeable differences between the methods used for *Listeria*.

Detailed method information for *Listeria* is provided in Appendix B2.

Instructions to Participants for this round requested that where a laboratory used two different methods for either *Salmonella* or *Listeria* testing, that results be reported separately for each method. However, in a majority of cases where more than one method was used only a single result was reported.

Speciation

Salmonella serotyping was not required for this round.

Thirty seven participants performed speciation of the *Listeria* for samples C and E. All of these participants correctly identified *L. monocytogenes* in the samples.

Twenty three of the participants that detected *Listeria* in sample A performed speciation. Eighteen of these correctly reported the presence of *L. innocua* in the sample. Another two reported "not *L. monocytogenes*" for the sample. One participant reported either *L. innocua* or *L. grayii*, subsp. *murrayii* in the sample, while another reported either *L. innocua* or *L. monocytogenes* in the sample. One laboratory incorrectly identified the isolate in sample A as *L. monocytogenes*.

Twenty five of the participants that detected *Listeria* in sample D performed speciation. Twenty three of these correctly reported the presence of *L. innocua* in the sample. The other two reported "not *L. monocytogenes*" for the sample.

Overall Laboratory Performance

The performance of the participants in this round of the Pathogens in Food Program was quite good for *Salmonella*. The overall failure rate for *Salmonella* of 2.7% is slightly higher than the 2.0% failure rate achieved in Round 11, but is better than the 3.6% failure rate achieved in Round 8 for *Salmonella* (see PTAC Report 398 and Report 459 for more details).

Since the determination of false results in this round of the Pathogens in Food Program for *Listeria* was only conducted for samples B, C and E, the performance of the participants for *Listeria* cannot be reliably compared with the performance of participants for *Listeria* in previous rounds. Nonetheless, it is very encouraging that no false results were reported by participants for samples B, C and E.

In comparing the failure rates in different rounds of the program, the levels of test organisms should also be taken into consideration. This information is available in the report issued for each round.

8. REFERENCE

Guide to NATA Proficiency Testing (2004).

This document can be found on the NATA website at http://www.nata.asn.au

APPENDIX A

Summary of Results

Section A1

Salmonella

A1.1

Salmonella Results

Lab Code	A	B	C	D	E	False Results
1	Present	Present	Absent	Present	Absent	
2	Present	Present	Absent	Present	Absent	
3	Present	Present	Absent	Present	Absent	
4	Present	Present	Absent	Present	Absent	
5	Present	Present	Absent	Present	Absent	
6	Present	Present	Absent	Present	Absent	
7A	Present	Present	Absent	Present	Absent	
7B	Present	Present	Absent	Present	Absent	
8	Present	Present	Absent	Present	Absent	
9	Present	Present	Absent	Present	Absent	
10	Present	Present	Absent	Present	Absent	
11	Present	Present	Absent	Present	Absent	
12	Present	Present	Absent	Present	Absent	
13	Present	Present	Absent	Present	Absent	
14	Present	Present	**Present**	Present	Absent	**1**
15	Present	Present	Absent	Present	Absent	
16	Present	Present	Absent	Present	Absent	
17	Present	Present	Absent	Present	Absent	
19	Present	Present	Absent	Present	Absent	
21	Present	Present	Absent	Present	Absent	
22	Present	Present	Absent	Present	Absent	
23A	Present	Present	Absent	Present	Absent	
23B	Present	Present	Absent	Present	Absent	
24	Present	Present	Absent	Present	Absent	
25	Present	Present	Absent	Present	Absent	
26	Present	Present	Absent	Present	Absent	
27	Present	Present	Absent	Present	Absent	
28	Present	Present	Absent	Present	Absent	
29	Present	Present	Absent	Present	Absent	
32	Present	Present	Absent	Present	Absent	
33	Present	Present	Absent	Present	Absent	
34	Present	Present	Absent	Present	Absent	
35	Present	Present	**Present**	**Absent**	**Present**	**3**
36	Present	Present	Absent	Present	**Present**	**1**
37	Present	Present	Absent	Present	Absent	
38	Present	Present	Absent	Present	Absent	
39	Present	Present	Absent	Present	Absent	
40	Present	Present	Absent	Present	Absent	
42	Present	Present	Absent	Present	Absent	
43	Present	Present	Absent	Present	Absent	
44	Present	Present	**Present**	Present	Absent	**1**
45	Present	Present	Absent	Present	Absent	
46	Present	Present	Absent	Present	Absent	
47	Present	Present	Absent	Present	Absent	
48	Present	Present	**Present**	Present	Absent	**1**
49	Present	Present	Absent	Present	Absent	
50	Present	Present	Absent	Present	Absent	

A1.2

Salmonella Results

Lab Code	A	B	C	D	E	False Results
51	Present	Present	Absent	Present	Absent	
52	Present	Present	Absent	Present	Absent	
53	Present	Present	Absent	Present	Absent	
54	Present	Present	Absent	Present	Absent	

Note:

A highlighted result (*i.e.* bold print) is a false result and should be investigated.

