

REPORT NO. 1084

**Pathogens In Food
Proficiency Testing Program
Round 37**

June 2018

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1. FOREWORD

This report summarises the results of round thirty-seven of a series of proficiency testing programs involving the analysis of different food types for the detection of a range of pathogens. This program is accredited to ISO/IEC 17043:2010 “*Conformity assessment - General requirements for proficiency testing*” by International Accreditation New Zealand (IANZ).

Proficiency Testing Australia conducted the program in April / May 2018. 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 Mrs S Mott, Global Proficiency Ltd (New Zealand). This report was authorised by Mrs K Cividin, PTA Quality Manager.

2. FEATURES OF THE PROGRAM

- (a) A total of nine laboratories received samples, all of which returned 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 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.
- (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* spp., *Listeria monocytogenes* (*L. monocytogenes*) and *Cronobacter* spp. 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 g of each sample for *Salmonella*, *Listeria* spp. and *Listeria monocytogenes* and 10 g of each sample for *Cronobacter* spp.

- Sample A contained *Salmonella* Senftenberg (H₂S negative strain) and *Listeria monocytogenes*.
- Sample B contained *Salmonella* Derby and *Listeria innocua*.
- Sample C contained *Cronobacter sakazakii*.
- Sample D contained *Salmonella* Adelaide and *Listeria monocytogenes*.
- Sample E contained *Cronobacter sakazakii*.

Other “typical” microflora was included in the samples (e.g. *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, etc.) The levels of *Salmonella* and *Listeria* in each sample were between 100 – 500 cfu per 25 g. The levels of *Cronobacter* in each sample were between 50 – 100 cfu per 10 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, 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 table on the following page.

Table A: False Results
(by laboratory code number)

Presence / Absence of <i>Salmonella</i> in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B		-
C	-	
D		-
E	-	
Presence / Absence of <i>Listeria</i> in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B		-
C	7	
D		-
E	-	
Presence / Absence of <i>L. monocytogenes</i> in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	7	
C	-	
D		-
E	-	
Presence / Absence of <i>Cronobacter</i> in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B	-	
C		-
D	-	
E		-

7. TECHNICAL COMMENTS

Response Rate

All nine of the laboratories that participated in the program submitted results for inclusion in the final report. All of these nine laboratories reported results for *Salmonella*. Seven of the nine participants (78%) reported results for *Listeria* spp. Six of the nine participants (67%) reported results for *Listeria monocytogenes*. Two of the nine participants (22%) reported results for *Cronobacter* spp.

Salmonella Results

The results submitted by participants for *Salmonella* are presented in Appendix A1.

There were no false positive or false negative results reported for any of the samples for *Salmonella*.

Three different techniques were used in the detection of *Salmonella* in this round including:

- **Culture methods - Confirmatory testing:**
Culture methods (AOAC 967.26 Chpt17/9-2012 and BAM) followed by confirmatory testing using biochemical testing, rapid kits and serotyping.
- **Culture methods – Immunoassay systems:**
Two methods including a culture method - AS 5013.10-2009 and a rapid method (not detailed), both followed by the use of the VIDAS® Immunoassay. The laboratory using the culture method undertook confirmatory testing using biochemical testing and serotyping.
- **Culture methods – Molecular techniques:**
Culture methods (one referenced as AS 5013.10 and ISO 6785; one referenced as AS 5013.10; another stated both AS/ISO and internal methods were used and one referenced BAM) followed by a Molecular technique. One laboratory stated using the 3M™ Molecular Detection System (MDS) followed by confirmatory testing using biochemical testing, rapid kits and serotyping, and four laboratories referenced using PCR systems - also followed by biochemical testing, rapid kits and serotyping for the confirmatory processes.

Details of the culture methods referenced are included below:

- AS5013.10:2009 - Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.; equivalent to ISO 6579:2002/Cor.1:2004*
- ISO 6785* – *Salmonella* in Milk and Milk Products
- AOAC 967.26: *Salmonella* in Processed Foods

***Note:** ISO 6579-1:2017 – Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. has superseded the following methods:

- ISO 6579:2002/Cor.1:2004
- ISO 6579:2002/Amd 1:2007
- ISO 6785:2001.

Subsequent detection of *Salmonella* following culturing/enrichment is reliant on the cells present being able to grow to levels that are within the detection limits or threshold level for the test, and method selection must consider the product type under test. Based on the culture method information provided, we would not expect to see any false-negative results for the *Salmonella* species included in the milk powder samples in this round.

