REPORT NO. 1137

Pathogens In Food

Proficiency Testing Program

Round 39

June 2019

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) and Global Proficiency Pty Ltd (Australia) for producing and distributing the samples.

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APPENDICES

1. FOREWORD

This report summarises the results of round thirty-nine 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 2019. 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 11 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 70 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.

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* Derby and *Listeria innocua*.
- Sample B contained *Cronobacter sakazakii*.
- Sample C contained *Cronobacter sakazakii*.
- Sample D contained *Salmonella* Senftenberg (H_2S negative strain) and *Listeria monocytogenes*.
- Sample E contained *Salmonella* Adelaide and *Listeria monocytogenes*.

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 *Salmonella* in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	-	
C	-	
D		11
E		-
Presence / Absence of *Listeria* in Whole Milk Powder		
Sample	False Positives	False Negatives
A		-
B	11	
C	-	
D		-
E		-
Presence / Absence of *L. monocytogenes* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B	-	
C	-	
D		9
E		-
Presence / Absence of *Cronobacter* in Whole Milk Powder		
Sample	False Positives	False Negatives
A	-	
B		-
C		-
D	-	
E	-	

7. TECHNICAL COMMENTS

Response Rate

Ten of the 11 laboratories (91%) that participated in the program submitted results for inclusion in the final report. All of these ten laboratories reported results for *Salmonella.* Eight of these ten laboratories (80%) reported results for *Listeria* spp. Seven of these ten laboratories (70%) reported results for *Listeria monocytogenes*. Three of these ten laboratories (30%) reported results for *Cronobacter* spp.

Salmonella Results

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

Laboratory code 11 reported a false negative for sample D. This sample contained an H_2S-negative strain of *Salmonella* Senftenberg and *Listeria monocytogenes*.

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

- **Culture methods - Confirmatory testing:**
  Culture methods (FDA-BAM – Chapter 5 and AOAC 967.26 – Chapter 17, Sub-Chapter 9) followed by confirmatory testing using biochemical testing, rapid kits and agglutination.
- **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 system. The laboratory using AS 5013.10-2009 undertook confirmatory testing using biochemical testing and serotyping.
- **Culture methods – Molecular techniques:**
  Culture methods (one participant referenced AS 5013.10 and ISO 6785, and one referenced ISO 6579-1) 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; another used the same system combined with the use of a rapid test kit tests in the confirmatory processes. Four participants stated they had used PCR systems (BAX (2) and Thermofisher Real-Time (2)), followed by the use of one, or a combination of biochemical testing, rapid kits and serotyping for the confirmatory processes.

Details of the culture methods referenced are included below:

- US Food & Drug Administration Bacteriological Analytical Manual (BAM): Chapter 5 – *Salmonella* (July 2018).
- AOAC 967.26 – *Salmonella* in processed foods**.**
- ISO 6785 – *Salmonella* in Milk and Milk Products

- AS 5013.10:2009 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.; equivalent to ISO 6579:2002/Cor.1:2004*****

***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.

The emergence of H_2S-negative *Salmonella* serotypes including *S. enterica* serovar Senftenberg, *S. enterica* serovar Typhimurium and *S. enterica* serovar Infantis, for example, is well documented (see References – 13), so additional steps may be required in the selective plating stages of the culture method to ensure H_2S-negative colonies are not overlooked. Some researchers recommend the use of chromogenic media, which are not reliant on the production of sulphide for the differentiation of *Salmonella* from other members of the Enterobacteriaceae.

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.

Laboratory code 11 reported a false positive for sample B for *Listeria* spp. This sample contained *Cronobacter sakazakii* only.

Laboratory code 9 reported a false negative for sample D for *Listeria monocytogenes*. This sample contained an H_2S negative strain of *Salmonella* Senftenberg and *Listeria monocytogenes*.

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

- **Culture methods - Confirmatory testing:**
  Culture methods (FDA-BAM Chapter 10 and APHA Chapter 35) followed by confirmatory testing using a rapid test kit.
- **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 (AS 5013.24.1-2009 and ISO 11290-1: 2017) followed by the 3M™ Molecular Detection System (MDS) with confirmatory testing using biochemical testing, haemolysis, CAMP test and rapid test kits. Three laboratories referenced culture methods (IS 14988 (ISO 11290-1); others not provided) followed by the use of PCR systems (BAX (2) and Thermofisher Real-Time (1)), with biochemical testing, the CAMP test and rapid test kits for the confirmatory testing processes.

Details of the culture methods referenced are included below:

- US Food & Drug Administration Bacteriological Analytical Manual (BAM): Chapter 10 – Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods (March 2017).
- 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**.**

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*.

- **Culture methods - Confirmatory testing:**
  Culture method ISO/TS 22964 – 2005 (year of method reference is actually 2006) / 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 in this proficiency program is *Enterobacter sakazakii* and, with the test being Presence / Absence only, it would still be expected to provide the correct results. A second laboratory referenced ISO 22964:2017 followed by the use of a chromogenic agar and rapid test kit.
- **Culture methods – Molecular techniques:**
  One laboratory stated they had used the PCR-BAX system (culture method details not provided), with chromogenic agar for the confirmatory testing processes.