A1.3

Salmonella Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	4	1	2	7
Total Results	51	51	51	51	51	255

Failure rate (Sample A) = $\frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 0 / 51
= 0%

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 51
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 4 / 51
= 7.8%

Failure rate (Sample D) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 1 / 51
= 2.0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 2 / 51
= 3.9%

Overall failure rate (*Salmonella)* = $\frac{\text{Total No of False Results}}{\text{Total No of Results}}$
= 7 / 255
= 2.7%

Section A2

Listeria

A2.1

Listeria Results

Lab Code	A	B	C	D	E	False Results
1	Absent	Absent	Present	Present	Present	
2	Absent	Absent	Present	Absent	Present	
3	Present	Absent	Present	Present	Present	
4	Present	Absent	Present	Present	Present	
6	Absent	Absent	Present	Absent	Present	
7A	Present	Absent	Present	Present	Present	
7B	Present	Absent	Present	Present	Present	
8	Absent	Absent	Present	Absent	Present	
9	Present	Absent	Present	Present	Present	
10	Present	Absent	Present	Absent	Present	
11	Present	Absent	Present	Absent	Present	
12	Absent	Absent	Present	Absent	Present	
14	Present	Absent	Present	Present	Present	
16	Absent	Absent	Present	Present	Present	
17	Present	Absent	Present	Present	Present	
19	Present	Absent	Present	Absent	Present	
21	Absent	Absent	Present	Present	Present	
22	Present	Absent	Present	Present	Present	
23A	Present	Absent	Present	Present	Present	
23B	Present	Absent	Present	Present	Present	
24	Present	Absent	Present	Present	Present	
25	Absent	Absent	Present	Present	Present	
26	Present	Absent	Present	Absent	Present	
27	Present	Absent	Present	Present	Present	
28	Present	Absent	Present	Present	Present	
29	Present	Absent	Present	Present	Present	
32	Absent	Absent	Present	Present	Present	
33	Present	Absent	Present	Present	Present	
34	Present	Absent	Present	Present	Present	
35	Absent	Absent	Present	Present	Present	
36	Absent	Absent	Present	Absent	Present	
37	Absent	Absent	Present	Absent	Present	
38	Present	Absent	Present	Present	Present	
39	Absent	Absent	Present	Present	Present	
40	Present	Absent	Present	Absent	Present	
41	Present	Absent	Present	Present	Present	
42	Absent	Absent	Present	Absent	Present	
43	Present	Absent	Present	Absent	Present	
45	Absent	Absent	Present	Present	Present	
47	Absent	Absent	Present	Absent	Present	
48	Present	Absent	Present	Present	Present	
50	Present	Absent	Present	Present	Present	
51	Absent	Absent	Present	Absent	Present	
52	Present	Absent	Present	Absent	Present	
53	Present	Absent	Present	Present	Present	
54	Present	Absent	Present	Present	Present	

Note:

Samples A and D contained *Listeria innocua*. Due to concerns about the homogeneity and stability of these samples for *Listeria*, “absent” results for *Listeria* for samples A and D have not been considered to be false negative results.

A2.3

Listeria Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results		0	0		0	0
Total Results		46	46		46	138

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 46
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 0 / 46
= 0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 0 / 46
= 0%

Overall failure rate (*Listeria)* = $\frac{\text{Total No of False Results}}{\text{Total No of Results}}$
= 0 / 138
= 0%

Note:

Due to concerns about the homogeneity of samples A and D for *Listeria*, failure rates for these samples have not been calculated.

Listeria Speciation

Lab Code	A	C	D	E
1	-	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
2	-	*L. monocytogenes*	-	*L. monocytogenes*
3	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
4	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
6	-	*L. monocytogenes*	-	*L. monocytogenes*
7A	Not *L. monocytogenes*	*L. monocytogenes*	Not *L. monocytogenes*	*L. monocytogenes*
7B	Not *L. monocytogenes*	*L. monocytogenes*	Not *L. monocytogenes*	*L. monocytogenes*
8	-	*L. monocytogenes*	-	*L. monocytogenes*
9	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
10	*L. monocytogenes/ L. innocua*	*L. monocytogenes*	-	*L. monocytogenes*
11	*L. innocua/ L. grayii* subsp. *murrayii*	*L. monocytogenes*	Not done	*L. monocytogenes*
12	Not done	Not done	Not done	Not done
14	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
16	-	-	-	-
17	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
19	Not done	Not done	Not done	Not done
21	Not done	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
22	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
23A	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
23B	*Listeria* spp	*L. monocytogenes*	*Listeria* spp	*L. monocytogenes*
24	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
25	Not done	Not done	Not done	Not done
26	*L. monocytogenes*	*L. monocytogenes*	-	*L. monocytogenes*
27	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
28	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
29	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
32	-	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
33	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
34	Not done	*L. monocytogenes*	Not done	*L. monocytogenes*
35	Not done	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
36	-	-	-	-
37	Not done	*L. monocytogenes*	Not done	*L. monocytogenes*
38	Not *L. monocytogenes*	*L. monocytogenes*	Not *L. monocytogenes*	*L. monocytogenes*
39	-	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
40	*L. innocua*	*L. monocytogenes*	-	*L. monocytogenes*
41	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
42	-	*L. monocytogenes*	-	*L. monocytogenes*
43	Not done	*L. monocytogenes*	Not done	*L. monocytogenes*
45	-	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
47	Not done	Not done	Not done	Not done

Listeria Speciation

Lab Code	A	C	D	E
48	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
50	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
51	Not done	Not done	Not done	Not done
52	Not done	*L. monocytogenes*	Not done	*L. monocytogenes*
53	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*
54	*L. innocua*	*L. monocytogenes*	*L. innocua*	*L. monocytogenes*