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.

Laboratory 7 reported a false positive for sample C for *Listeria* spp. and a false positive for sample B for *L. monocytogenes*. Laboratory 3 incorrectly reported that sample B contained *Listeria welshimeri*, whereas sample B actually contained *Listeria innocua*. *Listeria welshimeri* is closely related to *Listeria innocua* and, in standard biochemical tests, is differentiated by the carbohydrate tests, where *Listeria welshimeri* gives a positive xylose test and *Listeria innocua* is negative.

Three different techniques were used in the detection of *Listeria* / *L. monocytogenes* in this round including:

- **Culture methods - Confirmatory testing:**
Culture methods (AOAC 993.12 Chpt17/10-2012 and BAM followed by confirmatory testing using rapid kits.
- **Culture methods – Immunoassay systems:**
Culture method (AS 5013.24.1-2009) followed by the use of the VIDAS® Immunoassay system. Confirmatory testing undertaken included biochemical testing, haemolysis and the CAMP test.
- **Culture methods – Molecular techniques:**
Culture methods (one referenced AS 5013.24.1-2009) followed by a Molecular technique using the 3M™ Molecular Detection System (MDS) followed by confirmatory testing using biochemical testing, serotyping and Gram staining, and three laboratories referenced using PCR systems - also followed by biochemical testing, haemolysis and the CAMP test, rapid kits, and serotyping for the confirmatory processes.

Details of the culture methods referenced are included below:

- AS 5013.24.1-2009 Microbiology of food and animal feeding stuffs - Horizontal method for *Listeria monocytogenes*, which is an adoption with national modifications and reproduced from ISO 11290-1:1996, including Amendment 1:2004*
- AOAC 993.12 *Listeria monocytogenes* in milk and dairy products.

***Note:** ISO 11290-1: 2017 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method has superseded the following methods:

- ISO 11290-1:1996
- ISO 11290-1:1996 / Amd 1:2004
- AS 5013.17 was listed as a culture method, however this is the Australian method used for the preparation of milk products for microbiological testing: “Milk and milk products - General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination”.

Based on the culture method information provided, we would not expect to see any false-negative results for the *Listeria* species included in the milk powder samples in this round.

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

***Cronobacter* Results**

The results submitted by participants for *Cronobacter* are presented in Appendix A3.

There were no false positive or false negative results reported for any of the samples for *Cronobacter*.

Two laboratories submitted results in this round and both used different techniques for the detection of *Cronobacter* spp. including:

- **Culture methods - Confirmatory testing:**
Culture method ISO/TS 22964 – 2005 / AS 5013.13 – 2010* followed by confirmatory testing using chromogenic agar; pigmentation on TSA and MALDI-TOF rapid kit. Although this culture method has been superseded, as discussed below, the organism used for the positive samples is *Enterobacter sakazakii*, so it would still be expected to provide the correct results.
- **Culture methods – Molecular techniques:**
Full culture details not provided but the use of confirmatory testing using chromogenic and VRBG agars; pigmentation on TSA and PCR-BAX was stated as being used.

***Note:** AS 5013.13 (Int) – 2010 (ISO/TS 22964:2006): Milk and milk products - Detection of *Enterobacter sakazakii* expired 15 June 2012 and has since been superseded by AS 5013.13:2018: Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp. (ISO 22964:2017, MOD). This method does differ significantly in that the scope has been extended to include *Cronobacter* species; the selective enrichment and plating processes with regard to the media used and temperatures of incubation has changed, as have the confirmatory tests applied to presumptive isolates.

Detailed method information for *Cronobacter* is provided in Appendix B3.

8. REFERENCES

1. *Guide to Proficiency Testing Australia (2016)*. (This document is located on the PTA website at www.pta.asn.au under Programs / Documents).
2. ISO/IEC 17043: 2010 *Conformity assessment - General requirements for proficiency testing*.
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 5013.13: 2018 *Food microbiology – Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp. (ISO 22964:2017, MOD)*.
5. AS 5013.17: 2004 *Food microbiology – Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*.
6. 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)*.
7. AOAC 967.26 *Salmonella in processed foods. Detection*.
8. AOAC 993.12 *Listeria monocytogenes in milk and dairy products. Selective enrichment and isolation method*.
9. ISO 6579-1:2017 *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: Detection of Salmonella spp.*
10. ISO 6785: 2001 *Milk and milk products – Detection of Salmonella spp.*
11. ISO 11290-1: 2017 *Microbiology of the food chain – Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. – Part 1: Detection method*.
12. ISO 22964: 2017 *Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp.*
13. AS 5013.13:2018: *Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp. (ISO 22964:2017, MOD)*.

