

REPORT NO. 1177

**Non-Pathogens In Food
Proficiency Testing Program
Round 27**

January 2020

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Mrs 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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1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the twenty-seventh of an ongoing series of rounds, involving the microbiological analysis of food samples for a range of non-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 (PTA) conducted the exercise in October / November 2019. The aim of the program was to assess laboratories' ability 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 Mr P Briggs, PTA General Manager.

2. FEATURES OF THE PROGRAM

(a) Participating Laboratories

A total of eight laboratories participated in the program, two of which did not return results for inclusion in the final report.

(b) Documentation and Testing Methods

Laboratories were provided with two 30 g (approx.) whole milk powder samples, labelled PTA 1 and PTA 2, with two accompanying freeze-dried vials for microbiological analysis. The milk powder samples were provided in sealed foil laminate sachets. Participants were asked to perform tests for:

- Aerobic Plate Count (APC)
- Coliforms
- *Escherichia coli* (*E. coli*)
- Enterobacteriaceae
- Coagulase-positive *Staphylococci*
- *Bacillus cereus* (*B. cereus*)
- Yeasts
- Moulds
- Total Yeasts and Moulds

Laboratories were requested to perform the tests according to the *Instructions to Participants* provided and to record the results, along with an estimate of their measurement uncertainty (MU) for each result, on the accompanying *Results Sheets*, which were distributed with the samples. Copies of these documents appear in Appendix C.

(c) Laboratory Identification and Confidentiality

To ensure confidentiality, each laboratory was allocated a random code number. Reference to each laboratory in this report is by its code number. Please note that some laboratories reported more than one set of results and, therefore, these laboratories' code numbers (with letter) could appear several times in the same data set.

(d) Homogeneity Testing

Prior to sample distribution, randomly selected samples were analysed for homogeneity by Global Proficiency Ltd (New Zealand). Based on the results of this testing, the homogeneity of the samples was established (see Appendix B).

(e) Stability Testing

Stability testing was also performed on the samples by Global Proficiency Ltd (New Zealand). The analysis of the stability testing results showed that the samples were sufficiently stable for testing for the duration of the program (see Appendix B).

3. FORMAT OF THE APPENDICES

(a) Appendix A is divided into nine sections (A1–A9). These sections contain the analysis of results reported by laboratories for Aerobic Plate Count, Coliforms, *E. coli*, Enterobacteriaceae, Coagulase-positive *Staphylococci*, *B. cereus*, Yeasts, Moulds and Total Yeasts and Moulds.

Each section contains, where appropriate:

- i) a table of results reported by laboratories for each test, with estimates of their MUs, calculated z-scores and methods used;
- ii) a listing of the summary statistics; and
- iii) ordered z-score charts.

(b) Appendix B contains details of the homogeneity testing and stability testing.

(c) Appendix C contains copies of the *Instructions to Participants* and *Results Sheets*.

4. STATISTICAL DESIGN OF THE PROGRAM

Samples PTA 1 and PTA 2 were obtained from the Global Proficiency DairyChek Microbiology program. Approximate levels (in cfu/g) were as follows:

<u>Test</u>	<u>Sample PTA 1</u>	<u>Sample PTA 2</u>
Aerobic Plate Count	10,000	50,000
Coliforms	200	900
<i>E. coli</i>	0	900
Enterobacteriaceae	200	900
Coagulase-positive <i>Staphylococci</i>	100	600
<i>B. cereus</i>	1,500	9,000
Yeasts	400	0
Moulds	100	500

The summary statistics calculated for each test / sample consists of:

- *No. of Results*: the total number of results for that test / sample;
- *Median*: the middle value of the results;
- *Normalised IQR*: the normalised interquartile range of the results;
- *Uncertainty of the Median*: a robust estimate of the standard deviation of the *Median*;
- *Robust CV*: the robust coefficient of variation expressed as a percentage, *i.e.* $100 \times \text{Normalised IQR} / \text{Median}$;
- *Minimum*: the lowest laboratory result;
- *Maximum*: the highest laboratory result; and
- *Range*: the difference between the *Maximum* and *Minimum*.

The median is a measure of the centre of the data. The normalised IQR is a measure of the spread of the results. It is calculated by multiplying the interquartile range (IQR) by a correction factor, which converts the IQR to an estimate of the standard deviation. The IQR is the difference between the upper and lower quartiles (*i.e.* the values above and below which a quarter of the results lie, respectively).

For normally distributed data, the uncertainty of the median is approximated by:

$$\sqrt{\frac{\pi}{2}} \times \frac{\text{normIQR}}{\sqrt{n}}$$

where *normIQR* is the normalised IQR and *n* is the number of results.

In order to assess laboratories' testing performance, a robust statistical approach, using z-scores, was utilised. Z-scores give a measure of how far a result is from the consensus value (*i.e.* the median) and gives a "score" to each result relative to the other results in the group.

A z-score with an absolute value less than or equal to 2.0 is considered to be satisfactory, whereas, a z-score with an absolute value greater than or equal to 3.0 is considered to be an outlier and is marked by the symbol "§". Laboratories are also encouraged to review results which have an absolute z-score value between 2.0 and 3.0 (*i.e.* $2.0 < |\text{z-score}| < 3.0$). These results are considered to be questionable results.

Ordered z-score charts indicate each laboratory's robust z-score, in order of magnitude, marked with its laboratory code number. From these charts, each laboratory can readily compare its performance relative to the other laboratories. The ordered z-score charts in Appendix A are limited on the vertical axis to +3.0 and -3.0, so that outliers are clearly identifiable as those laboratories whose "bar" extends beyond the chart boundary.

For further details on the calculation and interpretation of robust z-scores and ordered z-score charts, please see the *Guide to Proficiency Testing Australia (2019)*.

5. OUTLIER RESULTS

The table on the next page summarises the results submitted by the participants for this round of the program and the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

Table A: Summary Statistics for All Tests

Test	Method	Summary Statistics	PTA 1	PTA 2
Aerobic Plate Count	Pour Plate / Petrifilm™	Number of Results	11	12
		Median	4.000	4.280
		Normalised IQR	0.152	0.354
		Uncertainty (Median)	0.057	0.128
Coliforms	Pour Plate / Petrifilm™	Number of Results	9	7
		Median	2.140	2.230
		Normalised IQR	0.061	0.207
		Uncertainty (Median)	0.026	0.098
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other	Number of Results	7	6
		Median	n/a	2.320
		Normalised IQR	n/a	0.228
		Uncertainty (Median)	n/a	0.117
Enterobacteriaceae	Pour Plate / Petrifilm™	Number of Results	9	9
		Median	2.080	2.230
		Normalised IQR	0.096	0.402
		Uncertainty (Median)	0.040	0.168
Coagulase-positive <i>Staphylococci</i>	Spread Plate / Petrifilm™	Number of Results	9	10
		Median	2.570	2.530
		Normalised IQR	0.122	0.201
		Uncertainty (Median)	0.051	0.079
<i>B. cereus</i>	Spread Plate	Number of Results	7	6
		Median	3.150	3.750
		Normalised IQR	0.126	0.202
		Uncertainty (Median)	0.060	0.103
Yeasts	All Methods Pooled	Number of Results	9	9
		Median	2.699	n/a
		Normalised IQR	0.125	n/a
		Uncertainty (Median)	0.052	n/a
Moulds	All Methods Pooled	Number of Results	9	10
		Median	1.903	1.991
		Normalised IQR	0.348	0.243
		Uncertainty (Median)	0.145	0.096
Total Yeasts and Moulds	All Methods Pooled	Number of Results	12	12
		Median	2.675	1.915
		Normalised IQR	0.119	0.133
		Uncertainty (Median)	0.043	0.048

Table B: Summary of Statistical Outliers and False Results

The following table lists the laboratories (by code number) that obtained outliers or false results for each test.