APPENDIX B

Summary of Methods

SECTION B1

Salmonella

Lab Code	***Salmonella* – Media**					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
1	Buffered peptone water	MKTTn	RVS broth	XLD, BSA	SPCA	Plate onto non-selective agar, O & H serotyping, 12A (Oxoid) ID kit, oxidase
2	Buffered peptone water	MSC broth	RVS broth	XLD	BGA	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, O serotyping, commercial ID kit
3	Buffered peptone water	MSC broth, RVS broth	M-broth	XLD, BSA	CLED	API 20E ID kit
4	Buffered peptone water	MSC broth	RV broth	XLD	BSA	Plate onto non-selective agar, L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping
5	Buffered peptone water	MKTTn	RVS broth	XLD	Chromogenic *Salmonella* (Oxoid)	Plate onto non-selective agar, TSI, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, Oxoid latex ID kit (H antigen only), Microbact 12A
6	Buffered peptone water	MKTT	RVS broth	XLD	BSA	Plate onto non-selective agar, L-lysine/LDC, B-galactosidase/ONPG, Oxoid Sal test kit (latex)
7A	Buffered peptone water	Not done	Not done	BAX PCR		Not done
7B	Buffered peptone water	RV broth	MSC broth	XLD	BSA	O & H serotyping, Glissuda slope
8	Buffered peptone water	RVS broth, MKTTn	M-broth	XLD, VIDAS SLM	BSA	O, Vi & H serotyping, API 20 ID kit
9	Buffered peptone water	MSC broth	RVS broth	XLD, BSA, DCA	XLD, BSA, DCA	Urea, L-lysine/LDC, O & H serotyping
10	Buffered peptone water	Tecra Immunocapture	Tecra Elisa VIA	XLD, MLCB	BSA	Plate onto non-selective agar, TYA, CLED, API 20E, gram stain, oxidase
11	Buffered peptone water, Elisa	MSC broth	RVS broth, M-broth for Elisa	XLD	BSA	L-lysine/LDC, B-galactosidase/ONPG, indole, O & H serotyping
13	Buffered peptone water	MSC broth	RVS broth	XLD	Brilliant Green Agar	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG
14	Buffered peptone water	MKTTn	RVS broth	XLD	Chromogenic *Salmonella* Agar	Plate onto non-selective agar, TSI, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, O & H serotyping, Denkia Seiken ID kit, Microbact 12A & OBIS *Salmonella*
15	Modified buffered peptone water	Not done	Not done	Tecra Unique *Salmonella*	XLD, Brilliant Green Agar with Sulphamandelate	L-lysine/LDC, B-galactosidase/ONPG, O serotyping, oxidase
16	Buffered peptone water	M-broth	Not done	XLD, BSA		Plate onto non-selective agar, commercial ID kit
17	Buffered peptone water	MSC broth	RVS broth	XLD	BGA	Microbact 24E ID kit

Lab Code	*Salmonella* – Media					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
19	Buffered peptone water	MSC broth	RVS broth	XLD	BGS	Plate onto non-selective agar, L-lysine/LDC, B-galactosidase/ONPG, O serotyping
21	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG, indole, O & H serotyping, API 20E ID kit
22	Buffered peptone water	MSC broth	RV broth	XLD	BSA	TSI, urea, L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping
23A	Buffered peptone water	MSC broth	RV broth	XLD	BSA	Plate onto non-selective agar, L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping, biochemical: DSSIU
23B	Buffered peptone water	MSC broth, RVS broth				
24	Buffered peptone water	RVS broth		Tecra		Plate onto selective agar XLD & chromogenic *Salmonella* agar, Microbact 12E
25	Buffered peptone water	Not done	Not done	A/BAX PCR	Not done	Not done
26	LB	RV (Bio)	M-broth	XLD	Not done	Plate onto non-selective agar, B-galactosidase/ONPG, Microbact 24E (Oxoid) ID kit
27	Buffered peptone water	MKTTn	RVS broth	XLD	BSA	Urea, L-lysine/LDC, B-galactosidase/ONPG, Microbact ID kit, serotyping
28	Buffered peptone water	MSC broth	RV broth	XLD	BSA	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, O & H serotyping, Microbact 12A ID kit
29	Buffered peptone water	RV broth	MSC broth	BSA	XLD	Plate onto non-selective agar, L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping
32.1	Buffered peptone water			MSRV		Plate onto non-selective agar, Microbact 24E ID kit (12A & 12B), *Salmonella* latex kit
32.2	Buffered peptone water	RV broth	M-broth		A: XLD, B: BSA	Plate onto non-selective agar, Microbact 24E ID kit (12A & 12B), *Salmonella* latex kit
32.3	Buffered peptone water	A: MSC broth, B: RVS broth		A: XLD, B: BSA		Plate onto non-selective agar, Microbact 24E ID kit (12A & 12B), *Salmonella* latex kit
33	Buffered peptone water	RVS broth	M-broth	VIDAS then plated onto XLD, SMID2		Plate onto non-selective agar, API 20E
34.1	Buffered peptone water	MKTTn	RVS broth	XLD	BSA	Plate onto non-selective agar, O & H serotyping, API 20E
34.2	Buffered peptone water	MSC broth	RV medium	XLD	BSA	Plate onto non-selective agar, O & H serotyping, API 20E

