REPORT NO. 813

Pathogens In Food

Proficiency Testing Program

Round 27

June 2013

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Ms S Mott, Global Proficiency Ltd (New Zealand). This assistance included providing input into the design of the program, technical advice and discussion of the final report. PTA also wishes to gratefully acknowledge Global Proficiency Ltd (New Zealand) for producing the samples and Global Proficiency Pty Ltd (Australia) for distributing the samples.

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APPENDICES

1. FOREWORD

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

Proficiency Testing Australia conducted the program in April / May 2013. The aim of the program was to assess laboratories' abilities to competently perform the nominated tests.

The Program Coordinator was Dr M Bunt and the Technical Adviser was Ms S Mott, Global Proficiency Ltd (New Zealand). This report was authorised by Ms W Fajloun, PTA Quality Coordinator.

2. FEATURES OF THE PROGRAM

(a) A total of ten laboratories received samples, one of which did not return results for inclusion in the final report.

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

(c) Laboratories were provided with five samples. Each sample consisted of a freeze-dried vial with an accompanying matrix. Each matrix consisted of a total of **25 – 30 g** (please refer to the General Comments section on page 6 of this report) of whole milk powder. Laboratories were also provided with "Instructions to Participants" and a "Results Sheet" (see Appendix D).

(d) 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 one laboratory reported more than one set of results and, therefore, this laboratory's 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 Sheets".

4. DESIGN OF THE PROGRAM

Participants were asked to determine the presence or absence of *Salmonella*, *Listeria*, *Listeria monocytogenes* (*L. monocytogenes*) and *Bacillus cereus* (*B. cereus*) in five samples of whole milk powder.

Each laboratory was provided with five samples labelled A, B, C, D and E and was requested to test **10 g** of each sample for each analysis (please refer to the General Comments section on page 6 of this report).

- Sample A contained *Salmonella* Derby and *Listeria monocytogenes*.
- Sample B contained *Bacillus cereus*.
- Sample C contained *Salmonella* Senftenberg (H_2S positive strain) and *Listeria innocua*.
- Sample D contained *Listeria monocytogenes*.
- Sample E contained *Salmonella* Adelaide and *Bacillus cereus*.

Other "typical" microflora were included in the samples (*e.g. Escherichia coli*, *Enterococcus faecalis*, etc.) The levels of *Salmonella* and *Listeria* in each sample were between **40 – 200** cfu per **10 g**. The level of *Bacillus cereus* in each sample was approximately **4 000** cfu per **10 g** (please refer to the General Comments section on page 6 of this report).

Microbiological samples for the Pathogens in Food program are manufactured according to Global Proficiency Ltd's Standard Operating Procedures.

5. HOMOGENEITY AND STABILITY TESTING

Prior to sample distribution, ten randomly selected samples from each matrix (A, B, C, D and E) were analysed for homogeneity by Global Proficiency Ltd (New Zealand). Based on the results of this testing, the homogeneity of the samples was established.

Stability testing was also performed on the samples by Global Proficiency Ltd (New Zealand). The results showed that the samples were sufficiently stable for testing for the duration 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 Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	-	
C		-
D	-	
E		5
Presence / Absence of *Listeria* in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	-	
C		-
D		-
E	-	
Presence / Absence of *Listeria monocytogenes* in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	-	
C	-	
D		-
E	-	
Presence / Absence of *Bacillus cereus* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B		-
C	-	
D	-	
E		-

7. TECHNICAL COMMENTS

Response Rate

Nine of the ten laboratories (90%) that received samples for the program submitted results for inclusion in the final report. All of these nine laboratories reported results for *Salmonella.* Eight of these nine laboratories (89%) reported results for *Listeria*. Six of these nine laboratories (67%) reported results for *Bacillus cereus*. Seven of the eight laboratories (88%) that submitted results for *Listeria* also tested for *Listeria monocytogenes*. One participant (laboratory 4) submitted two sets of results, each from a different analyst.

Salmonella Results

The results submitted by participants for *Salmonella* are presented in Appendix A1. Laboratory 5 reported a false negative result for sample E, which contained *Salmonella* Adelaide in addition to *Bacillus cereus*.

Six laboratories used a Culture method with three stating they had followed an AS 5013 method; one referenced FDA-BAM, another ISO 6785 and another laboratory referenced SNI 2897:2008 (National Standards Indonesia). Of these six, one laboratory used the culture techniques from positive results gained from the molecular technique; the 3M MDS DNA probe system.