Test	Method	Outliers		False Results	
		Sample PTA 1	Sample PTA 2	Sample PTA 1	Sample PTA 2
Aerobic Plate Count	Pour Plate / Petrifilm™	-	7 (x2)	-	-
Coliforms	Pour Plate / Petrifilm™	-	6A	-	7
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other		7	-	-
Enterobacteriaceae	Pour Plate / Petrifilm™	-	7 (x2)	-	-
Coagulase-positive <i>Staphylococci</i>	Spread Plate / Petrifilm™	1	1	-	-
<i>B. cereus</i>	Spread Plate	1	-	-	-
Yeasts	All Methods Pooled	-		-	-
Moulds	All Methods Pooled	1, 7	4B, 7	5	-
Total Yeasts and Moulds	All Methods Pooled	-	7	-	-

Notes for Tables A and B:

1. The results reported are for log₁₀ (cfu/g).
2. All the methods used by the participants were pooled when analysing the results.
3. The summary statistics reported (including the number of results) and z-scores were calculated from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples, for Aerobic Plate Count, Coliforms, *E. coli*, Enterobacteriaceae, Coagulase-positive *Staphylococci*, *B. cereus* and Total Yeasts and Moulds.
4. The summary statistics reported (including the number of results) and z-scores were calculated from the participants' results for Yeasts and Moulds.
5. Target CVs were used to calculate the z-scores for at least one of the samples for Aerobic Plate Count, Coliforms, *E. coli*, Enterobacteriaceae, Coagulase-positive *Staphylococci* and Moulds.
6. Sample PTA 1 did not contain *E. coli*.
7. Sample PTA 2 did not contain Yeasts.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 27 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *Klebsiella pneumoniae* as the only Coliform / Enterobacteriaceae organism present in the sample, whereas sample PTA 2 contained *E. coli* as the Coliform / Enterobacteriaceae organism present.

Both sample PTA 1 and PTA 2 contained a species of *Penicillium* to contribute to the Mould count, and sample PTA 1 contained a species of *Candida* to contribute to the Yeast count.

Both samples PTA 1 and PTA 2 contained *Bacillus cereus* and *Staphylococcus aureus* species. Other bacterial species were included to contribute to the Aerobic Plate Count, but not interfere with the tests for the indicator organisms.

Consensus values (medians) derived from participants' results, are used as the assigned values in this program. These values are not metrologically traceable to an external reference.

The summary statistics, uncertainties of the assigned values, outliers and false results identified for each of the tests / methods analysed are reported in Tables A and B on the previous pages. Complete details of the statistical analyses and the methods used by laboratories for testing appear in Appendix A.

6.1 Return Rate

Six of the eight laboratories (75%) that participated in the program submitted results for inclusion in the final report. Of these six laboratories, five (83%) submitted more than one result for each sample, for at least one of the tests, often using the same method. One of these six laboratories (17%) submitted results for all nine tests. The return rate for all tests is as follows:

• Aerobic Plate Count	6 out of 6	100%
• Coliforms	5 out of 6	83%
• <i>E. coli</i>	2 out of 6	33%
• Enterobacteriaceae	4 out of 6	67%
• Coagulase-positive <i>Staphylococci</i>	4 out of 6	67%
• <i>B. cereus</i>	2 out of 6	33%
• Yeasts	5 out of 6	83%
• Moulds	6 out of 6	100%
• Total Yeasts and Moulds	4 out of 6	67%

6.2 Performance Summary

One or more statistical outliers or false results were reported by five of the six laboratories (83%) that submitted results for this round of the Non-Pathogens in Food program. For comparison, 89% of the participants in Round 26 of the Non-Pathogens in Food program reported outliers or false results (see Report No. 1150 for more details).

A total of 132 results were analysed in this round of the program. Of these results, 16 (12%) were identified as outliers or false results. For comparison, 21% of the results analysed in Round 26 of the Non-Pathogens in Food program were outliers or false results (see Report No. 1150 for more details).

6.3 Aerobic Plate Count

Five of the six laboratories that undertook testing for Aerobic Plate Count tested using Pour Plate, including three laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results. The results for the Pour Plate and Petrifilm™ methods were pooled and analysed against the Pour Plate and Petrifilm™ results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 3.8% and 8.3%, for samples PTA 1 and PTA 2, respectively. The robust CV of 8.3% for sample PTA 2 was considered inappropriate to evaluate the performance of the participants in this round, so a target CV was used to calculate the z-scores for sample PTA 2. The target CV chosen was 3.0%. This value was chosen because it is more consistent with the robust CVs obtained in previous rounds of this program for Aerobic Plate Count.

Laboratory code 7 (using Petrifilm™) reported two outliers for sample PTA 2. There were no outliers reported for sample PTA 1.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Aerobic Plate Count test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
APC - Pour Plate / Petrifilm™	4.000 ± 0.057	4.280 ± 0.128
APC - Pour Plate	4.010 ± 0.079	4.280 ± 0.144

As can be seen, the medians obtained for each method compared very well.

One laboratory reported MUs associated with their test results in this round for Aerobic Plate Count, as log₁₀ cfu/g values, which overlapped the median and associated standard error (se) for each sample.

6.4 Coliforms

A total of five laboratories submitted results for Coliforms. Four laboratories tested using Pour Plate (VRBA), including three laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™. The Pour Plate and Petrifilm™ results were pooled and analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 2.9% and 9.3%, for samples PTA 1 and PTA 2, respectively. Neither of these robust CVs were considered appropriate to evaluate the performance of the participants in this round, so target CVs were used to calculate the z-scores for both samples. The target CVs chosen were 5.3% and 7.1% for samples PTA 1 and PTA 2, respectively. These values were chosen because they are more consistent with the robust CVs obtained in previous rounds of this program for Coliforms.

Laboratory code 6A (using Pour Plate) reported an outlier for sample PTA 2. There were no outliers reported for sample PTA 1.

Laboratory code 7 (using Petrifilm™) reported a false negative result (<3 cfu/g) for sample PTA 2, which should be investigated further as this sample contained *E. coli*.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Coliforms test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Coliforms - Pour Plate	2.140 ± 0.026	2.230 ± 0.098

None of the laboratories reported MUs associated with their test results in this round for Coliforms.

6.5 *E. coli*

Two laboratories submitted results for *E. coli*. Of these two laboratories, one submitted two sets of results using the Pour Plate method, while the other tested using Petrifilm™. The results for the Pour Plate and Petrifilm™ methods were pooled and analysed against the pooled Pour Plate and HGMF results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CV for sample PTA 2 this round was 9.8%. This value was considered inappropriate to evaluate the performance of the participants in this round, so a target CV was used to calculate the z-scores for sample PTA 2. The target CV chosen was 5.0%. This value was chosen because it is more consistent with the robust CVs obtained in previous rounds of this program for *E. coli*.

Laboratory code 7 (using Petrifilm™) reported an outlier for sample PTA 2. The result was lower than expected. Sample PTA 1 did not contain *E. coli*.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the *E. coli* test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
<i>E. coli</i> - Pour Plate / HGMF	-	2.320 ± 0.117

None of the laboratories reported MUs associated with their test results in this round for *E. coli*.