Lab Code	*Salmonella* – Media					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
35	Buffered peptone water	RVS broth	MKTTn	XLD	Hektoen	Plate onto non-selective agar, O & H serotyping, SM1D2
36	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	Plate onto non-selective agar, TSI, L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping
37	Buffered peptone water	MSC broth	RVS broth	VIDAS SLM, suspects plated onto XLD	VIDAS SLM, suspects plated onto BSA	Plate onto non-selective agar, API 20E ID kit
38	Lactose broth	RVS broth	M-broth	Tecra Elisa	Not done	Not done
39	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, O & H serotyping
40	Buffered peptone water	RVS broth	SEL	XLD	BSA	L-lysine/LDC, B-galactosidase/ONPG, O & H serotyping, Microbact 12A Oxoid ID kit
42	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	Plate onto non-selective agar, urea, L-lysine/LDC, B-galactosidase/ONPG, VP, indole, oxidase test, O & H serotyping, Microbact 24E (biochemical testing)
43	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	H serotyping, Microbact ID kit
44	Buffered peptone water	Modified Rappaport Vassiliadis medium	Modified Lysine Deoxycholate medium	Modified Lysine Iron Cystine Neutral Red medium	Modified Brilliant Green medium	Plate onto non-selective agar, Oxoid *Salmonella* Latex ID kit
45	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	urea, L-lysine/LDC, B-galactosidase/ONPG, indole, O & H serotyping
46	Buffered peptone water	Tecra Unique for *Salmonella*	Tecra Unique for *Salmonella*	Tecra Unique for *Salmonella*	Tecra Unique for *Salmonella*	Not done
47	Buffered peptone water	MSC broth, RV broth	M-broth	Not done	Not done	Not done
48	Buffered peptone water	MSC broth	RVS broth	XLD	BSA	O & H serotyping, Glissuda
49	Buffered peptone water	RVS broth	MKTTn	XLD	SCA, MLCB	Plate onto non-selective agar, O & H serotyping, API 20Eyy
50	Buffered peptone water	MSC broth	RVS broth	Lysine Mannitol Glycerol	Brilliant Green Agar	Serobact ID kit, Microbact 24E
51	Buffered peptone water	Not done	Not done	VIDAS ICS (ICS broth)		Not done
52	Buffered peptone water	RVS broth	M-broth	XLD, VIDAS *Salmonella* (Biomerieux)	BSA, BAX (PCR) *Salmonella*	Plate onto non-selective agar, O, Vi & H serotyping, Microbact (Oxoid) ID kit

Lab Code	*Salmonella* – **Media**					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
53	Buffered peptone water	MSC broth, M-broth → VIDAS SLM	RV broth, M-broth → VIDAS SLM	XLD	Hektoen Enteric Agar	Plate onto non-selective agar, API 10S
54	Buffered peptone water	RVS broth	MKTTn	XLD	SM2	Plate onto non-selective agar, O, Vi & H serotyping, API 20E (biochemical)

Abbreviations: BGA, brilliant green agar; BHI, brain-heart infusion broth; BSA, bismuth sulfite agar; CLED, cystine lactose electrolyte deficient agar; DCA, desoxycholate agar; LDC, lysine decarboxylase; MSC, mannitol selenite cystine; MSRV, mannitol selenite/ Rappaport-Vassiliadis; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; RV, Rappaport Vassiliadis; TSI, triple sugar iron agar; XLD, xylose lysine desoxycholate agar.

Lab Code	*Salmonella* – Incubation Temperature (°C)						*Salmonella* – Duration of Incubation (hours)					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
1	37	37	41.5±1	37	37	37	18	24	24	XLD: 24, BSA:48	24	24
2		37	42	37	37			24	24	24	24	
3	37	37, 42	42	37	37	37	24	18	24	24, 48	18	18
4	37	37	42	37	37	37	24	24	24	18, 24	24	18, 24
5	35	35	42	35	35	35	18	24	24	24	48	24
6	37	37	42	37	37	37	18	24	24	24	24,48	24
7A	37						18					
7B	37	42	37	37	37	37	18	18	18	24	48	24
8	37	MKTTn: 37, RVS: 42	42	XLD: 37	BSA: 37	37	18	6.8 then 12	18	24	48	18
9	37	37	42	37	37	37	24	24	24	24	24	24
10	37	37		37	37	37	24	5		24	24, 48	24
11	37	37	42	37	37	37	18	24	24	24	48	24
13	37	37	42	37	37	37	24	24	24	24	24	24
14	37	37	41.5	37	37	37	24	24	24	24		24
15	36			36	36	36	18			~6	24	24
16	37	37		37			48	24		24, 48		
17	37	37	42	37	37	37	24	24	24	24	24	24
19	37	37	42	37	37	37	18, 24	24	24	24	24	24

Lab Code	*Salmonella* – Incubation Temperature (°C)						*Salmonella* – Duration of Incubation (hours)					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
21	37	37	42	37	37	37	18	24	24	24	48	24
22	37	37	42	37	37	37	24	24	24	24	48	24
23A	37	37	42	37	37	37	18	24	24	24	24	24
23B												
24	37	42					24	24				
25	37											
26	37	42	37	37		37	18	7	16	24		18
27	37	37	42	37	37	37	18, 24	24	24	24	48	24
28	37	37	42	37	37	37	18	18	18	24	48	18
29	37	42	37	37	37	37	18	24	24	24	24	24
32.1	37			42		37	20			24		24
32.2	37	42	42		A & B: 37	37	20	6	20		A: 24, B: 48	24
32.3	37	A: 37, B: 42		A & B: 37		37	20	A & B: 24		A: 24, B: 48		24
33	37	42	42	37	37	37	24	6-8, 16-20	16-20	24	24	24
34.1	37	37	42	37	37	37	18	24	24	24	48	24
34.2	37	37	42	37			18	24	24	24	24	
35	37	42	37	37	37	37	24	24	24	24	24	24
36	37	37	42	37	37	37	24	24	24	24	48	24

