pt:a proficiency testing australia

REPORT NO. 836

Pathogens In Food
Proficiency Testing Program
Round 28

November 2013

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TABLE OF CONTENTS

1. FOREWORD	**1**
2. FEATURES OF THE PROGRAM	**1**
3. FORMAT OF THE APPENDICES	**1**
4. DESIGN OF THE PROGRAM	**2**
5. HOMOGENEITY AND STABILITY TESTING	**2**
6. FALSE RESULTS	**2**
TABLE A: False Results	3
7. TECHNICAL COMMENTS	**4**
8. REFERENCES	**7**

APPENDICES

APPENDIX A	
Summary of Results	
Salmonella	A1.1
Listeria	A2.1
Staphylococcus aureus	A3.1
APPENDIX B	
Summary of Methods	
Salmonella	B1.1
Listeria	B2.1
Staphylococcus aureus	B3.1
APPENDIX C	
Homogeneity and Stability Testing	C1.1
APPENDIX D	
Instructions to Participants	D1.1
Results Sheets	D2.1

1. FOREWORD

This report summarises the results of round twenty-eight 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 September / October 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 eleven laboratories received samples, all of which returned results.

(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 60 g of whole milk powder. Laboratories were also provided with "Instructions to Participants" and "Results Sheets" (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 *Staphylococcus aureus* (*S. aureus*) 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 25 grams of each sample for each analysis.

- Sample A was a negative sample. It did not contain *Salmonella*, *Listeria* or *S. aureus*.
- Sample B contained *Salmonella* Adelaide.
- Sample C contained *Salmonella* Senftenberg (H_2S negative strain), *Listeria innocua* and *S. aureus*.
- Sample D contained *Salmonella* Derby, *L. monocytogenes* and *S. aureus*.
- Sample E contained *L. monocytogenes* and *S. aureus*.

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 100 – 500 cfu per 25 g. The level of *Staphylococcus aureus* in each sample was approximately 10 000 cfu per 25 g.

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	6	
B		10
C		6, 10
D		6, 10
E	-	

Presence / Absence of *Listeria* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	10	
B	-	
C		10
D		10
E		10

Presence / Absence of *L. monocytogenes* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B	-	
C	-	
D		-
E		-

Presence / Absence of *S. aureus* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B	3	
C		-
D		10
E		10

7. TECHNICAL COMMENTS

Response Rate

All of the eleven laboratories that participated in the program submitted results for inclusion in the final report. All of the eleven participants reported results for *Salmonella.* Six of the eleven participants (55%) reported results for *Listeria*. Five of these six laboratories (83%) also tested for *L. monocytogenes*. Nine of the eleven participants (82%) reported results for *S. aureus*. One participant (laboratory 5) 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 6 reported a false positive result for sample A (this sample contained *E. coli*) and false negative results for samples C and D. Laboratory 10 reported false negative results for samples B, C and D.

Two laboratories submitted reports where two methods were quoted as being used.

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

Three laboratories reported using molecular techniques; two laboratories used the 3M™ Molecular Detection Assay Salmonella system, and the third quoted PCR.

Six laboratories used a Culture method, with two stating they had followed the FDA Bacteriological Analytical Manual: Chapter 5; one laboratory referenced AS 5013.10: 2009; another quoted ISO 6579: 2002, a fifth stated they had used ISO 6785: 2001 and the sixth laboratory did not include a method reference. The two ISO methods differ in that ISO 6785: 2001 is for the detection of *Salmonella* in Milk and Milk Products, and ISO 6579: 2002 is a horizontal method for the detection of *Salmonella* in Food and Animal Feeding Stuffs. Although this proficiency round offered testing in whole milk powder, it is expected that results could be fully duplicated with either method.

Five laboratories reported using the API 20E rapid test kit in the confirmation stage of the testing process, and another laboratory stated the Vitek® rapid kit had been used. Four laboratories also used O or O and H serotyping in the confirmatory process, although one laboratory indicated there were some issues with reading agglutination in this round (this laboratory reported two false negative results and one false positive result in this round).

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 *L. monocytogenes* are presented in Appendix A2. For the *Listeria* species test, laboratory 10 reported a false positive result for sample A (this sample contained *Bacillus cereus*) and false negative results for samples C, D and E (this laboratory did not report results for *L. monocytogenes*). There were no false results submitted for *L. monocytogenes*.

Two laboratories submitted reports where more than one method was used in the testing process.