6.6 Enterobacteriaceae

A total of four laboratories submitted results for Enterobacteriaceae. Three of these laboratories tested using Pour Plate, with all three submitting two sets of results. One laboratory submitted two sets of results using Petrifilm™. The Pour Plate and Petrifilm™ results were pooled and analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 4.6% and 18.0%, for samples PTA 1 and PTA 2, respectively. Neither of these robust CVs were considered appropriate to evaluate the performance of the participants in this round, so target CVs were used to calculate the z-scores for both samples. The target CVs chosen were 7.5% for both samples. These values were chosen because they are more consistent with the robust CVs obtained in previous rounds of this program for Enterobacteriaceae.

Laboratory code 7 (using Petrifilm™) reported two outliers for sample PTA 2. There were no outliers reported for sample PTA 1.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Enterobacteriaceae test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Enterobacteriaceae - Pour Plate	2.080 ± 0.040	2.230 ± 0.168

None of the laboratories reported MUs associated with their test results in this round for Enterobacteriaceae.

6.7 Coagulase-positive *Staphylococci*

Three of the four laboratories that submitted results for Coagulase-positive *Staphylococci* tested using Spread Plate, including one laboratory that submitted two sets of results. One laboratory submitted two sets of results using Petrifilm™. The Spread Plate and Petrifilm™ results were pooled and analysed against the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 4.8% and 7.9%, for samples PTA 1 and PTA 2, respectively. The robust CV of 7.9% for sample PTA 2 was considered inappropriate to evaluate the performance of the participants in this round, so a target CV was used to calculate the z-scores for sample PTA 2. The target CV chosen was 4.8%, which is the same as the robust CV obtained for the sample PTA 1 results.

Laboratory code 1 (using Spread Plate) reported outlier results for both samples, with results higher than expected.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Coagulase-positive *Staphylococci* test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Coagulase-positive <i>Staphylococci</i> - Spread Plate	2.570 ± 0.051	2.530 ± 0.079

None of the laboratories reported MUs associated with their test results in this round for Coagulase-positive *Staphylococci*.

6.8 *B. cereus*

A total of two laboratories tested the samples for *B. cereus*. Both of these laboratories tested using the Spread Plate method (using MYP agar), with one submitting two sets of results. These Spread Plate results were analysed against the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 4.0% and 5.4%, for samples PTA 1 and PTA 2, respectively. These values compare well with the robust CVs obtained in previous rounds of this program for *B. cereus*.

Laboratory code 1 (using the Spread Plate method) reported an outlier for sample PTA 1. There were no outliers reported for sample PTA 2.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the *B. cereus* test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
<i>B. cereus</i> - Spread Plate	3.150 ± 0.060	3.750 ± 0.103

None of the laboratories reported MUs associated with their test results in this round for *B. cereus*.

6.9 Yeasts

A total of five laboratories submitted results for Yeasts. Three laboratories tested using Pour Plate, including one laboratory that submitted two sets of results. Three laboratories tested using Spread Plate, including two laboratories that submitted two sets of results. All the methods were pooled for analysis.

The robust CV of 4.6% for sample PTA 1 for this round compares well with the robust CVs obtained in previous rounds of this program for Yeasts.

There were no outlier results reported for sample PTA 1. Sample PTA 2 did not contain Yeasts.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Yeasts test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Yeasts - Pour Plate / Spread Plate	2.699 ± 0.052	-

None of the laboratories reported MUs associated with their test results in this round for Yeasts.

6.10 Moulds

A total of six laboratories submitted results for Moulds. Four laboratories tested using Pour Plate, including one laboratory that submitted two sets of results. Three laboratories tested using Spread Plate, including two laboratories that submitted two sets of results. All the methods were pooled for analysis.

The robust CVs for the results for this round were 18.3% and 12.2%, for samples PTA 1 and PTA 2, respectively. Neither of these robust CVs were considered appropriate to evaluate the performance of the participants in this round, so target CVs were used to calculate the z-scores for both samples. The target CVs chosen were 8.6% for both samples. These values were chosen because they are more consistent with the robust CVs obtained in previous rounds of this program for Moulds.

Laboratory code 1 (using the Pour Plate method) reported an outlier for sample PTA 1. Laboratory code 4B (using the Spread Plate method) reported an outlier for sample PTA 2. Laboratory code 7 (using Petrifilm™) reported outliers for both samples.

Laboratory code 5 (using the Pour Plate method) reported a false negative result for sample PTA 1.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Moulds test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Moulds - Pour Plate / Spread Plate	1.903 ± 0.145	1.991 ± 0.096

One laboratory reported MUs associated with their test results in this round for Moulds, as log₁₀ cfu/g values. It is recommended that laboratory code 1 re-examines their test results or their MU calculations for sample PTA 1 as their result was further from the median than their stated uncertainty (taking into consideration the uncertainty associated with the median).

6.11 Total Yeasts and Moulds

A total of four laboratories submitted results for Total Yeasts and Moulds. Three laboratories tested using Pour Plate, including one laboratory that submitted two sets of results. Two laboratories tested using Spread Plate, including one laboratory that submitted two sets of results. All the methods were pooled and analysed against the Spread Plate and Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs for the results for this round were 4.4% and 7.0%, for samples PTA 1 and PTA 2, respectively. These values compare well with the robust CVs obtained in previous rounds of this program for Total Yeasts and Moulds.

Laboratory code 7 (using Petrifilm™) reported an outlier for sample PTA 2. There were no outliers reported for sample PTA 1.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 3 of this report), which was calculated for each test and / or method within a test. For the Total Yeasts and Moulds test, the median and associated standard error (se) for each sample (expressed in log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
Total Yeasts and Moulds - Pour Plate / Spread Plate	2.675 ± 0.043	1.915 ± 0.048
Total Yeasts and Moulds - Pour Plate	2.650 ± 0.043	1.930 ± 0.042

None of the laboratories reported MUs associated with their test results in this round for Total Yeasts and Moulds.

For the plating techniques used for Yeast and Mould testing, five different types of media were used in this round including RBCA (Rose Bengal-Chloramphenicol agar), DRBCA (Dichloran-Rose Bengal-Chloramphenicol agar) – both RBCA and DRBCA were used via both the Pour Plate and Spread Plate techniques; YDCA (Yeast Extract-Dextrose-Chloramphenicol agar), PDA (Potato Dextrose agar) and DG-18 (Dichloran Glycerol). The media differ in the antibiotics and selective and differential agents incorporated in the medium, and the final pH to inhibit bacterial growth, as well as the inclusion of other compounds to inhibit spreading moulds.

7. REFERENCES

1. *Guide to Proficiency Testing Australia (2019)*. (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 5013.2 (2007) *Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of Bacillus cereus – Colony-count technique at 30°C (ISO 7932: 2004, MOD)*.
4. AS 5013.4 (2009) *Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique*.
5. AS 5013.5 (2016) *Food microbiology – Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Colony count at 30°C by the pour plate technique*.
6. AS 5013.9 (2009) *Food microbiology – Examination for specific organisms – Coliforms and Escherichia coli by the triplicate tube detection method*.
7. 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*.
8. AS 5013.15 (2006) *Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive Escherichia coli – Most probable number technique*.
9. AS 5013.29 (2009) *Food microbiology – Examination for specific organisms – Colony count of yeasts and moulds*.
10. ISO 6611 (2004) / IDF 94 (2004) *Milk and milk products – Enumeration of colony-forming units of yeasts and/or moulds – Colony-count technique at 25 degrees C*.
11. ISO 6888-1:1999/Amd.2:2018 *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*.
12. ISO 7932 (2004) *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Bacillus cereus – Colony-count technique at 30 degrees C*.
13. ISO 16649-2 (2001) *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli – Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*.
14. ISO 21528-2 (2017) *Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae – Part 2: Colony-count technique*.