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	
6	Present	Present	Absent	Present	Absent	
7	Present	Present	Absent	Present	Absent	
8A	Present	Present	Absent	Present	Absent	
8B	Present	Present	Absent	Present	Absent	
10	Present	Present	Absent	Present	Absent	
11	Present	Present	Absent	Present	Absent	

A1.2

Salmonella Failure Rate

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

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

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

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

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

$$\begin{aligned}\text{Failure rate (Sample E)} &= \frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}} \\ &= 0 / 10 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Overall failure rate} &= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}} \\ \text{(Salmonella)} &= 0 / 50 \\ &= 0\%\end{aligned}$$

Section A2

Listeria

A2.1

Listeria Results

Lab Code	A	B	C	D	E	False Results
1	Present	Present	Absent	Present	Absent	1
2	Present	Present	Absent	Present	Absent	
3	Present	Present	Absent	Present	Absent	
4	Present	Present	Absent	Present	Absent	
7	Present	Present	Present	Present	Absent	
8A	Present	Present	Absent	Present	Absent	
8B	Present	Present	Absent	Present	Absent	
11	Present	Present	Absent	Present	Absent	

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	0	0	1	0	0	1
Total Results	8	8	8	8	8	40

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

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

$$\begin{aligned}\text{Failure rate (Sample C)} &= \frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}} \\ &= 1 / 8 \\ &= 12.5\%\end{aligned}$$

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

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

$$\begin{aligned}\text{Overall failure rate} &= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}} \\ \text{(Listeria)} &= 1 / 40 \\ &= 2.5\%\end{aligned}$$

A2.3

Listeria monocytogenes Results

Lab Code	A	B	C	D	E	False Results
1	Present	Absent	Absent	Present	Absent	1
3	Present	Absent	Absent	Present	Absent	
4	Present	Absent	Absent	Present	Absent	
7	Present	Present	Absent	Present	Absent	
8A	Present	Absent	Absent	Present	Absent	
11	Present	Absent	Absent	Present	Absent	

Note:

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

A2.4

Listeria monocytogenes Failure Rate

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	1	0	0	0	1
Total Results	6	6	6	6	6	30

$$\begin{aligned}
 \text{Failure rate (Sample A)} &= \frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}} \\
 &= 0 / 6 \\
 &= 0\%
 \end{aligned}$$

$$\begin{aligned}
 \text{Failure rate (Sample B)} &= \frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}} \\
 &= 1 / 6 \\
 &= 16.7\%
 \end{aligned}$$

$$\begin{aligned}
 \text{Failure rate (Sample C)} &= \frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}} \\
 &= 0 / 6 \\
 &= 0\%
 \end{aligned}$$

$$\begin{aligned}
 \text{Failure rate (Sample D)} &= \frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}} \\
 &= 0 / 6 \\
 &= 0\%
 \end{aligned}$$

$$\begin{aligned}
 \text{Failure rate (Sample E)} &= \frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}} \\
 &= 0 / 6 \\
 &= 0\%
 \end{aligned}$$

$$\begin{aligned}
 \text{Overall failure rate} &= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}} \\
 \text{(*L. monocytogenes*)} &= 1 / 30 \\
 &= 3.3\%
 \end{aligned}$$

Section A3

Cronobacter

A3.1

***Cronobacter* Results**

Lab Code	A	B	C	D	E	False Results
1	Absent	Absent	Present	Absent	Present	
4	Absent	Absent	Present	Absent	Present	