Two laboratories stated they had used a cultural method; one referenced AS 5013.24.1: 2009 and the other ISO 11290-1: 1996. AS 5013.24.1: 2009 is aligned to ISO 11290-1: 1996 with modifications to the control cultures used in the testing process (specific cultures are stipulated). A third laboratory reported using culture methods in the confirmation of *L. monocytogenes*.

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

Two laboratories reported using molecular techniques; one used the 3M™ Molecular Detection Assay system and another used PCR.

Three laboratories used biochemical testing in the confirmation process. One laboratory reported using the FOOD-system (system for detection and presumptive identification of pathogenic microorganisms from food). The CAMP test was undertaken by two laboratories.

One laboratory did not provide method information.

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

Staphylococcus aureus Results

The results submitted by participants for *S. aureus* are presented in Appendix A3. Laboratory 3 reported a false positive result for sample B (this sample contained *Bacillus cereus, E. coli* and *Salmonella*). Laboratory 10 reported false negative results for samples D and E.

Of the nine laboratories that submitted results for *S. aureus*, seven laboratories reported using Baird-Parker agar. One laboratory submitted results where two methods were quoted as being used (3M™ Petrifilm™ Staph Express and MPN using TSB).

One laboratory did not submit method information.

The method references reported by the participants included:

- AS 5013.12.1: 2004 (equivalent to ISO 6888-1: 1999 and ISO 6888-1: 1999 Amdt 1: 2003).
- ISO 6888-1: 1999 (Enumeration using Baird-Parker agar).
- ISO 6888-3: 2004 (Detection and MPN technique). The laboratory using this method stated Baird-Parker agar had been used, but also quoted Chapman agar (this agar is also known as Mannitol Salt agar).
- FDA-BAM, Chapter 12 (Laboratory used Baird-Parker agar).
- AOAC 975.55 (Laboratory used 3M™ Petrifilm™ Staph Express and MPN using TSB).

As this program offers presence / absence testing for *S. aureus*, it is fully expected that all the above methods should produce identical results.

Four laboratories performed confirmatory testing using the tube/coagulase test; one laboratory used Rabbit Plasma Fibrinogen agar, one laboratory used the Staf-Sistem 18-R (a rapid kit for speciation of staphylococcal isolates) and one laboratory used Baird-Parker agar to confirm the Petrifilm™ results.

One laboratory reported that the colonies appearing on the Baird-Parker agar were white in colour for all samples. This could indicate either an issue with the concentration of potassium tellurite in the medium, or may be a brand of agar with reduced tellurite levels in consideration of some *S. aureus* strains sensitivity to tellurite. The laboratory did not report any false results.

Detailed method information for *S. aureus* is provided in Appendix B3.

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

3. *AS 5013.12.1: 2004 Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Technique using Baird-Parker agar medium.*

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).*

5. *ISO 6579: 2002 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.*

6. *ISO 6785: 2001 Milk and milk products - Detection of Salmonella spp.*

7. *ISO 11290-1: 1996 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method.*

8. *ISO 6888-1: 1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium.*

9. *ISO 6888-3: 2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 3: Detection and MPN technique for low numbers.*

APPENDIX A

Summary of Results

Section A1

Salmonella

A1.1

Salmonella Results

Lab Code	A	B	C	D	E	False Results
1	Absent	Present	Present	Present	Absent	
2	Absent	Present	Present	Present	Absent	
3	Absent	Present	Present	Present	Absent	
4	Absent	Present	Present	Present	Absent	
5A	Absent	Present	Present	Present	Absent	
5B	Absent	Present	Present	Present	Absent	
6	**Present**	Present	**Absent**	**Absent**	Absent	**3**
7	Absent	Present	Present	Present	Absent	
8	Absent	Present	Present	Present	Absent	
9	Absent	Present	Present	Present	Absent	
10	Absent	**Absent**	**Absent**	**Absent**	Absent	**3**
11	Absent	Present	Present	Present	Absent	

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	1	1	2	2	0	6
Total Results	12	12	12	12	12	60

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

= 1 / 12

= 8.3%

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

= 1 / 12

= 8.3%

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

= 2 / 12

= 16.7%

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

= 2 / 12

= 16.7%

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

= 0 / 12

= 0%

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

= 6 / 60

= 10.0%

Section A2

Listeria

A2.1

***Listeria* Results**

Lab Code	A	B	C	D	E	False Results
1	Absent	Absent	Present	Present	Present	
4	Absent	Absent	Present	Present	Present	
5A	Absent	Absent	Present	Present	Present	
5B	Absent	Absent	Present	Present	Present	
9	Absent	Absent	Present	Present	Present	
10	**Present**	Absent	**Absent**	**Absent**	**Absent**	**4**
11	Absent	Absent	Present	Present	Present	