Lab Code	*Salmonella* – Incubation Temperature (°C)						*Salmonella* – Duration of Incubation (hours)					
	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation	Non-selective	Selective 1	Selective 2	Detection/ Isolation 1	Detection/ Isolation 2	Confirmation
37	37	37	42	37	37	37	18	18	18	24	48	24
38	35	42	35				18	18	6			
39	37	37	42	37	37	37	18	18	18	18	18	18
40	35	42	35	35	35	35	24	24	24	48	48	24
42	37	37	42	37	37	37	24	24	24	24	24, 48	24
43	37	37	42	37	37	37	24	24	24	24	48	24
44	35	41	41	41	41	35	18	24	24	24	24	24
45	37	37	42	37	37	37	24	24	24	24	48	24
46	42						18					
47	37	MSC: 37, RV: 42					18	6.5 each	16			
48	37	37	42	37	37		18	24	24	24	24	
49	37	42	37	37	37	37	18	24	24	24	24	24
50	37	37	42	37	37	37	24	24	24	24	24	24
51	37			42			18			5		
52	37	42	42	37	37		18	8	18	24	48	
53	37	MSC: 6-8, 37	42	37	37	37	18	24	24			
54	37	42	37	37	37	37	18	24	24	24	24	24

Notes:

1. For laboratory 5, samples A and D did not react with Oxoid *Salmonella* latex (H antigen only).

2. 7A refers to the BAX PCR results for laboratory 7.

3. 7B refers to the culture results for laboratory 7.

4. Laboratory 12 did not provide any method information for *Salmonella*.

5. Laboratory 23B used PCR on 2% enrichment broth.

SECTION B2

Listeria

Lab Code	***Listeria* – Media**						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirmation
1	Not done	Half Fraser broth + supplement SR 166M (Oxoid)	Buffered *Listeria* enrichment broth + supplement SR 141E (Oxoid)	Oxford agar	Not done	Oxoid Clearview *Listeria* Device (AOAC/ AFNOR approved)	Plate onto non-selective agar, β haemolysis, 12L (Oxoid) ID kit, sheep blood agar
2	UVM	Not done	Full strength Fraser broth	Oxford agar subbed from non-selective enrichment and secondary selective enrichment			Plate onto non-selective agar, β haemolysis, motility test, sugar fermentation tests, latex test, serological test
3	Not done	Buffered *Listeria* enrichment broth	BLEB	Oxford agar	Not done	VIDAS *Listeria*	CAMP test, API *Listeria* ID kit
4	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests, API ID kit, catalase, gram stain
6		Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, Oxoid Microbact *Listeria* ID kit, Henry's Illumination, catalase, gram stain
7A	Not done	Half Fraser broth	MOPS - BLEB	Not done	Not done	BAX PCR	Not done
7B	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, CAMP test, serological test
8		Half Fraser broth	Full strength Fraser broth	VIDAS LIS	Oxford agar	VIDAS LIS	β haemolysis, *Listeria* API ID kit
9	Not done	Buffered *Listeria* enrichment broth	Full strength Fraser broth	LSA		Tecra	DNA probe
10		Buffered *Listeria* enrichment broth	Not done	Oxford agar	Rapid L mono agar (Biorad) (Chromogenic)	Tecra, Geneprobe	Plate onto non-selective agar, β haemolysis, CAMP test, API *Listeria* ID kit, gram stain, catalase
11	Not done	Half Fraser broth, Elisa	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests, catalase, gram stain
14		Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, CAMP test, motility test
16	Not done	Buffered *Listeria* enrichment broth	Not done	Oxford agar	Palcam agar	Tecra Unique	Microbact *Listeria* ID kit
17		Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		
19	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Not done	BAX (L. monocytogenes)	Plate onto non-selective agar, β haemolysis, CAMP test, sugar fermentation tests, API ID kit

Lab Code	*Listeria* – Media						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirmation
21	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests
22	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	β haemolysis, CAMP test, motility test, sugar fermentation tests
23A	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	PCR	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests
24	Not done	Buffered *Listeria* enrichment broth	Full strength Fraser broth			Tecra	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, Microbact 12L ID kit
25	Not done	Half Fraser broth	MOPS - BLEB	Not done	Not done	A/BAX PCR	Not done
26	Not done	Buffered *Listeria* enrichment broth	Not done	Oxford agar	Not done		Plate onto non-selective agar, Microbact 12L (Oxoid) ID kit
27	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, API *Listeria* ID kit
28	Not done	Half Fraser broth	Full strength Fraser broth	LSA	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, Microbact 12L ID kit, gram stain, catalase
29	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	VIDAS LIS	Plate onto non-selective agar, β haemolysis, API *Listeria* ID kit
32.1		Half Fraser broth		Oxford agar			Plate onto non-selective agar, β haemolysis, motility test, Microbact 12L ID kit
32.2		Half Fraser broth	Full strength Fraser broth	Oxford agar			Plate onto non-selective agar, β haemolysis, motility test, Microbact 12L ID kit
33	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	ELFA kit VIDAS	Plate onto non-selective agar, motility test, *Listeria* API ID kit, catalase, gram stain, Henry's Illumination
34	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		β haemolysis, CAMP test, sugar fermentation tests
35	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, CAMP test, sugar fermentation tests
36		Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests
37	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar, Chromogenic media - rapid L. mono agar	VIDAS LIS, suspects plated onto selective agars	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests, gram stain, catalase