Two laboratories reported using the VIDAS® immunoassay system with one laboratory submitting two reports via this technology.

Six laboratories stated they had used biochemical testing in the confirmatory testing process. Five laboratories reported using rapid kits, which included API 20E and a latex kit. The majority of laboratories also used O or O and H serotyping in the confirmatory process.

One laboratory did not provide method information.

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

Listeria Results

The results submitted by participants for *Listeria* and *Listeria monocytogenes* are presented in Appendix A2. There were no false results submitted for *Listeria* or *Listeria monocytogenes*.

Five laboratories stated they had used a cultural method; three referenced AS 5013 (two stated they had used AS 5013.24.1), and two referenced an ISO method (one ISO 11920 (Food and Animal Feeding Stuffs), the other ISO 10560 (Milk and Milk Products)). Of these five, one laboratory used the culture techniques from positive results gained from the molecular technique; the 3M MDS DNA probe system, and another used the culture techniques from positive results gained from the molecular technique; PCR-BAX Q10.

Two laboratories reported using the VIDAS® immunoassay system with one laboratory submitting two reports via this technology.

Four laboratories used biochemical testing in the confirmation process. Three laboratories reported using rapid kits; the *Listeria* API ID kit, Microbact 12L and OBIS (Oxoid Biochemical Identification System). The CAMP test and assessment for production of β-haemolysis was undertaken by three laboratories.

One laboratory did not provide method information.

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

Bacillus cereus Results

The results submitted by participants for *Bacillus cereus* are presented in Appendix A3. There were no false results submitted for *Bacillus cereus*.

All laboratories used plating methods for detection of the organism. Two laboratories reported using MYP agar; two used BCSA and one used PEMBA (and submitted two reports).

In the confirmation process, laboratories reported using spore and lipid stains, haemolysis and biochemical tests.

One laboratory did not provide method information.

Detailed method information for *Bacillus cereus* is provided in Appendix B3.

General Comments

In this round, the sample provider inadvertently dispatched the incorrect matrix size; 25 g sachets of whole milk powder were provided instead of the 60 g sachets as required for the program.

This resulted in some laboratories requesting extra sachets of matrix to be sent, and others adjusting the matrix to enrichment broth ratios and, therefore, the inoculum level added. An amended set of Instructions was emailed to some of the participants to enable testing via the ratio adjustments (please refer to Appendix D1).

Although certainly not satisfactory, both options were still considered comparable given the program is Presence/Absence testing and not enumerative. Examination of final round results supported this, with only one outlying result submitted, for a false negative result for *Salmonella* on a sample containing *Salmonella* Adelaide (the laboratory correctly reported positive *Salmonella* in samples A and C).

The provider has advised that a full investigation has been undertaken, resulting in the generation of a non-conformance report, with preventive action to ensure this does not occur again.

8. REFERENCES

1. *Guide to Proficiency Testing Australia (2012)*. (This document is located on the PTA website at www.pta.asn.au under Programs / Documents).

2. *AS 5013.2: 2007 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Bacillus cereus - Colony-count technique at 30ºC (ISO 7932:2004, MOD).*

3. *AS 5013.10: 2009 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp (ISO 6579:2002, MOD).*

4. *AS 5013.24.1: 2009 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Detection method (ISO 11290-1:1996, MOD).*

APPENDIX A

Summary of Results

Section A1

Salmonella

A1.1

Salmonella Results

Lab Code	A	B	C	D	E	False Results
1	Present	Absent	Present	Absent	Present	
2	Present	Absent	Present	Absent	Present	
4A	Present	Absent	Present	Absent	Present	
4B	Present	Absent	Present	Absent	Present	
5	Present	Absent	Present	Absent	**Absent**	**1**
6	Present	Absent	Present	Absent	Present	
7	Present	Absent	Present	Absent	Present	
8	Present	Absent	Present	Absent	Present	
9	Present	Absent	Present	Absent	Present	
10	Present	Absent	Present	Absent	Present	

Note:

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

A1.2

Salmonella Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	0	0	1	1
Total Results	10	10	10	10	10	50

Failure rate (Sample A) = $\frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 0 / 10
= 0%

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 10
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 0 / 10
= 0%

Failure rate (Sample D) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 0 / 10
= 0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 1 / 10
= 10.0%

Overall failure rate (*Salmonella*) = $\frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 1 / 50
= 2.0%