APPENDIX A

Summary of Results

Section A1

Aerobic Plate Count

A1.1

Milk Powder – Aerobic Plate Count, Pour Plate / Petrifilm™ (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	15000	4.18	±0.134	20000	4.30	±0.134	1.16	0.16	PP	PCA
3A	19000	4.28	-	39000	4.59	-	1.83	2.42	PP	PCA
3B	20000	4.30	-	38000	4.58	-	1.98	2.33	PP	PCA
4A	7700	3.89	-	11200	4.05	-	-0.75	-1.80	PP	PCA
4B	7000	3.85	-	11200	4.05	-	-1.02	-1.80	PP	PCA
5	14000	4.15	-	37000	4.57	-	0.96	2.24	PP	PCA
5	19000	4.28	-	39000	4.59	-	1.83	2.42	Pfm	-
6A	24000	4.38	-	43000	4.63	-	2.50	2.75	PP	PCA
6B	22000	4.34	-	41000	4.61	-	2.25	2.59	PP	PCA
7	12400	4.09	-	53000	4.72	-	0.61	3.46 §	Pfm	-
7	13800	4.14	-	48000	4.68	-	0.92	3.12 §	Pfm	-

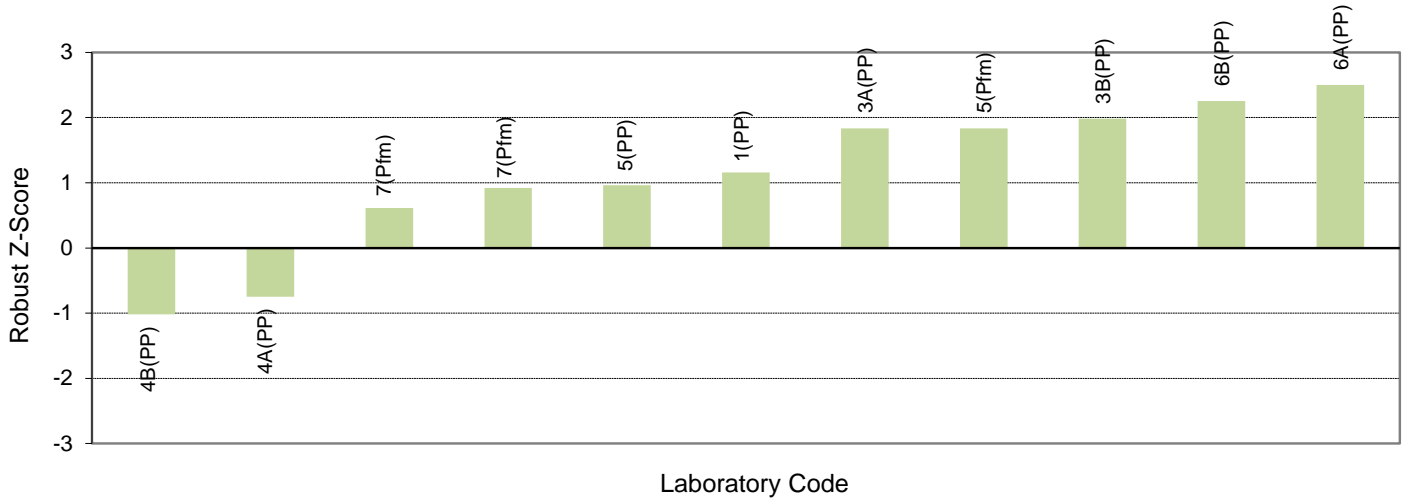
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	11	12
Median	4.000	4.280
Normalised IQR	0.152	0.354
Uncertainty (Median)	0.057	0.128
Robust CV	3.8%	8.3%
Target SD	-	0.128
Target CV	-	3.0%
Minimum	3.78	3.89
Maximum	4.20	4.64
Range	0.42	0.75

Notes:

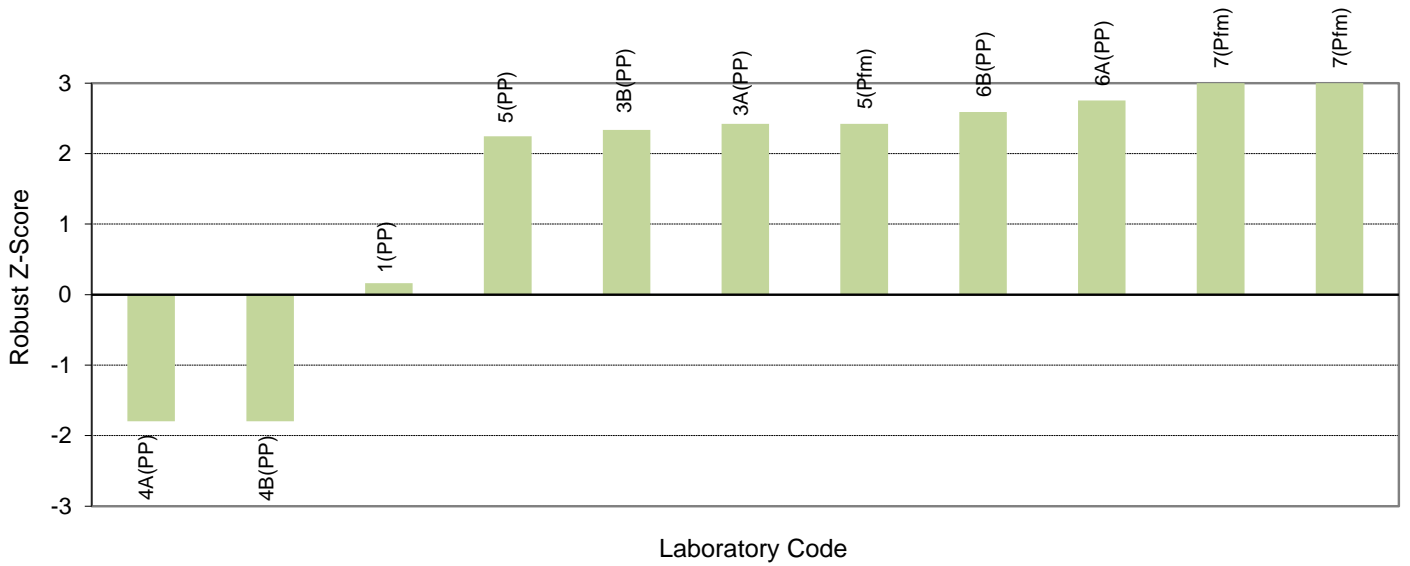
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- The MU estimates reported by laboratory code 1 are in log₁₀ cfu/g.
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Aerobic Plate Count results.
- A target CV was used to calculate the robust z-scores for sample PTA 2. The target CV chosen was 3.0%.
- The target SD was obtained for sample PTA 2 by multiplying the target CV by the median. This value was used to calculate the z-scores for sample PTA 2. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
- Z-scores and summary statistics (including the number of results) for Aerobic Plate Count were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
- The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A1.2

Milk Powder - Aerobic Plate Count, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Aerobic Plate Count, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A2

Coliforms

A2.1

Milk Powder – Coliforms, Pour Plate / Petrifilm™ (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
3A	140	2.15	-	200	2.30	-	0.05	0.45	PP	VRBA
3B	160	2.20	-	200	2.30	-	0.57	0.45	PP	VRBA
4A	100	2.00	-	140	2.15	-	-1.23	-0.53	PP	VRBA
4B	120	2.08	-	130	2.11	-	-0.54	-0.73	PP	VRBA
5	180	2.26	-	300	2.48	-	1.02	1.56	PP	VRBA
5	200	2.30	-	470	2.67	-	1.42	2.79	Pfm	-
6A	100	2.00	-	20	1.30	-	-1.23	-5.87 §	PP	VRBA
6B	150	2.18	-	200	2.30	-	0.32	0.45	PP	VRBA
7	230	2.36	-	< 3 †	-	-	1.95	-	Pfm	-