Lab Code	*Listeria* – **Media**						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirmation
38	Not done	Buffered *Listeria* enrichment broth	Full strength Fraser broth	Oxford agar	Not done	Tecra VIA	DNA probe
39	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, motility test, Microbact 12L (Oxoid) ID kit, catalase, gram stain
40	Not done	Buffered *Listeria* enrichment broth	Full strength Fraser broth	Oxford agar	Palcam agar		β haemolysis, motility test, sugar fermentation tests, BBL Crystol ID kit
41	Not done	Buffered *Listeria* enrichment broth	Not done	Oxford agar	Not done	Not done	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, API *Listeria* ID kit
42	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	Not done	Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests, Microbact *Listeria* 12L ID kit, gram stain, catalase
43	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	BAX/PCR	Plate onto non-selective agar, β haemolysis, rapid L. mono
45	Not done	Buffered *Listeria* enrichment broth	Not done	Oxford agar	Not done		Plate onto non-selective agar, β haemolysis, CAMP test, motility test, sugar fermentation tests
47	Buffered peptone water	Not done	Not done	Agar *Listeria* according to OHaviani & Agosti (ALOA)	Palcam agar	Not done	Not done
48	Not done	Buffered *Listeria* enrichment broth	Not done	Oxford agar			DNA probe, API *Listeria* ID kit
50	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Microbact 12L ID kit
51	Not done	Half Fraser broth	Full strength Fraser broth	Not done	Not done	Mini VIDAS	Not done
52	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar	ELFA kit VIDAS *Listeria*, BAX (PCR) Genus *Listeria*	Plate onto non-selective agar, CAMP test, motility test, Microbact (Oxoid) ID kit, BAX (PCR) L. monocytogenes
53	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar (only for samples positive by VIDAS)	Palcam agar (only for samples positive by VIDAS)	VIDAS LIS	Plate onto non-selective agar, API *Listeria* ID kit
54	Not done	Half Fraser broth	Full strength Fraser broth	Oxford agar	Palcam agar		Plate onto non-selective agar, β haemolysis, motility test, sugar fermentation tests, Microbact *Listeria* ID kit

Lab Code	*Listeria* – Incubation Temperature (°C)							*Listeria* – Duration of Incubation (hours)						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.
1		30	30	37			37		21	21	48			24
2	30		35	35				24		40	24			
3		30	30	30, 37		37	37		24	24	48		2	24
4		30	37	37	37		25, 37		24	48	48	48		24
6		30	37	37	37				24	48	24, 48	24, 48		
7A		30	37						24	24				
7B		30	37	37	37		30, 37		24	48	48	48		24
8		30	30		37		37		24	24		48		24
9		30	30	37					24	24	48			
10		30		37	37		37		48		72	24		24
11		30	37	37	37		37		24	48	48	48		24
14		30	30	37	37		37		24	24	24, 48	24, 48		24, 48
16		30		37	37				48		24,48	24,48		
17		30	37	37	37				24	48	48	48		
19		30	37	37			37		24	48	48			24
21		37	37	37	37		25 (motility), 37		24	48	48	48		24, β haemolysis: 48, sugars & motility: 5 days

Lab Code	*Listeria* – Incubation Temperature (°C)							*Listeria* – Duration of Incubation (hours)						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.
22		30	37	37	37		37		24	48	48	48		24, sugars: up to 7 days
23A		30	37	37	37		motility: 22, others: 37		24	48	48	48		plate, CAMP & motility: 24 β haemolysis: 48 sugars: 5 days
24		30	30						24	24				
25		30	35						24	24				
26		37		37			37		24		48			24
27		30	37	37	37		37		24	48	48	48		24
28		30	37	37	37		37		24	48	48	48		24
29		30	30	37	37		37		24	24	24	24		24
32.1		30		37			30		48		48			24
32.2		30	30	37			30		24	24	48			24
33		30	30	37	37		37		24	24	48	48		24
34		30	37	37	37		37		24	48	24, 48	24, 48		24
35		30	30	37	37		37		24	24	48	48		48
36		37	37	37	37		37		48	48	48	48		24
37		30	30	37	37		β haemolysis, CAMP, sugars: 37		24	24	48	24, 48		β haemolysis, CAMP: 24 sugars: 7 days
38		30	30	30					24	22	24			

Lab Code	*Listeria* – Incubation Temperature (°C)							*Listeria* – Duration of Incubation (hours)						
	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.	Non-selective	Primary	Second Selective	Agar Plating 1	Agar Plating 2	Other Detection	Confirm.
39		30	37	37	37		37		24	48	48	48		24
40		30	35	37	37		35		48	24	48	48		24
41		30		37			API *Listeria*: 37		48		48			24
42		30	37	37	37		37		24	48	24, 48	24, 48		24
43		30	37	37	37	30	37		24	24	48	48	24	24
45		30		37			37		48		48			CAMP: 24, sugars: 5 days
47	20			37	37			1			48	48		
48		30		37			37		48		48			24
49														
50		30	37	37	37		37		24	48	48	48		24
51		30	30						24	24				
52		30	30	37	37				24	24	48	48		
53		30	30	37	37		37		24	24	48	48		24
54	30	37	37	37	37		various	24	48	48	48	48		various

Notes:

1. 7A refers to the BAX PCR results for laboratory 7.

2. 7B refers to the culture results for laboratory 7.

3. Laboratory 12 did not provide any method information for *Listeria*.

4. Laboratory 17 used another method with BLEB as a secondary selective enrichment at 30^{o}C / 24 hours with Oxoid Rapid Elisa kit.

5. Laboratory 23B used PCR on half Fraser broth.

APPENDIX C

Homogeneity and Stability Testing

HOMOGENEITY TESTING RESULTS

Ten samples from each matrix (A/D, B and C/E) were randomly chosen and set aside for testing by IFM Quality Services Pty Ltd to confirm that the samples were homogeneous. Homogeneity testing was completed and the results were analysed prior to sample dispatch. Australian Standard methods (1766) were followed for *Salmonella* and *Listeria* tests. The results are tabulated below.

Note that, in the following tables, "*Listeria* spp." results mean *Listeria* species other than *Listeria monocytogenes*.