A3.2

***Cronobacter* Failure Rate**

No. of Results	Sample					Total
	A	B	C	D	E	
False Results	0	0	0	0	0	0
Total Results	2	2	2	2	2	10

$$\begin{aligned}\text{Failure rate (Sample A)} &= \frac{\text{No. of False Results (A)}}{\text{Total No. of Results (A)}} \\ &= 0 / 2 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Failure rate (Sample B)} &= \frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}} \\ &= 0 / 2 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Failure rate (Sample C)} &= \frac{\text{No. of False Results (C)}}{\text{Total No. of Results (C)}} \\ &= 0 / 2 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Failure rate (Sample D)} &= \frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}} \\ &= 0 / 2 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Failure rate (Sample E)} &= \frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}} \\ &= 0 / 2 \\ &= 0\%\end{aligned}$$

$$\begin{aligned}\text{Overall failure rate} &= \frac{\text{Total No. of False Results}}{\text{Total No. of Results}} \\ \text{(Cronobacter)} &= 0 / 10 \\ &= 0\%\end{aligned}$$

APPENDIX B

Summary of Methods

SECTION B1

Salmonella

B1.1

Lab Code	<i>Salmonella</i> Method Information	
	Detection	Confirmation
1	Rapid Method - Immunoassay (VIDAS), Molecular Technique (BAX 07 PCR Dupont)	Biochemical tests, O & H serotyping
2	Culture Method (AOAC 967.26, Chapter 17, Sub Chapter 9, 19th Edition, 2012)	Microgen GN-ID rapid kit, agglutination testing (Microgen <i>Salmonella</i>)
3	Culture Method (AS / ISO + internal methods), Molecular Technique (Thermofisher Piko Real-Time PCR)	API 20E rapid kit
4	Culture Method (AS 5013.10 - 2009), Rapid Method - Immunoassay (VIDAS)	Biochemical tests, O & H serotyping
6	Culture Method (AOAC 967.26)	API 20E rapid kit
7	Culture Method (AS 5013.10), Molecular Technique (BAX <i>Salmonella</i> PCR)	Biochemical tests, O & H serotyping
8A	Culture Method (BAM Method)	Biochemical tests
8B	Culture Method (BAM Method), Molecular Technique (PCR)	-
10	Rapid Method - Immunoassay (VIDAS)	-
11	Culture Method (AS 5013.10 / ISO 6785), Molecular Technique (3M Molecular Detection System)	Biochemical tests, API 20E rapid kit, O & H serotyping

SECTION B2

Listeria

B2.1

Lab Code	<i>Listeria</i> Method Information	
	Detection	Confirmation
1	Molecular Technique (BAX 07 PCR Dupont)	Biochemical tests
2	Culture Method (AOAC 993.12, Chapter 17, Sub Chapter 10, 19th Edition, 2012)	Microgen <i>Listeria</i> ID rapid kit
3	Culture Method (AS / ISO + internal methods), Molecular Technique (Thermofisher Piko Real-Time PCR)	β haemolysis, <i>Listeria</i> API ID rapid kit
4	Culture Method (AS 5013.24.1 - 2009), Rapid Method - Immunoassay (VIDAS)	Biochemical tests, CAMP test, β -haemolysis
7	Culture Method (AS 5013.17), Molecular Technique (BAX <i>Listeria</i> PCR)	Biochemical tests, CAMP test, β -haemolysis
8A	Culture Method (BAM Method)	-
8B	Culture Method (BAM Method)	-
11	Culture Method (AS 5013.24.1 - 2009), Molecular Technique (3M Molecular Detection System)	Biochemical tests, CAMP test, β -haemolysis

Comments:

1. Laboratory 1 reported that the Catalase test was performed for biochemical confirmation.
2. Laboratory 3 reported that sample B contained *Listeria welshimeri*.

SECTION B3

Cronobacter

B3.1

Lab Code	<i>Cronobacter</i> Method Information	
	Detection	Confirmation
1	Molecular Technique (BAX 07 PCR Dupont)	Chromogenic agar, Yellow pigmentation on TSA, PCR Confirmation (BAX 07 PCR Dupont)
4	Culture Method (ISO/TS 22964 - 2005 / AS 5013.13 - 2010)	Chromogenic agar, Yellow pigmentation on TSA, MALDI-TOF rapid kit

Comments:

Laboratory 1 reported that VRBGA (Violet Red Bile Glucose Agar) was used for confirmation, along with Chromogenic agar and TSA.

APPENDIX C

Homogeneity and Stability Testing

C1.1

HOMOGENEITY TESTING RESULTS

Five 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. 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 *Cronobacter*, the method of testing was enumeration via spread plate technique using the chromogenic agar ESIA. The samples were verified using a New Zealand method based on ISO/TS 22964 / IDF/RM 210: 2006 (E): Milk and milk products – Detection of *Enterobacter sakazakii* (samples were verified prior to the issue of AS 5013.13:2018: Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp. (ISO 22964: 2017, MOD)).