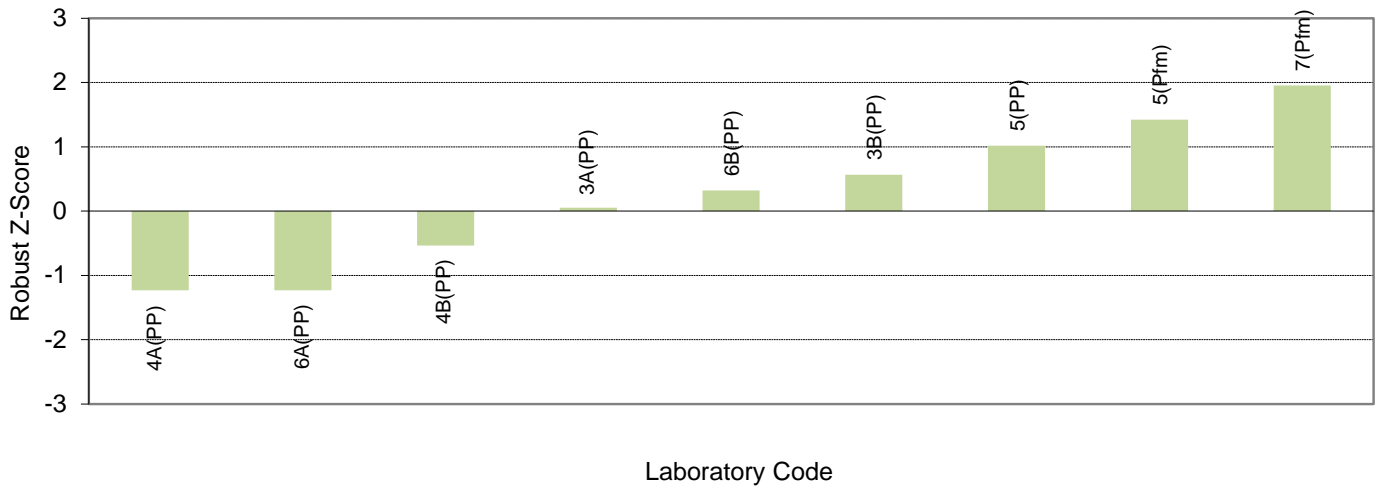
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	7
Median	2.140	2.230
Normalised IQR	0.061	0.207
Uncertainty (Median)	0.026	0.098
Robust CV	2.9%	9.3%
Target SD	0.113	0.158
Target CV	5.3%	7.1%
Minimum	1.96	1.85
Maximum	2.32	2.63
Range	0.36	0.78

Notes:

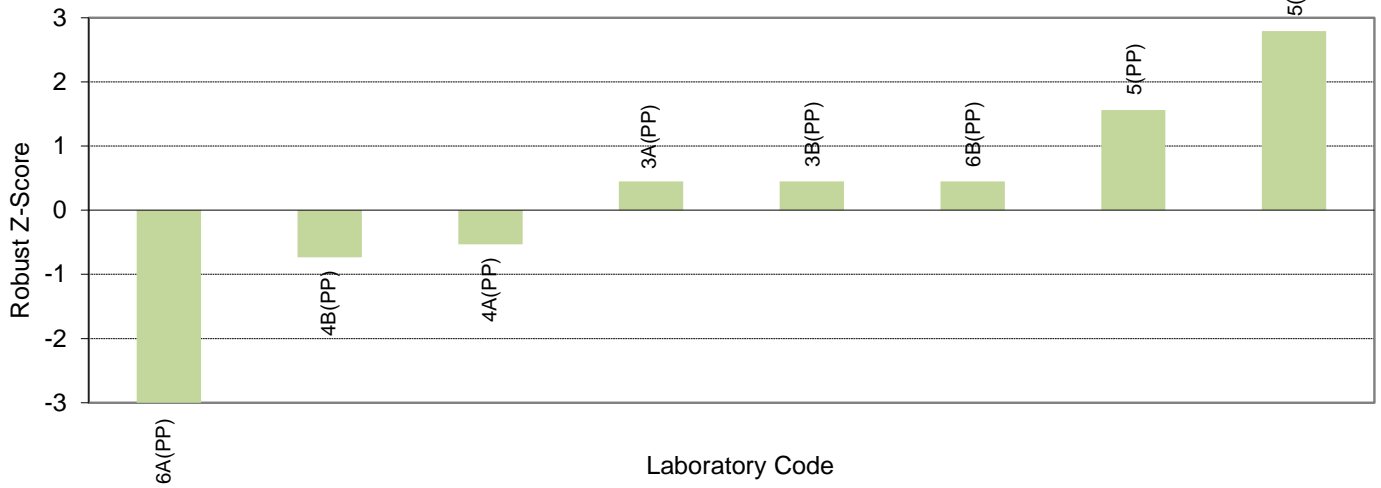
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- † denotes a false negative result.
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
- Target CVs were used to calculate the robust z-scores for both samples. The target CVs chosen were 5.3% and 7.1% for samples PTA 1 and PTA 2, respectively.
- The target SDs were obtained for each sample by multiplying the target CV by the median. These values were used to calculate the z-scores for each sample. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
- Z-scores and summary statistics (including the number of results) for Coliforms were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
- The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A2.2

Milk Powder - Coliforms, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Coliforms, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A3

E. coli

A3.1

Milk Powder – *E. coli*, Pour Plate / Petrifilm™ (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
3A	< 10	-	-	180	2.26	-	-	-0.56	PP	VRBA MUG
3B	< 10	-	-	180	2.26	-	-	-0.56	PP	VRBA MUG
7	< 3	-	-	60	1.78	-	-	-4.67 §	Pfm	-

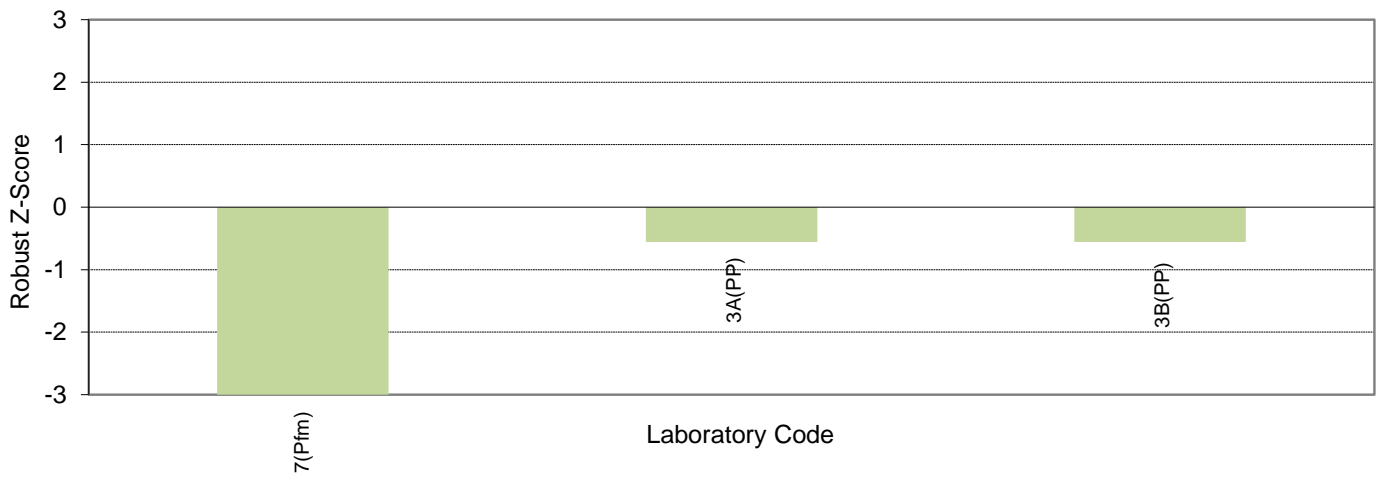
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	7	6
Median	n/a	2.320
Normalised IQR	n/a	0.228
Uncertainty (Median)	n/a	0.117
Robust CV	n/a	9.8%
Target SD	n/a	0.116
Target CV	n/a	5.0%
Minimum	n/a	2.26
Maximum	n/a	2.78
Range	n/a	0.52