Samples A/D (containing *Salmonella* Adelaide and *Listeria innocua*)

Samples A/D – tested 19/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2692	Detected	Detected	Not detected
05/2693	Detected	Detected	Not detected
05/2694	Detected	Detected	Not detected
05/2695	Detected	Detected	Not detected
05/2696	Detected	Detected	Not detected
05/2697	Detected	Detected	Not detected
05/2698	Detected	Detected #	Not detected
05/2699	Detected	Detected	Not detected
05/2700	Detected	Detected	Not detected
05/2701	Detected	Detected	Not detected

Very poor growth. Only very weak growth after second enrichment (no typical colonies after 24h enrichment).

Sample B (containing *Salmonella* Bredeney)

Sample B – tested 21/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2731	Detected	Not detected	Not detected
05/2732	Detected	Not detected	Not detected
05/2733	Detected	Not detected	Not detected
05/2734	Detected	Not detected	Not detected
05/2735	Detected	Not detected	Not detected
05/2736	Detected	Not detected	Not detected
05/2737	Detected	Not detected	Not detected
05/2738	Detected	Not detected	Not detected
05/2739	Detected	Not detected	Not detected
05/2740	Detected	Not detected	Not detected

Samples C/E (containing *Listeria monocytogenes*)

Samples C/E – tested 20/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2708	Not detected	Not detected	Detected
05/2709	Not detected	Not detected	Detected
05/2710	Not detected	Not detected	Detected
05/2711	Not detected	Not detected	Detected
05/2712	Not detected	Not detected	Detected
05/2713	Not detected	Not detected	Detected
05/2714	Not detected	Not detected	Detected
05/2715	Not detected	Not detected	Detected
05/2716	Not detected	Not detected	Detected
05/2717	Not detected	Not detected	Detected

Based on the above testing results, the homogeneity of samples B, C and E was established. The *Listeria* spp. result for IFM sample number 05/2698 from matrix A/D indicated a problem with homogeneity of A/D. NATA therefore requested additional testing at the stability time frame (see stability testing results).

STABILITY TESTING RESULTS

To determine whether the samples used for this program were stable, NATA requested that three samples from matrices B and C/E be randomly chosen and set aside for testing by IFM Quality Services Pty Ltd. Because of concern over the homogeneity testing results for the samples from matrix A/D, NATA requested that five samples from this matrix be randomly chosen and set aside for stability testing by IFM Quality Services Pty Ltd.

The stability testing took place on 1 November 2005, which was one week after sample distribution. The results are tabulated below.

Note that, in the following tables, "*Listeria* spp." results mean *Listeria* species other than *Listeria monocytogenes*.

Samples A/D (containing *Salmonella* Adelaide and *Listeria innocua*)

Samples A/D – tested 1/11/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2815	Detected	Detected	Not detected
05/2816	Detected	*	Not detected
05/2817	Detected	Detected	Not detected
05/2818	Detected	Detected	Not detected
05/2819	Detected	*	Not detected

* *Bacillus*-like organisms overgrew the plates. Therefore, no *Listeria* was isolated.

Sample B (containing *Salmonella* Bredeney)

Sample B – tested 1/11/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2820	Detected	Not detected	Not detected
05/2821	Detected	Not detected	Not detected
05/2822	Detected	Not detected	Not detected

Samples C/E (containing *Listeria monocytogenes*)

Samples C/E – tested 1/11/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2823	Not detected	Not detected	Detected
05/2824	Not detected	Not detected	Detected
05/2825	Not detected	Not detected	Detected

Based on these results, samples B, C and E were considered to be stable for both *Salmonella* and *Listeria* during the period that this proficiency testing program was conducted. Samples A and D were also considered to be stable for *Salmonella.* Stability of samples A and D for *Listeria*, however, could not be established.

TRIP CONTROL TESTING RESULTS

IFM Quality Services Pty Ltd used a number of trip control samples for this program. These samples were returned to IFM by the courier after being in uncontrolled temperature conditions for 3 days. The samples were then tested. The results are tabulated below.

Note that, in the following tables, "*Listeria* spp." results mean *Listeria* species other than *Listeria monocytogenes*.

Samples A/D (containing *Salmonella* Adelaide and *Listeria innocua*)

Samples A/D – tested 31/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2806	Detected	Not detected	Not detected
05/2807	Detected	Detected	Not detected
05/2808	Detected	Detected	Not detected
05/2809	Detected	Detected	Not detected

Sample B (containing *Salmonella* Bredeney)

Sample B – tested 31/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2810	Detected	Not tested	Not detected
05/2811	Detected	Not tested	Not detected

Samples C/E (containing *Listeria monocytogenes*)

Samples C/E – tested 31/10/05			
IFM Lab No.	*Salmonella* / 5g	*Listeria* spp. / 5g	*L. monocytogenes* / 5g
05/2812	Not detected	Not detected	Detected
05/2813	Not detected	Not detected	Detected

Based on the homogeneity results, stability results and trip control results, NATA decided the *Listeria* results for samples A and D in this round of the program should not be analysed, as homogeneity and stability could not be established.

APPENDIX D

Instructions to Participants

and

Results Sheets

NATA PATHOGENS IN FOOD (ROUND 13) PROFICIENCY TESTING PROGRAM

INSTRUCTIONS TO PARTICIPANTS

On receipt of samples:

Open the container immediately and check the contents are in order

- Return the contents to the original packaging.
- Transfer the samples to a refrigerator (2-5°C) for storage prior to testing.
- Protect the samples from light.