The results are tabulated below.

Sample A (containing <i>Salmonella</i> Senftenberg and <i>L. monocytogenes</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
4	Detected	Detected	Detected	Not detected
9	Detected	Detected	Detected	Not detected
16	Detected	Detected	Detected	Not detected
43	Detected	Detected	Detected	Not detected
66	Detected	Detected	Detected	Not detected

Sample B (containing <i>Salmonella</i> Derby and <i>Listeria innocua</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
9	Detected	Detected	Not detected	Not detected
13	Detected	Detected	Not detected	Not detected
23	Detected	Detected	Not detected	Not detected
29	Detected	Detected	Not detected	Not detected
57	Detected	Detected	Not detected	Not detected

Sample C (containing <i>Cronobacter sakazakii</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
21	Not detected	Not detected	Not detected	Detected
23	Not detected	Not detected	Not detected	Detected
29	Not detected	Not detected	Not detected	Detected
36	Not detected	Not detected	Not detected	Detected
46	Not detected	Not detected	Not detected	Detected

C1.2

Sample D (containing <i>Salmonella</i> Adelaide and <i>L. monocytogenes</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
9	Detected	Detected	Detected	Not detected
12	Detected	Detected	Detected	Not detected
55	Detected	Detected	Detected	Not detected
62	Detected	Detected	Detected	Not detected
70	Detected	Detected	Detected	Not detected

Sample E (containing <i>Cronobacter sakazakii</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
11	Not detected	Not detected	Not detected	Detected
13	Not detected	Not detected	Not detected	Detected
30	Not detected	Not detected	Not detected	Detected
40	Not detected	Not detected	Not detected	Detected
43	Not detected	Not detected	Not detected	Detected

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

C2.1

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 4 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 (containing <i>Salmonella</i> Senftenberg and <i>L. monocytogenes</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
29	Detected	Detected	Detected	Not detected
34	Detected	Detected	Detected	Not detected
69	Detected	Detected	Detected	Not detected

Sample B (containing <i>Salmonella</i> Derby and <i>Listeria innocua</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
40	Detected	Detected	Not detected	Not detected
41	Detected	Detected	Not detected	Not detected
50	Detected	Detected	Not detected	Not detected

Sample C (containing <i>Cronobacter sakazakii</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
7	Not detected	Not detected	Not detected	Detected
9	Not detected	Not detected	Not detected	Detected
22	Not detected	Not detected	Not detected	Detected

Sample D (containing <i>Salmonella</i> Adelaide and <i>L. monocytogenes</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
8	Detected	Detected	Detected	Not detected
16	Detected	Detected	Detected	Not detected
20	Detected	Detected	Detected	Not detected

Sample E (containing <i>Cronobacter sakazakii</i>)				
Sample	<i>Salmonella</i>	<i>Listeria</i>	<i>L. monocytogenes</i>	<i>Cronobacter</i>
24	Not detected	Not detected	Not detected	Detected
27	Not detected	Not detected	Not detected	Detected
29	Not 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

**PROFICIENCY TESTING AUSTRALIA
PATHOGENS IN FOOD PROGRAM – ROUND 37**
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*, *Listeria* and *Cronobacter* as per instructions below.
- An extra 60 g whole milk powder sample, labelled Spare, has also been provided in case more milk powder sample is needed for testing.
- 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.
- 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.

***Salmonella* & *Listeria* testing:**

4. Aseptically open the sachets. Weigh out 25 g powder for each of the *Salmonella* and *Listeria* tests to be performed. Add 225 mL enrichment broth. Mix to dissolve the milk powder. Add 0.5 mL of the rehydrated vial contents to each enrichment and proceed as per your Standard method.
5. 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* or *Listeria* are not detected in a sample then this should be indicated by filling in (●) in the circle alongside “absent”.

D1.2

***Cronobacter* testing:**

6. Aseptically open the sachets. Weigh out 10 g of the powder. Add 90 mL (pre) enrichment broth. Mix to dissolve the milk powder. Add 0.5 mL of the rehydrated vial contents and proceed as per your Standard method.
7. Report results as presence or absence per 10 gram of sample in Table A of the supplied Results Sheets by filling in (●) in the appropriate circles. If *Cronobacter* is not detected in a sample then this should be indicated by filling in (●) in the circle alongside “absent”.