Note:

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

A2.2

Listeria Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	1	0	1	1	1	4
Total Results	7	7	7	7	7	35

Failure rate (Sample A) $= \frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}}$
= 1 / 7
= 14.3%

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

Failure rate (Sample C) $= \frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}}$
= 1 / 7
= 14.3%

Failure rate (Sample D) $= \frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 1 / 7
= 14.3%

Failure rate (Sample E) $= \frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 1 / 7
= 14.3%

Overall failure rate (*Listeria*) $= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 4 / 35
= 11.4%

A2.3

Listeria monocytogenes Results

Lab Code	A	B	C	D	E	False Results
1	Absent	Absent	Absent	Present	Present	
4	Absent	Absent	Absent	Present	Present	
5A	Absent	Absent	Absent	Present	Present	
5B	Absent	Absent	Absent	Present	Present	
9	Absent	Absent	Absent	Present	Present	
10	Not done	Not done	Not done	Not done	Not done	
11	Absent	Absent	Absent	Present	Present	

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	6	6	6	6	6	30

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

= 0 / 6

= 0%

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

= 0 / 6

= 0%

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

= 0 / 6

= 0%

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

= 0 / 6

= 0%

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

= 0 / 6

= 0%

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

= 0 / 30

= 0%

Section A3

Staphylococcus aureus

A3.1

Staphylococcus aureus Results

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

Note:

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

A3.2

Staphylococcus aureus Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	1	0	1	1	3
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) $= \frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$

= 1 / 10

= 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) $= \frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$

= 1 / 10

= 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 (*S. aureus)* $= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$

= 3 / 50

= 6.0%

APPENDIX B

Summary of Methods

SECTION B1

Salmonella

Lab Code	*Salmonella* Method Information	
	Detection	Confirmation
1	Culture Method (AS 5013.10 - 2009)	Biochemical tests, O & H serotyping
2	Culture Method (ISO 6579: 2002)	Biochemical tests
4	Rapid Method - Immunoassay (VIDAS)	Biochemical tests, API 20E rapid kit, O & H serotyping, XLD, SMID2 (Chrom Salm)
5A	Rapid Method - Immunoassay (VIDAS)	API 20E rapid kit
5B	Rapid Method - Immunoassay (VIDAS)	API 20E rapid kit
6	Culture Method (FDA Bacteriological Analytical Manual: Chapter 5)	Biochemical tests
7	Molecular Technique (Isothermal amplification of nucleic acid sequence)	-
8	Culture Method (FDA BAM Chapter 5 Nov 2011)	Biochemical tests, API 20E rapid kit
9	Rapid Method - Immunoassay (VIDAS), Molecular Technique (PCR)	Vitek rapid kit, O & H serotyping, O39
10	Culture Method (ISO 6785: 2001 - Pre-enrichment - Enrichment - Isolation - Confirmation Technique)	Isolation on Hektoen agar and Chromatic *Salmonella*, confirmation on rapid 20E
11	Culture Method, Rapid Method - Immunoassay (3M MDS), Molecular Technique (3M MDS)	Biochemical tests, API 20E rapid kit, O serotyping, O & H serotyping

Comments:

1. Laboratory 3 tested the samples for *Salmonella* but did not report the methods they used for their *Salmonella* testing.
2. Laboratory 6 had challenges with serotyping, as agglutination could not clearly be observed, so this component was not used when they interpreted their results.
3. The molecular technique used by laboratory 7 was 3M™ Molecular Detection Assay *Salmonella*.
4. Laboratory 10 had a positive response on the enrichment medium for samples A and E and a positive response on the isolation medium for sample A.

SECTION B2

Listeria

B2.1

Lab Code	*Listeria* Method Information	
	Detection	Confirmation
1	Culture Method (AS 5013.24.1 - 2009)	Biochemical tests, CAMP test, β-haemolysis
4	Rapid Method - Immunoassay (VIDAS)	Biochemical tests, β-haemolysis, *Listeria* API ID rapid kit, catalase, Gram stain, motility
5A	Rapid Method - Immunoassay (VIDAS)	Catalase, motility and Gram stain
5B	Rapid Method - Immunoassay (VIDAS)	Catalase, motility and Gram stain
9	Rapid Method - Immunoassay (VIDAS), Molecular Technique (PCR)	-
10	Culture Method (ISO 11290-1: 1996)	Isolation on Oxford agar. Confirmation by FOOD - system (system for detection and presumptive identification of pathogenic microorganisms from food stuffs)
11	Culture Method (for L. mono identification), Rapid Method - Immunoassay, Molecular Technique (3M MDS)	Biochemical tests, CAMP test, β-haemolysis