Section A2

Listeria

A2.1

Listeria Results

Lab Code	A	B	C	D	E	False Results
1	Present	Absent	Present	Present	Absent	
2	Present	Absent	Present	Present	Absent	
4A	Present	Absent	Present	Present	Absent	
4B	Present	Absent	Present	Present	Absent	
6	Present	Absent	Present	Present	Absent	
7	Present	Absent	Present	Present	Absent	
8	Present	Absent	Present	Present	Absent	
9	Present	Absent	Present	Present	Absent	
10	Present	Absent	Present	Present	Absent	

A2.2

Listeria Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	0	0	0	0
Total Results	9	9	9	9	9	45

Failure rate (Sample A) = $\frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 0 / 9
= 0%

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 9
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 0 / 9
= 0%

Failure rate (Sample D) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 0 / 9
= 0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 0 / 9
= 0%

Overall failure rate (*Listeria)* = $\frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 0 / 45
= 0%

A2.3

Listeria monocytogenes Results

Lab Code	A	B	C	D	E	False Results
1	Present	Absent	Absent	Present	Absent	
4A	Present	Absent	Absent	Present	Absent	
4B	Present	Absent	Absent	Present	Absent	
6	Present	Absent	Absent	Present	Absent	
7	Present	Absent	Absent	Present	Absent	
8	Present	Absent	Absent	Present	Absent	
9	Present	Absent	Absent	Present	Absent	
10	Present	Absent	Absent	Present	Absent	

A2.4

Listeria monocytogenes Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	0	0	0	0
Total Results	8	8	8	8	8	40

Failure rate (Sample A) = $\frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 0 / 8
= 0%

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 8
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 0 / 8
= 0%

Failure rate (Sample D) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 0 / 8
= 0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 0 / 8
= 0%

Overall failure rate (*L. monocytogenes)* = $\frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 0 / 40
= 0%

Section A3

Bacillus cereus

A3.1

Bacillus cereus Results

Lab Code	A	B	C	D	E	False Results
1	Absent	Present	Absent	Absent	Present	
4A	Absent	Present	Absent	Absent	Present	
4B	Absent	Present	Absent	Absent	Present	
6	Absent	Present	Absent	Absent	Present	
8	Absent	Present	Absent	Absent	Present	
9	Absent	Present	Absent	Absent	Present	
10	Absent	Present	Absent	Absent	Present	

A3.2

Bacillus cereus Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	0	0	0	0
Total Results	7	7	7	7	7	35

Failure rate (Sample A) = $\frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 0 / 7
= 0%

Failure rate (Sample B) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 0 / 7
= 0%

Failure rate (Sample C) = $\frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 0 / 7
= 0%

Failure rate (Sample D) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 0 / 7
= 0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 0 / 7
= 0%

Overall failure rate (*B. cereus*) = $\frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 0 / 35
= 0%

APPENDIX B

Summary of Methods

SECTION B1

Salmonella

Lab Code	***Salmonella* Method Information**	
	Detection	Confirmation
1	Culture Method (SNI 2897: 2008)	Biochemical tests, *Salmonella* latex test rapid kit, O & H serotyping
2	Culture Method (AS 5013.11.5 - 2012)	API 20E rapid kit, O & H serotyping
4A	Rapid Method - Immunoassay (VIDAS)	API 20E rapid kit
4B	Rapid Method - Immunoassay (VIDAS)	API 20E rapid kit
5	Culture Method (as per FDA BAM)	Biochemical tests
6	Culture Method (AS 5013.10 - 2009)	Biochemical tests, O & H serotyping
7	Rapid Method - Immunoassay (VIDAS)	Biochemical tests, API 20E rapid kit, O & H serotyping
8	Culture Method (ISO 6785 Milk and milk products - Detection of *Salmonellae*), Molecular Technique (3M MDS)	Biochemical tests, API 20E rapid kit, O & H serotyping
9	Culture Method (AS 5013)	Biochemical tests, O serotyping

Comments:

1. Laboratory 7 plated out ex VIDAS on XLD and Chrom ID.
2. The cultural method for laboratory 8 was performed in full from MDS positive results.
3. Laboratory 10 did not provide any information on the method they used for *Salmonella* testing.