Prior to testing please note:

- Five samples (labelled A, B, C, D, E) each containing 11g of flavoured pudding mix are to be tested for the presence/absence of *Salmonella* and *Listeria* as per instructions below.
- Samples are for laboratory use only.
- Store your samples in the original packaging between 2-5°C until testing commences.
- Testing is to commence on 25 October 2005 or as soon as possible after this date. Testing must not commence after 1 November 2005.
- Where practical your laboratory is encouraged to test different samples using different analysts.
- Laboratories are required to perform all tests for which NATA accreditation is held or sought and are welcome to report results for any remaining tests.
- *Listeria* speciation is not mandatory but is encouraged and can be reported in the "*Listeria* Species" boxes in Table A.
- *Salmonella* serotyping is not required.
- Your laboratory has been allocated the code number shown on the attached Results and Method Information Sheets.
- Please return the shipping containers (box plus plastic inner) using the pre-paid bag provided.

Instructions

You have been supplied with flavoured pudding mix in plastic sachets.

1. Aseptically open the sachets. Weigh out 5g for each test to be performed (*Salmonella*, *Listeria*).
2. Add **225mL** enrichment broth and proceed as per your laboratory test method.
3. The Australian Standard Methods are the preferred test methods.
4. Report results as presence or absence per 5 gram of sample in Table A of the supplied Results Sheets.
5. Report all method information in Tables B and C of the supplied Results Sheets by filling in (•) in the appropriate circles. If two different methods are used for a test report each result separately (copy and use a separate Results Sheet for each method).

Please return results **no later than 9 November 2005** to:

Mark Bunt
National Association of Testing Authorities, Australia
7 Leeds Street
RHODES NSW 2138

Telephone: (02) 9736 8222
Fax: (02) 9743 6664 OR (02) 9743 5311

NATA "Pathogens in Food" Round 13 Proficiency Testing Program

RESULTS SHEET

Date samples arrived	Date testing began	Signature

Laboratory Code: ☐

Table A: Results

	Presence / Absence per 5 gram of sample				
Results	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**
Salmonella	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent
Listeria	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent	❍ Present ❍ Absent
Listeria Species	❍ Not done ❍ Done Indicate species _____	❍ Not done ❍ Done Indicate species _____	❍ Not done ❍ Done Indicate species _____	❍ Not done ❍ Done Indicate species _____	❍ Not done ❍ Done Indicate species _____

Please indicate your results by filling in (•) in the appropriate circles above. If *Salmonella* or *Listeria* are not detected in a sample then this should be indicated by filling in (•) in the circle alongside "absent". Failing to fill in a circle for any sample will be considered an "unsatisfactory" result.

D2.1

Table B: Method Information: *Salmonella*

Laboratory Code ☐

Please indicate the methodology used by filling in (•) in the appropriate circles below:

Method	Non-selective Enrichment	Selective Enrichment Medium 1	Selective Enrichment Medium 2	Detection/Isolation		Confirmation of suspect *Salmonella*
Media	❍ Not done ❍ Buffered peptone water ❍ Other (specify) _____	❍ Not done ❍ MSC broth ❍ RVS broth ❍ MKTTn ❍ M-broth ❍ Other (specify) _____	❍ Not done ❍ MSC broth ❍ RVS broth ❍ MKTTn ❍ M-broth ❍ Other (specify) _____	❍ Not done PLATING Medium 1 ❍ XLD ❍ BSA ❍ Other (specify) _____	❍ Not done PLATING Medium 2 ❍ XLD ❍ BSA ❍ Other (specify) _____	❍ Not done ❍ Plate onto non-selective agar BIOCHEMICAL ❍ TSI ❍ Urea ❍ L-lysine / LDC ❍ B-galactosidase/ ONPG ❍ VP ❍ Indole SEROTYPING ❍ O ❍ Vi ❍ H ❍ Commercial ID kit (specify) _____ ❍ Other (specify) _____
Incubation Temperature	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC	❍ 35ºC ❍ 37ºC ❍ 42ºC ❍ Other _____ ºC
Duration of Incubation	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other_____	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other_____	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other_____	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other_____	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other_____	❍ 18 hours ❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other _____

D2.2

Table C: Method Information: *Listeria*

Laboratory Code ☐

Please indicate the methodology used by filling in (•) in the appropriate circles below:

Method	Non-selective Enrichment	Primary Selective Enrichment	Secondary Selective Enrichment	Selective Agar Plating Medium 1	Selective Agar Plating Medium 2	Other Detection Methods	Confirmation of suspect *Listeria*
Media	❍ Not done ❍ Tryptone soy broth with yeast extract ❍ Other (specify) ______________	❍ Not done ❍ Buffered *Listeria* enrichment broth ❍ Half Fraser broth ❍ UVM-I ❍ Other (specify) ______________	❍ Not done ❍ Full strength Fraser broth ❍ UVM-II ❍ Other (specify) ______________	❍ Not done ❍ Oxford agar ❍ Other agar (specify) ______________	❍ Not done ❍ Palcam agar ❍ Other agar (specify) ______________	❍ Not done ❍ Elisa kit (specify type) ______________ ❍ DNA probe (specify type) ______________ ❍ Other detection methods (specify type) ______________	❍ Not done ❍ Plate onto non-selective agar ❍ β haemolysis ❍ CAMP test ❍ Motility test ❍ Growth in anaerobic conditions ❍ Sugar fermentation tests ❍ Latex test ❍ DNA probe ❍ Serological test ❍ Commercial ID kit (specify) ______________ ❍ Other (specify) ______________
Incubation Temperature	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC	❍ 25ºC ❍ 30ºC ❍ 37ºC ❍ Other _____ ºC
Duration of Incubation	❍ 4 hours ❍ 24 hours ❍ 48 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________	❍ 24 hours ❍ 48 hours ❍ 72 hours ❍ Other __________

D2.3