Comments:

1. For laboratory 10, only isolation on Oxford agar was possible, without confirmation.

SECTION B3

Staphylococcus aureus

B3.1

Lab Code	*Staphylococcus aureus* Method Information					
	Method Reference	Medium		Plating / Inoculation Details		Confirmation
		Plating Method	MPN Method	Plating Method	MPN Method	
1	AS 5013.12.1 - 2004	Baird-Parker	Not done	1 mL over 3 plates	-	Tube Coagulase
2	ISO 6888-1: 1999	Baird-Parker	-	0.1 mL per plate	-	Coagulase test
3	AOAC 975.55	3M™ Petrifilm™ Staph Express	Trypticase Soy broth	1.0 mL (Petrifilm™)	0.1 g (1 mL of 10^{-1}), 0.01 g (1 mL of 10^{-2}), 0.001 g (1 mL of 10^{-3})	Baird-Parker
5A	-	Baird-Parker	-	0.1 mL per plate	-	Tube Coagulase
5B	-	Baird-Parker	-	0.1 mL per plate	-	Tube Coagulase
6	FDA Bacteriological Analytical Manual: Chapter 12	Baird-Parker	Not done	1 mL over 3 plates	-	Tube Coagulase
9	AS 5013.12.3 (2004), AS 5013.12.1 (2004)	Baird-Parker, Modified Giolitti & Cantoni broth (AS 5013)	-	1 mL over 3 plates	-	Rabbit Plasma Fibrinogen agar
10	ISO 6888-3: 2004	Baird-Parker	-	1 mL over 3 plates	-	STAF 18-R
11	-	Baird-Parker	-	1 mL over 3 plates	-	-

Comments:

1. Laboratory 3 used Baird-Parker agar to confirm the results of their Petrifilm^{TM} testing.
2. Laboratory 4 tested the samples for *Staphylococcus aureus* but did not report the methods they used for their *Staphylococcus aureus* testing.
3. The colonies observed on Baird-Parker agar by laboratory 6 appeared white in colour for all samples.
4. For laboratory 10, isolation on Chapman agar gave *Staphylococcus* spp, after Gram staining of all samples.

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 13 August 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 ISO 6579: 2002 (E). For *Listeria*, the method of testing was enumeration via spread plate technique using PALCAM agar. The samples were verified using ISO 11290-1: 1996 / Amdt 1: 2004. For *Staphylococcus aureus*, the method of testing was enumeration via spread plate technique using Baird-Parker agar. The samples were verified using AS 5013.12.1: 2004.

The results are tabulated below.

Sample A (negative sample)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
10	Not detected	Not detected	Not detected	Not detected
30	Not detected	Not detected	Not detected	Not detected
43	Not detected	Not detected	Not detected	Not detected
47	Not detected	Not detected	Not detected	Not detected
58	Not detected	Not detected	Not detected	Not detected
66	Not detected	Not detected	Not detected	Not detected
81	Not detected	Not detected	Not detected	Not detected
95	Not detected	Not detected	Not detected	Not detected
102	Not detected	Not detected	Not detected	Not detected
118	Not detected	Not detected	Not detected	Not detected

Sample B (containing *Salmonella* Adelaide)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
18	Detected	Not detected	Not detected	Not detected
21	Detected	Not detected	Not detected	Not detected
32	Detected	Not detected	Not detected	Not detected
36	Detected	Not detected	Not detected	Not detected
50	Detected	Not detected	Not detected	Not detected
53	Detected	Not detected	Not detected	Not detected
67	Detected	Not detected	Not detected	Not detected
70	Detected	Not detected	Not detected	Not detected
89	Detected	Not detected	Not detected	Not detected
112	Detected	Not detected	Not detected	Not detected

C1.2

Sample C (containing *Salmonella* Senftenberg, *Listeria innocua* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
7	Detected	Detected	Not detected	Detected
15	Detected	Detected	Not detected	Detected
26	Detected	Detected	Not detected	Detected
36	Detected	Detected	Not detected	Detected
37	Detected	Detected	Not detected	Detected
67	Detected	Detected	Not detected	Detected
70	Detected	Detected	Not detected	Detected
80	Detected	Detected	Not detected	Detected
112	Detected	Detected	Not detected	Detected
113	Detected	Detected	Not detected	Detected