SECTION B2

Listeria

Lab Code	***Listeria* Method Information**	
	Detection	Confirmation
1	Culture Method (Oxoid *Listeria* Method refers to ISO 11920)	Biochemical tests, OBIS (Oxoid Biochemical Identification System) rapid kit, Cowan and Steels manual for the identification of medical bacteria, third ed.
2	Culture Method (ISO 10560 - 1993)	Microbact 12L rapid kit
4A	Rapid Method - Immunoassay (VIDAS)	Catalase test, gram stain, motility
4B	Rapid Method - Immunoassay (VIDAS)	Catalase test, motility, gram stain
6	Culture Method (AS 5013.24.1 - 2009)	Biochemical tests, CAMP test, β haemolysis
7	Rapid Method - Immunoassay (VIDAS)	β haemolysis, *Listeria* API ID rapid kit, catalase, gram, motility
8	Culture Method (AS 5013.24.1 - 2009), Molecular Technique (3M MDS)	Biochemical tests, CAMP test, β haemolysis
9	Culture Method (AS 5013), Molecular Technique (PCR BAX Q10)	Biochemical tests, CAMP test, β haemolysis

Comments:

1. Laboratory 7 plated out ex VIDAS on OAA.
2. The cultural method for laboratory 8 was performed in full from MDS positive results. The pre-enrichment media used by laboratory 8 for their cultural method was 3M MLRB.
3. Laboratory 10 did not provide any information on the method they used for *Listeria* testing.

SECTION B3

Bacillus cereus

B3.1

Lab Code	***Bacillus cereus* Method Information**				
	Medium		**Plating / Inoculation Details**		**Confirmation**
	Plating Method	MPN Method	Plating Method	MPN Method	
1	Brilliance Bacillus Cereus Selective Agar	Not done	1 mL over 5 plates with spreader method	-	Haemolysis, gram stain, nitrate reduction, Voges Proskauer (VP), sugar fermentation tests, anaerobic condition, catalase
4A	PEMBA	Not done	1 mL over 3 plates	-	Spore stain / Lipid stain
4B	PEMBA	Not done	1 mL over 3 plates	-	Spore stain / Lipid stain
6	BCSA	Not done	1 mL over 3 plates	-	Spore stain / Lipid stain
8	MYP	-	1 mL over 3 plates	-	Haemolysis
9	MYP	-	1 mL over 3 plates	-	Haemolysis

Comments:

1. The colony gram inoculation plating method used by laboratory 1, with 1 mL over 3 plates, was too crowded so the colony blocked. They used 1 mL over 5 plates and 0.1 mL per plate with the spreader method.
2. Laboratory 10 did not provide any information on the method they used for *Bacillus cereus* testing.

APPENDIX C

Homogeneity and Stability Testing

HOMOGENEITY TESTING RESULTS

Ten samples from each matrix (A, B, C, D and E) were randomly chosen and tested by Global Proficiency Ltd (New Zealand) to confirm that the samples were homogeneous. Homogeneity testing was performed on 20 March 2013. The results were analysed prior to sample dispatch.

For *Salmonella*, the method of testing was enumeration via spread plate technique using XLD agar. The samples were verified using AS 5013.10: 2009 (ISO equivalent – ISO 6579: 2002). For *Listeria*, the method of testing was enumeration via spread plate technique using PALCAM agar. The samples were verified using AS 5013.24.1: 2009 (ISO equivalent – ISO 11290-1: 1996, MOD). For *Bacillus cereus*, the method of testing was enumeration via spread plate technique using MYP agar. The samples were verified using AS 5013.2: 2007.

The results are tabulated below.

Sample A (containing *Salmonella* Derby and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
15	Detected	Detected	Detected	Not detected
20	Detected	Detected	Detected	Not detected
36	Detected	Detected	Detected	Not detected
43	Detected	Detected	Detected	Not detected
51	Detected	Detected	Detected	Not detected
71	Detected	Detected	Detected	Not detected
80	Detected	Detected	Detected	Not detected
84	Detected	Detected	Detected	Not detected
88	Detected	Detected	Detected	Not detected
110	Detected	Detected	Detected	Not detected

Sample B (containing *Bacillus cereus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
11	Not detected	Not detected	Not detected	Detected
23	Not detected	Not detected	Not detected	Detected
31	Not detected	Not detected	Not detected	Detected
39	Not detected	Not detected	Not detected	Detected
61	Not detected	Not detected	Not detected	Detected
75	Not detected	Not detected	Not detected	Detected
82	Not detected	Not detected	Not detected	Detected
87	Not detected	Not detected	Not detected	Detected
103	Not detected	Not detected	Not detected	Detected
117	Not detected	Not detected	Not detected	Detected