Notes:

- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the *E. coli* results.
- A target CV was used to calculate the robust z-scores for sample PTA 2. The target CV chosen was 5.0%.
- The target SD was obtained for sample PTA 2 by multiplying the target CV by the median. This value was used to calculate the z-scores for sample PTA 2. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
- Z-scores and summary statistics (including the number of results) for *E. coli* were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
- The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
- Sample PTA 1 did not contain *E. coli*.

A3.2

Milk Powder - *E.coli*, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A4

Enterobacteriaceae

A4.1

Milk Powder – Enterobacteriaceae, Pour Plate / Petrifilm™ (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
3A	190	2.28	-	220	2.34	-	1.27	0.67	PP	VRBGA
3B	190	2.28	-	200	2.30	-	1.27	0.42	PP	VRBGA
4A	105	2.02	-	100	2.00	-	-0.38	-1.38	PP	VRBD
4B	90	1.95	-	80	1.90	-	-0.81	-1.95	PP	VRBD
6A	170	2.23	-	160	2.20	-	0.96	-0.15	PP	VRBGA
6B	200	2.30	-	200	2.30	-	1.42	0.42	PP	VRBGA
7	100	2.00	-	50	1.70	-	-0.51	-3.18 §	Pfm	-
7	250	2.40	-	30	1.48	-	2.04	-4.50 §	Pfm	-

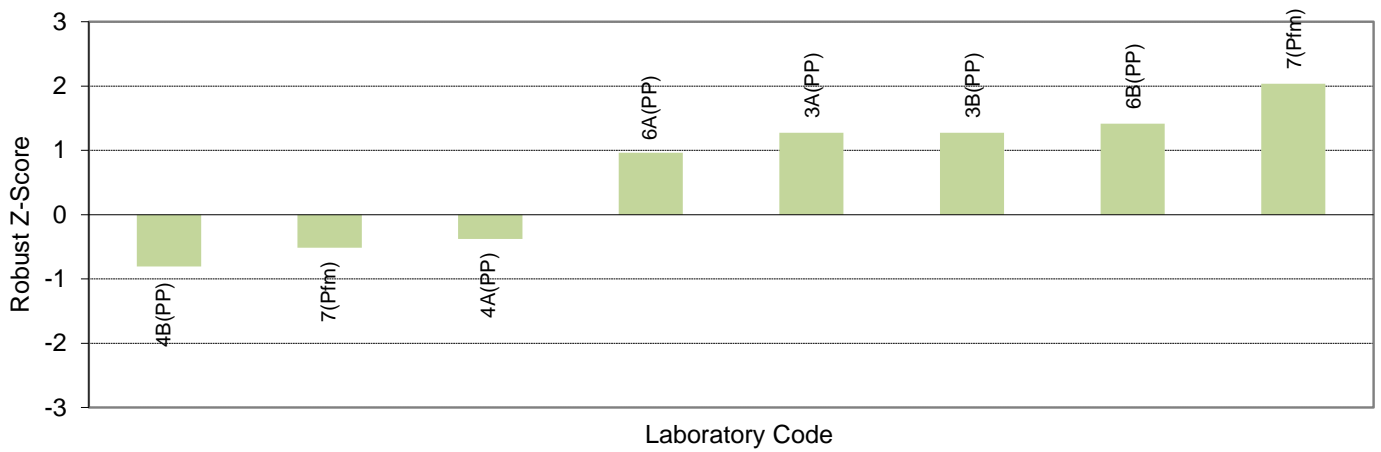
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	9
Median	2.080	2.230
Normalised IQR	0.096	0.402
Uncertainty (Median)	0.040	0.168
Robust CV	4.6%	18.0%
Target SD	0.156	0.167
Target CV	7.5%	7.5%
Minimum	1.68	1.60
Maximum	2.18	2.77
Range	0.50	1.17

Notes:

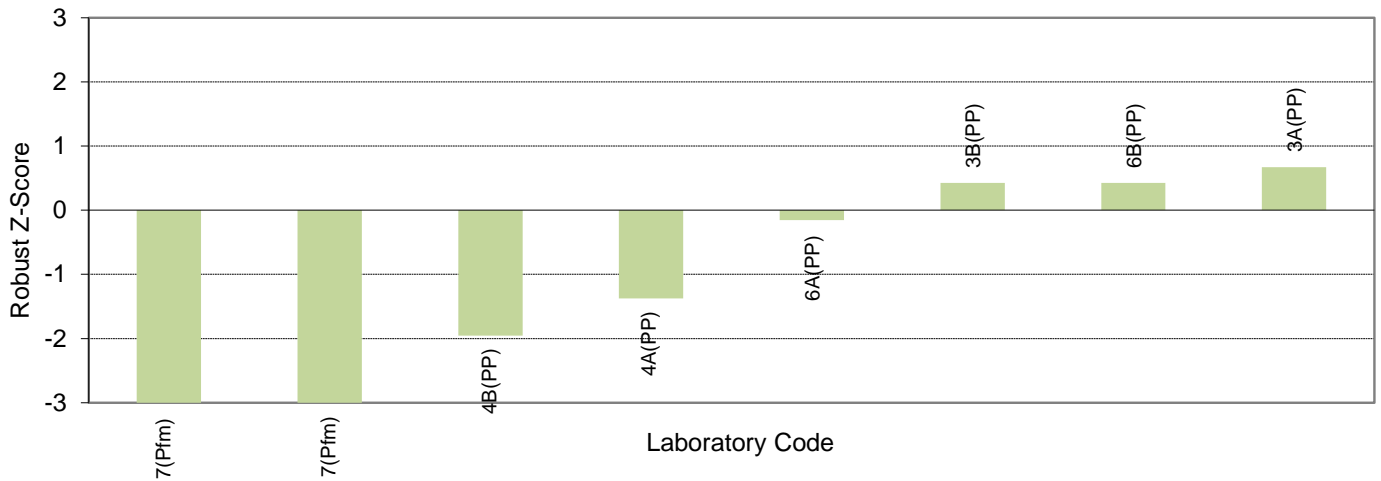
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Enterobacteriaceae results.
- Target CVs were used to calculate the robust z-scores for both samples. The target CVs chosen were 7.5% and 7.5% for samples PTA 1 and PTA 2, respectively.
- The target SDs were obtained for each sample by multiplying the target CV by the median. These values were used to calculate the z-scores for each sample. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
- Z-scores and summary statistics (including the number of results) for Enterobacteriaceae were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
- The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A4.2

Milk Powder - Enterobacteriaceae, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Enterobacteriaceae, Pour Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A5