Sample D (containing *Salmonella* Derby, *L. monocytogenes* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
23	Detected	Detected	Detected	Detected
29	Detected	Detected	Detected	Detected
49	Detected	Detected	Detected	Detected
50	Detected	Detected	Detected	Detected
53	Detected	Detected	Detected	Detected
96	Detected	Detected	Detected	Detected
102	Detected	Detected	Detected	Detected
111	Detected	Detected	Detected	Detected
115	Detected	Detected	Detected	Detected
118	Detected	Detected	Detected	Detected

Sample E (containing *L. monocytogenes* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
2	Not detected	Detected	Detected	Detected
32	Not detected	Detected	Detected	Detected
34	Not detected	Detected	Detected	Detected
36	Not detected	Detected	Detected	Detected
38	Not detected	Detected	Detected	Detected
65	Not detected	Detected	Detected	Detected
70	Not detected	Detected	Detected	Detected
88	Not detected	Detected	Detected	Detected
91	Not detected	Detected	Detected	Detected
107	Not detected	Detected	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 and stored at ambient temperature for 3 days. Samples were then tested (using the same media as detailed in the homogeneity section) by Global Proficiency Ltd (New Zealand). The results are tabulated below.

Sample A (negative sample)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
21	Not detected	Not detected	Not detected	Not detected
41	Not detected	Not detected	Not detected	Not detected
67	Not detected	Not detected	Not detected	Not detected

Sample B (containing *Salmonella* Adelaide)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
24	Detected	Not detected	Not detected	Not detected
66	Detected	Not detected	Not detected	Not detected
109	Detected	Not detected	Not detected	Not detected

Sample C (containing *Salmonella* Senftenberg, *Listeria innocua* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
20	Detected	Detected	Not detected	Detected
47	Detected	Detected	Not detected	Detected
92	Detected	Detected	Not detected	Detected

Sample D (containing *Salmonella* Derby, *L. monocytogenes* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
72	Detected	Detected	Detected	Detected
77	Detected	Detected	Detected	Detected
109	Detected	Detected	Detected	Detected

Sample E (containing *L. monocytogenes* and *S. aureus*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*S. aureus*
17	Not detected	Detected	Detected	Detected
79	Not detected	Detected	Detected	Detected
119	Not detected	Detected	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

PROFICIENCY TESTING AUSTRALIA
PATHOGENS IN FOOD PROGRAM – ROUND 28

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 60 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 *Staphylococcus aureus* 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 *S. aureus* 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 25 g for each of the *Salmonella* and *Listeria* tests to be performed. Add 225 mL enrichment broth. Mix to dissolve the milk powder. Add 1 mL of the rehydrated vial contents and continue as per your Standard method.
   Please note: *S. aureus* testing: Aliquots are to be removed from the initial non-selective *Salmonella* enrichment once prepared. If following the plating procedure for *S. aureus*, levels have been designed to suit the inoculum volume of 1 mL (over 3 plates) if following AS 5013.12.1 – 2004.
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 *S. aureus* 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 Thursday 10 October 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 28) 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
Staphylococcus aureus	❍ 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 Method Information 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: ____

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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: *Staphylococcus aureus* (Presence/Absence)

Laboratory Code [ ]

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

Method (please indicate method reference below):	**Plating Method**	**Most Probable Number Method**	**Confirmation**
____________ ____________ ____________ ____________	❍ Not done ❍ Baird-Parker ❍ Rabbit Plasma Fibrinogen agar ❍ 3M™ Petrifilm™ Staph Express ❍ Other (please specify) ____________	❍ Not done ❍ Modified Giolitti & Cantoni broth ❍ Trypticase Soy broth ❍ Other (please specify) ____________	❍ Not done ❍ Baird-Parker ❍ Rabbit Plasma Fibrinogen agar ❍ Tube Coagulase ❍ Thermonuclease test ❍ Latex Agglutination (please specify type) ____________ ❍ Other (please specify) ____________
Plating / Inoculation Details	❍ **1 mL over 3 plates (recommended)** ❍ 1.0 mL (Petrifilm™) ❍ 0.1 mL per plate ❍ Other (please specify) ____________	❍ 1 g (10 mL of 10^{-1}) ❍ 0.1 g (1 mL of 10^{-1}) ❍ 0.01 g (1 mL of 10^{-2}) ❍ Other (please specify) ____________	

Comments: ____

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