***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 5013.24.1: 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).*
6. *AOAC 967.26 Salmonella in processed foods. Detection.*
7. *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.*
8. *ISO 6785: 2001 Milk and milk products – Detection of Salmonella spp.*
9. *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.*
10. *ISO 22964: 2017 Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp.*
11. *US Food & Drug Administration Bacteriological Analytical Manual (BAM): Chapter 5 – Salmonella (July 2018).*
12. *US Food & Drug Administration Bacteriological Analytical Manual (BAM): Chapter 10 – Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods (March 2017)*
13. *J Clin Microbiol. 2014 Jul; 52(7): 2557–2565. Emergence and Prevalence of Non-H_2S-Producing Salmonella enterica Serovar Senftenberg Isolates Belonging to Novel Sequence Type 1751 in China.*

APPENDIX A

Summary of Results

Section A1

Salmonella

A1.1

Salmonella Results

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

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	1	0	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) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$
= 1 / 10
= 10.0%

Failure rate (Sample E) = $\frac{\text{No. of False Results (E)}}{\text{Total No. of Results (E)}}$
= 0 / 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	Absent	Present	Present	
4	Present	Absent	Absent	Present	Present	
5	Present	Absent	Absent	Present	Present	
6	Present	Absent	Absent	Present	Present	
7	Present	Absent	Absent	Present	Present	
9	Present	Absent	Absent	Present	Present	
10	Present	Absent	Absent	Present	Present	
11	Present	**Present**	Absent	Present	Present	**1**

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	1	0	0	0	1
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) = $\frac{\text{No. of False Results (B)}}{\text{Total No. of Results (B)}}$
= 1 / 8
= 12.5%

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 (*Listeria)* = $\frac{\text{Total No. of False Results}}{\text{Total No. of Results}}$
= 1 / 40
= 2.5%

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	
6	Absent	Absent	Absent	Present	Present	
7	Absent	Absent	Absent	Present	Present	
9	Absent	Absent	Absent	**Absent**	Present	**1**
10	Absent	Absent	Absent	Present	Present	
11	Absent	Absent	Absent	Present	Present	

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	0	0	1	0	1
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) = $\frac{\text{No. of False Results (D)}}{\text{Total No. of Results (D)}}$

= 1 / 7

= 14.3%

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

= 0 / 7

= 0%

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

= 1 / 35

= 2.9%

Section A3

Cronobacter

A3.1

Cronobacter Results

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

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	3	3	3	3	3	15

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

= 0 / 3

= 0%

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

= 0 / 3

= 0%

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

= 0 / 3

= 0%

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

= 0 / 3

= 0%

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

= 0 / 3

= 0%

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

= 0 / 15

= 0%

APPENDIX B

Summary of Methods

SECTION B1

Salmonella

Lab Code	***Salmonella* Method Information**	
	Detection	Confirmation
1	Culture Method (AS 5013.10 - 2009), Rapid Method - Immunoassay (VIDAS)	Biochemical tests, O & H serotyping
3	Molecular Techniques (7500 Fast Real-Time PCR Thermo Fisher, Taq Man DNA probe)	VITEK Compact 2 with GN Card rapid kit, agglutination testing (Pro Lab Diagnostics Inc. Monovalent antisera O55), cross confirmed by Chromogenic agar
4	Culture Method (ISO 6579-1: 2017), Molecular Technique (3M Molecular Detection Assay 2 (LAMP))	VITEK 2 rapid kit
5	Culture Method (AOAC 967.26, Chapter 17, Sub Chapter 9, 20th Edition, 2016)	Microgen GN-ID rapid kit, agglutination testing (Microgen *Salmonella*)
6	Molecular Technique (Thermofisher Piko Real-Time PCR)	API 20E rapid kit
7	Molecular Technique (BAX Q7 PCR)	Biochemical tests (TSI, LIA), O & H serotyping
8	Rapid Method - Immunoassay (VIDAS)	-
9	Culture Method, Molecular Technique (BAX PCR)	O & H serotyping
10	Culture Method (AS 5013.10 / ISO 6785), Molecular Technique (3M Molecular Detection System)	Biochemical tests, API 20E rapid kit, O & H serotyping
11	Culture Method (BAM Method Chapter 5)	Biochemical tests, API 20E rapid kit

SECTION B2

Listeria

B2.1

Lab Code	***Listeria* Method Information**	
	Detection	Confirmation
1	Culture Method (AS 5013.24.1 - 2009), Rapid Method - Immunoassay (VIDAS)	Biochemical tests, CAMP test, β-haemolysis
4	Culture Method (ISO 11290-1: 2017), Molecular Technique (3M Molecular Detection Assay 2 (LAMP))	*Listeria* API ID rapid kit, VITEK 2 rapid kit
5	Culture Method (Compendium Methods, 5th Edition, Chapter 35, 2015)	Microgen *Listeria* ID rapid kit
6	Molecular Technique (Thermofisher Piko Real-Time PCR)	β-haemolysis, *Listeria* API ID rapid kit
7	Culture Method (ISO method), Molecular Technique (BAX Q7 PCR)	BAX PCR assay
9	Culture Method, Molecular Technique (BAX PCR)	Biochemical tests, CAMP test, β-haemolysis
10	Culture Method (AS 5013.24.1 - 2009), Molecular Technique (3M Molecular Detection System)	Biochemical tests, CAMP test, β-haemolysis, Microbact 12L rapid kit
11	Culture Method (BAM Method Chapter 10)	-