Coagulase-positive *Staphylococci*

A5.1

Milk Powder – Coagulase-positive *Staphylococci*, Spread Plate / Petrifilm™ (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	1100	3.04	-	810	2.91	-	3.85 §	3.12 §	SP	BPA
3A	390	2.59	-	490	2.69	-	0.17	1.32	SP	BPA
3B	400	2.60	-	500	2.70	-	0.26	1.39	SP	BPA
5	530	2.72	-	300	2.48	-	1.26	-0.44	SP	BPA
7	500	2.70	-	400	2.60	-	1.05	0.59	Pfm	-
7	300	2.48	-	430	2.63	-	-0.76	0.85	Pfm	-

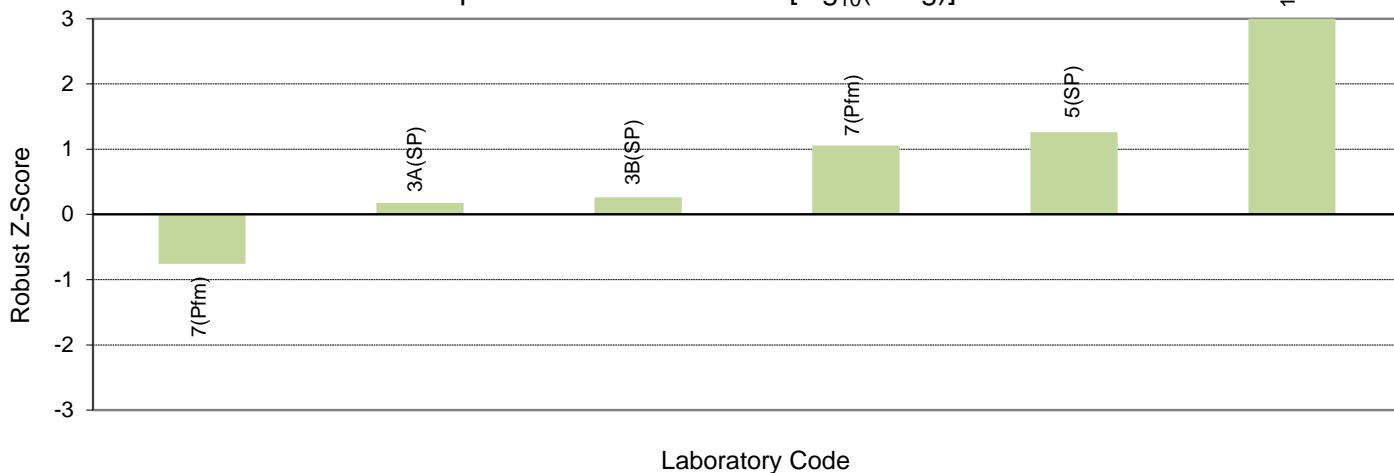
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	10
Median	2.570	2.530
Normalised IQR	0.122	0.201
Uncertainty (Median)	0.051	0.079
Robust CV	4.8%	7.9%
Target SD	-	0.121
Target CV	-	4.8%
Minimum	2.41	2.23
Maximum	2.89	3.04
Range	0.48	0.81

Notes:

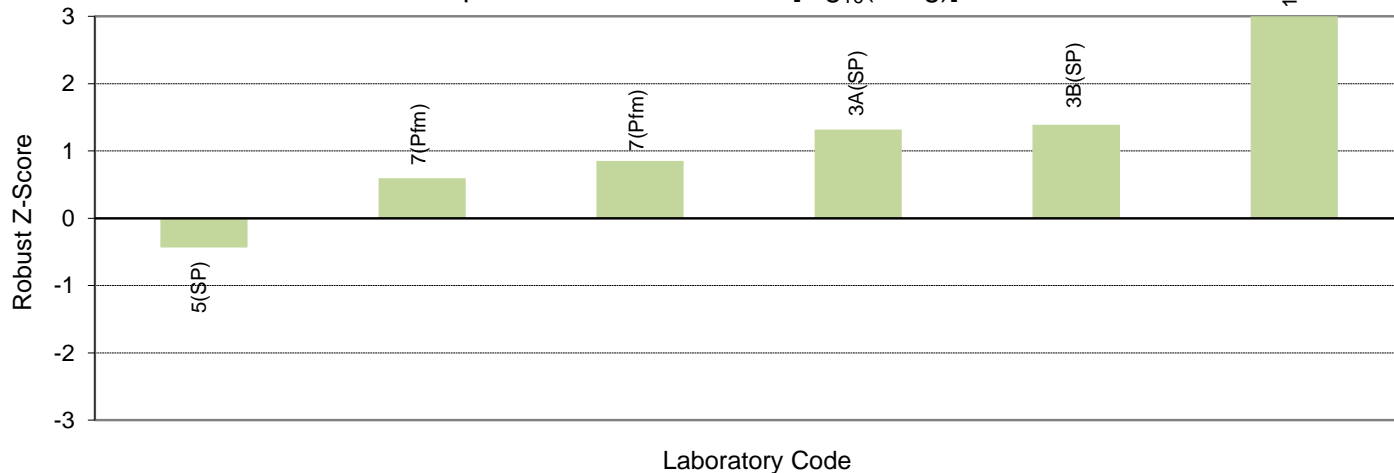
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, SP = Spread Plate and Pfm = Petrifilm™.
3. The Spread Plate and Petrifilm™ methods were pooled when analysing the Coagulase-positive *Staphylococci* results.
4. A target CV was used to calculate the robust z-scores for sample PTA 2. The target CV chosen was 4.8%.
5. The target SD was obtained for sample PTA 2 by multiplying the target CV by the median. This value was used to calculate the z-scores for sample PTA 2. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
6. Z-scores and summary statistics (including the number of results) for Coagulase-positive *Staphylococci* were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
7. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A5.2

Milk Powder - Coagulase-positive *Staphylococci*,
Spread Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Coagulase-positive *Staphylococci*,
Spread Plate / Petrifilm™ [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A6

Bacillus cereus

A6.1

Milk Powder – *Bacillus cereus*, Spread Plate (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	9300	3.97	-	15000	4.18	-	6.49 §	2.11	SP	MYP
3A	1600	3.20	-	4000	3.60	-	0.43	-0.73	SP	MYP
3B	1550	3.19	-	4000	3.60	-	0.32	-0.73	SP	MYP

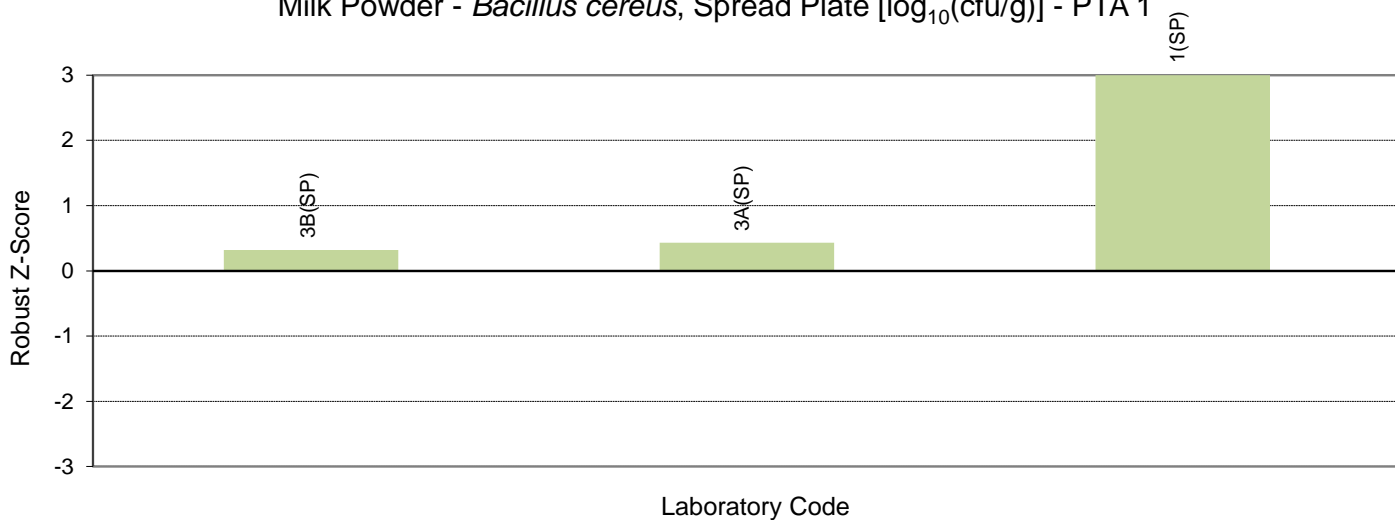
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	7	6
Median	3.150	3.750
Normalised IQR	0.126	0.202
Uncertainty (Median)	0.060	0.103
Robust CV	4.0%	5.4%
Minimum	3.00	3.43
Maximum	3.31	3.92
Range	0.31	0.49