Method reporting:

8. 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 Friday 27 April 2018** 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 37) Proficiency Testing Program

RESULTS SHEET

Date samples arrived	Sample temperature	Date testing began	Signature

Laboratory Code:

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Table A: Results

Test	Sample A	Sample B	Sample C	Sample D	Sample E
<i>Salmonella</i>	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done
<i>Listeria</i>	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done
<i>Listeria monocytogenes</i>	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done
<i>Cronobacter</i>	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done	<input type="radio"/> Present <input type="radio"/> Absent <input type="radio"/> Not done

Laboratory Code

Table B: Method Information: *Salmonella*

Please indicate the Method Information used by filling in (●) in the appropriate circles below:

Detection	Confirmation
<p><input type="radio"/> Culture Method (please specify method reference)</p> <p>_____</p> <p><input type="radio"/> Rapid Methods - Immunoassay</p> <p style="margin-left: 20px;"><input type="radio"/> TECRA</p> <p style="margin-left: 20px;"><input type="radio"/> VIDAS</p> <p><input type="radio"/> Other (please specify)</p> <p>_____</p> <p><input type="radio"/> Molecular Techniques (please specify system used)</p> <p style="margin-left: 20px;"><input type="radio"/> PCR_____</p> <p style="margin-left: 20px;"><input type="radio"/> DNA probe_____</p> <p style="margin-left: 20px;"><input type="radio"/> Other (please state)</p> <p>_____</p>	<p><input type="radio"/> Biochemical tests</p> <p><input type="radio"/> Rapid kits</p> <p style="margin-left: 20px;"><input type="radio"/> API 20E</p> <p style="margin-left: 20px;"><input type="radio"/> Microbact 12E</p> <p style="margin-left: 20px;"><input type="radio"/> Other (please specify)</p> <p>_____</p> <p><input type="radio"/> Serotyping</p> <p style="margin-left: 20px;"><input type="radio"/> O</p> <p style="margin-left: 20px;"><input type="radio"/> O & H</p> <p><input type="radio"/> Agglutination testing (please specify kit)</p> <p>_____</p> <p><input type="radio"/> Other (please specify)</p> <p>_____</p>

Comments:

Laboratory Code

Table C: Method Information: *Listeria*

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

Detection	Confirmation
<input type="radio"/> Culture Method (please specify method reference) <hr/> <input type="radio"/> Rapid Methods - Immunoassay <ul style="list-style-type: none"> <input type="radio"/> TECRA <input type="radio"/> VIDAS <input type="radio"/> Other (please specify) <hr/> <input type="radio"/> Molecular Techniques (please specify system used) <ul style="list-style-type: none"> <input type="radio"/> PCR <hr/> <input type="radio"/> DNA probe <hr/> <input type="radio"/> Other (please state) <hr/>	<input type="radio"/> Biochemical tests <ul style="list-style-type: none"> <input type="radio"/> CAMP test <input type="radio"/> β-haemolysis <input type="radio"/> Rapid kits <ul style="list-style-type: none"> <input type="radio"/> <i>Listeria</i> API ID <input type="radio"/> Microbact 12L <input type="radio"/> Other (please specify) <hr/> <input type="radio"/> Serological testing (please specify) <hr/> <input type="radio"/> Other (please specify) <hr/>

Comments:

Laboratory Code

Table D: Method Information: *Cronobacter*

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

Detection	Confirmation
<input type="radio"/> Culture Method (please specify method reference) _____ <input type="radio"/> Rapid Methods - Immunoassay <input type="radio"/> TECRA <input type="radio"/> VIDAS <input type="radio"/> Other (please specify) _____ <input type="radio"/> Molecular Techniques (please specify system used) <input type="radio"/> PCR _____ <input type="radio"/> Other (please specify) _____	<input type="radio"/> Chromogenic agar <input type="radio"/> Yellow pigmentation on TSA <input type="radio"/> Biochemical tests <input type="radio"/> Rapid kits <input type="radio"/> Rapid ID 32E <input type="radio"/> VITEK 2.0 <input type="radio"/> Other (please specify) _____ <input type="radio"/> PCR Confirmation (please specify system used) _____ <input type="radio"/> Other (please specify) _____

Comments:

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