SECTION B3

Cronobacter

B3.1

Lab Code	***Cronobacter* Method Information**	
	Detection	Confirmation
1	Culture Method (ISO/TS 22964 - 2005 Milk and Milk Products, AS 5013.13 - 2010)	Chromogenic agar, Yellow pigmentation on TSA, MALDITOF rapid kit
4	Culture Method (ISO 22964: 2017)	Chromogenic agar, VITEK 2.0 rapid kit
7	Molecular Technique (BAX Q7 PCR)	Chromogenic agar, BAX PCR assay

APPENDIX C

Homogeneity and Stability Testing

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 modification of the New Zealand method based on ISO/TS 22964 / IDF/RM 210: 2006 (E): Milk and milk products – Detection of *Enterobacter sakazakii*.

The results are tabulated below.

Sample A (containing *Salmonella* Derby and *Listeria innocua*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
10	Detected	Detected	Not detected	Not detected
11	Detected	Detected	Not detected	Not detected
12	Detected	Detected	Not detected	Not detected
17	Detected	Detected	Not detected	Not detected
27	Detected	Detected	Not detected	Not detected

Sample B (containing *Cronobacter sakazakii*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
10	Not detected	Not detected	Not detected	Detected
13	Not detected	Not detected	Not detected	Detected
15	Not detected	Not detected	Not detected	Detected
18	Not detected	Not detected	Not detected	Detected
28	Not detected	Not detected	Not detected	Detected

Sample C (containing *Cronobacter sakazakii*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
10	Not detected	Not detected	Not detected	Detected
13	Not detected	Not detected	Not detected	Detected
15	Not detected	Not detected	Not detected	Detected
18	Not detected	Not detected	Not detected	Detected
28	Not detected	Not detected	Not detected	Detected

Sample D (containing *Salmonella* Senftenberg and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
7	Detected	Detected	Detected	Not detected
8	Detected	Detected	Detected	Not detected
9	Detected	Detected	Detected	Not detected
20	Detected	Detected	Detected	Not detected
22	Detected	Detected	Detected	Not detected

Sample E (containing *Salmonella* Adelaide and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
4	Detected	Detected	Detected	Not detected
19	Detected	Detected	Detected	Not detected
20	Detected	Detected	Detected	Not detected
26	Detected	Detected	Detected	Not detected
27	Detected	Detected	Detected	Not 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 (containing *Salmonella* Derby and *Listeria innocua*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
7	Detected	Detected	Not detected	Not detected
13	Detected	Detected	Not detected	Not detected
15	Detected	Detected	Not detected	Not detected

Sample B (containing *Cronobacter sakazakii*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
9	Not detected	Not detected	Not detected	Detected
12	Not detected	Not detected	Not detected	Detected
16	Not detected	Not detected	Not detected	Detected

Sample C (containing *Cronobacter sakazakii*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
9	Not detected	Not detected	Not detected	Detected
12	Not detected	Not detected	Not detected	Detected
16	Not detected	Not detected	Not detected	Detected

Sample D (containing *Salmonella* Senftenberg and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
3	Detected	Detected	Detected	Not detected
16	Detected	Detected	Detected	Not detected
21	Detected	Detected	Detected	Not detected

Sample E (containing *Salmonella* Adelaide and *Listeria monocytogenes*)				
Sample	*Salmonella*	*Listeria*	*L. monocytogenes*	*Cronobacter*
1	Detected	Detected	Detected	Not detected
3	Detected	Detected	Detected	Not detected
21	Detected	Detected	Detected	Not 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 39

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 70 g of whole milk powder, are to be tested for the presence / absence of *Salmonella*, *Listeria* and *Cronobacter* as per instructions below.
- 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”.

***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 Monday 29 April 2019** to:

Mark Bunt
Proficiency Testing Australia
PO Box 7507 Telephone: +61 2 9736 8397 (1300 782 867)
Silverwater NSW 2128 Fax: +61 2 9743 6664
AUSTRALIA Email: mbunt@pta.asn.au

D2.1

PTA Pathogens in Food (Round 39) 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
Cronobacter	❍ 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: *Cronobacter*

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_____ ❍ Other (please specify) _____	❍ Chromogenic agar ❍ Yellow pigmentation on TSA ❍ Biochemical tests ❍ Rapid kits ❍ Rapid ID 32E ❍ VITEK 2.0 ❍ Other (please specify) _____ ❍ PCR Confirmation (please specify system used) _____ ❍ Other (please specify) _____

Comments: _____

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