C1.2

Sample C (containing *Salmonella* Senftenberg and *Listeria innocua*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
3	Detected	Detected	Not detected	Not detected
22	Detected	Detected	Not detected	Not detected
37	Detected	Detected	Not detected	Not detected
42	Detected	Detected	Not detected	Not detected
45	Detected	Detected	Not detected	Not detected
71	Detected	Detected	Not detected	Not detected
81	Detected	Detected	Not detected	Not detected
86	Detected	Detected	Not detected	Not detected
110	Detected	Detected	Not detected	Not detected
113	Detected	Detected	Not detected	Not detected

Sample D (containing *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
4	Not detected	Detected	Detected	Not detected
24	Not detected	Detected	Detected	Not detected
33	Not detected	Detected	Detected	Not detected
51	Not detected	Detected	Detected	Not detected
57	Not detected	Detected	Detected	Not detected
77	Not detected	Detected	Detected	Not detected
84	Not detected	Detected	Detected	Not detected
94	Not detected	Detected	Detected	Not detected
104	Not detected	Detected	Detected	Not detected
112	Not detected	Detected	Detected	Not detected

Sample E (containing *Salmonella* Adelaide and *Bacillus cereus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
27	Detected	Not detected	Not detected	Detected
35	Detected	Not detected	Not detected	Detected
40	Detected	Not detected	Not detected	Detected
41	Detected	Not detected	Not detected	Detected
48	Detected	Not detected	Not detected	Detected
49	Detected	Not detected	Not detected	Detected
51	Detected	Not detected	Not detected	Detected
64	Detected	Not detected	Not detected	Detected
82	Detected	Not detected	Not detected	Detected
97	Detected	Not detected	Not detected	Detected

Based on the above testing results, the homogeneity of the samples was established.

STABILITY TESTING RESULTS

To determine whether the samples used for this program were stable, three samples from each matrix (A, B, C, D and E) were randomly chosen, stored under ambient conditions for 3 days to simulate average transit conditions and tested by Global Proficiency Ltd (New Zealand). The results are tabulated below.

Sample A (containing *Salmonella* Derby and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
69	Detected	Detected	Detected	Not detected
85	Detected	Detected	Detected	Not detected
119	Detected	Detected	Detected	Not detected

Sample B (containing *Bacillus cereus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
9	Not detected	Not detected	Not detected	Detected
108	Not detected	Not detected	Not detected	Detected
110	Not detected	Not detected	Not detected	Detected

Sample C (containing *Salmonella* Senftenberg and *Listeria innocua*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
17	Detected	Detected	Not detected	Not detected
25	Detected	Detected	Not detected	Not detected
108	Detected	Detected	Not detected	Not detected

Sample D (containing *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
50	Not detected	Detected	Detected	Not detected
66	Not detected	Detected	Detected	Not detected
101	Not detected	Detected	Detected	Not detected

Sample E (containing *Salmonella* Adelaide and *Bacillus cereus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*B. cereus*
22	Detected	Not detected	Not detected	Detected
30	Detected	Not detected	Not detected	Detected
113	Detected	Not detected	Not detected	Detected

Based on these results, the samples were considered to be stable during the period that this proficiency testing program was conducted.

APPENDIX D

Instructions to Participants
and
Results Sheets

D1.1

PROFICIENCY TESTING AUSTRALIA
PATHOGENS IN FOOD PROGRAM – ROUND 27

AMENDED INSTRUCTIONS TO PARTICIPANTS

On receipt of samples:

Open the container immediately and check the contents are in order

- Record the temperature of the samples.
- 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 25-30 g of whole milk powder are to be tested for the presence / absence of *Salmonella* and *Listeria* as per instructions below. Testing for the presence / absence of *Bacillus cereus* is also offered in this round. If you have this capability, you are encouraged to perform the test.
- Samples are for laboratory use only.
- Store your samples in the original packaging between 2-5 °C until testing commences.
- It is strongly recommended that testing is initiated within 48 hours of receipt of the samples.
- Where practical your laboratory is encouraged to test different samples using different analysts.
- Laboratories should perform all testing using their routine test methods.
- *Listeria* speciation is not mandatory, but is encouraged.
- *Salmonella* serotyping is not required.
- Confirmatory testing for *B. cereus* is required.
- Your laboratory has been allocated the code number shown on the attached Results and Method Information Sheets.

Instructions

You have been supplied with freeze dried vials and accompanying whole milk powder matrices in foil laminate sachets. Please find below instructions for the re-hydration and preparation of the freeze-dried vials and steps for the preparation of the matrix.

1. Re-hydrate the freeze-dried matrix by adding 3.0 mL of sterile diluent (*e.g.* 0.1% (w/v) peptone and 0.85% (w/v) NaCl (ISO 6887-1)) at room temperature.
2. Allow standing at room temperature for 10 minutes.
3. Mix the vial contents using a vortex mixer for 15 seconds.
4. Aseptically open the sachets. Weigh out 10 g for each of the *Salmonella* and *Listeria* tests to be performed. Add 90 mL enrichment broth. Mix to dissolve the milk powder. Add 0.5 mL of the rehydrated vial contents and continue as per your Standard method.
   Please note: *B. cereus* testing: Aliquots are to be removed from the initial non-selective *Salmonella* enrichment once prepared. If following the plating procedure for *B. cereus*, levels have been designed to suit the inoculum volume of 1 mL (over 3 plates) if following AS 5013. 2 – 2007.
5. Proceed as per your laboratory test method. The Australian Standard Methods are the preferred test methods.
6. Report results as presence or absence per 25 gram of sample in Table A of the supplied Results Sheets by filling in (•) in the appropriate circles. If *Salmonella*, *Listeria* or *B. cereus* are not detected in a sample then this should be indicated by filling in (•) in the circle alongside "absent".
7. Report all method information in Tables B, C and D of the supplied Results Sheets by filling in (•) in the appropriate circles. If more than one method is used for a test report each result separately (copy and use a separate Results Sheet for each method).

Please return results **no later than Wednesday 8 May 2013** to:

Mark Bunt
Proficiency Testing Australia
PO Box 7507
Silverwater NSW 2128
AUSTRALIA

Telephone: +61 2 9736 8397 (1300 782 867)
Fax: +61 2 9743 6664
Email: mbunt@pta.asn.au

D2.1

PTA Pathogens in Food (Round 27) Proficiency Testing Program

RESULTS SHEET

Date samples arrived	Sample temperature	Date testing began	Signature

Laboratory Code: ☐

Table A: Results

Test	Sample A	Sample B	Sample C	Sample D	Sample E
Salmonella	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done
Listeria	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done
Listeria monocytogenes	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done
Bacillus cereus	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done	❍ Present ❍ Absent ❍ Not done

Table B: Method Information: *Salmonella*

Laboratory Code ☐

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

Detection	**Confirmation**
❍ **Culture Method** (please specify method reference) _____ ❍ **Rapid Methods - Immunoassay** ❍ TECRA ❍ VIDAS ❍ Other (please specify) _____ ❍ **Molecular Techniques** (please specify system used) ❍ PCR_____ ❍ DNA probe_____ ❍ Other (please state)	❍ Biochemical tests ❍ Rapid kits ❍ API 20E ❍ Microbact 12E ❍ Other (please specify) _____ ❍ Serotyping ❍ O ❍ O & H ❍ Agglutination testing (please specify kit) _____ ❍ Other (please specify) _____

Comments: _____

_____

_____

Table C: Method Information: *Listeria*

Laboratory Code ☐

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

Detection	**Confirmation**
❍ **Culture Method** (please specify method reference) _____ ❍ **Rapid Methods - Immunoassay** ❍ TECRA ❍ VIDAS ❍ Other (please specify) _____ ❍ **Molecular Techniques** (please specify system used) ❍ PCR_____ ❍ DNA probe_____ ❍ Other (please state)	❍ Biochemical tests ❍ CAMP test ❍ β-haemolysis ❍ Rapid kits ❍ *Listeria* API ID ❍ Microbact 12L ❍ Other (please specify) _____ ❍ Serological testing (please specify) _____ ❍ Other (please specify) _____

Comments: _____

_____

_____

Table D: Method Information: *Bacillus cereus* (Presence/Absence)

Laboratory Code ☐

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

Method	Plating Method	Most Probable Number Method	Confirmation
Medium	❍ MYP ❍ PEMBA ❍ BCSA ❍ Other (specify) _____	❍ Not done ❍ Trypticase Soy Polymixin broth ❍ Other (specify) _____	❍ Not done ❍ Haemolysis ❍ Spore stain / Lipid stain ❍ Gram stain ❍ Nitrate reduction ❍ Voges Proskauer (VP) ❍ Sugar fermentation tests ❍ Other (please specify) _____
Plating / Inoculation Details	❍ **1 mL over 3 plates (recommended)** ❍ 0.1 mL per plate ❍ Other (please specify) _____	❍ 0.1 g (1 mL of 10^{-1}) ❍ 0.01 g (1 mL of 10^{-2}) ❍ 0.001 g (1 mL of 10^{-3}) ❍ Other (please specify) _____	

Comments: _____

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----- End of report -----