Note:

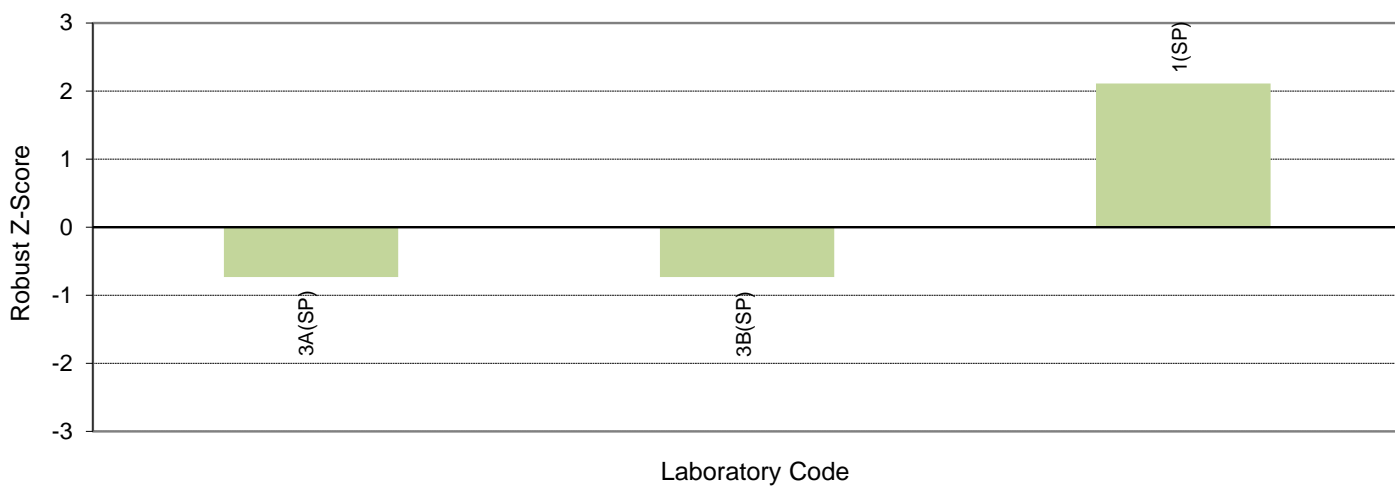
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, SP = Spread Plate.
3. Z-scores and summary statistics (including the number of results) for *Bacillus cereus* were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
4. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A6.2

Milk Powder - *Bacillus cereus*, Spread Plate [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - *Bacillus cereus*, Spread Plate [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A7

Yeasts

A7.1

Milk Powder – Yeasts, All Methods Pooled (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
3A	510	2.71	-	< 10	-	-	0.07	-	SP	RBA
3B	500	2.70	-	<10	-	-	0.00	-	SP	RBC
4A	230	2.36	-	0	-	-	-2.70	-	SP	DRBC
4B	230	2.36	-	0	-	-	-2.70	-	SP	DRBC
5	360	2.56	-	< 10	-	-	-1.14	-	PP	PDA
6A	500	2.70	-	< 10	-	-	0.00	-	PP	RBCA
6B	500	2.70	-	< 10	-	-	0.00	-	SP	RBCA
7	500	2.70	-	< 10	-	-	0.00	-	PP	DRBC
7	600	2.78	-	< 10	-	-	0.63	-	PP	DRBC

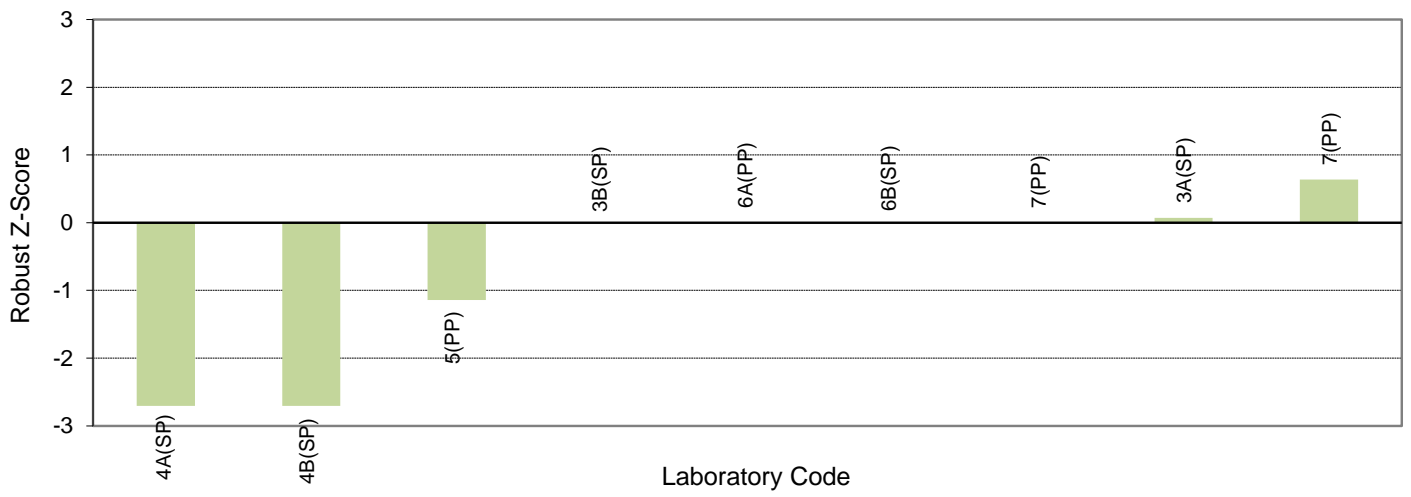
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	9
Median	2.699	n/a
Normalised IQR	0.125	n/a
Uncertainty (Median)	0.052	n/a
Robust CV	4.6%	n/a
Minimum	2.36	n/a
Maximum	2.78	n/a
Range	0.42	n/a

Notes:

1. For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
2. All the methods were pooled when analysing the Yeasts results.
3. Z-scores and summary statistics (including the number of results) for Yeasts were calculated from the participants' results.
4. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
5. Sample PTA 2 did not contain Yeasts.

A7.2

Milk Powder - Yeasts, All Methods Pooled [$\log_{10}(\text{cfu/g})$] - PTA 1



Section A8

Moulds

A8.1

Milk Powder – Moulds, All Methods Pooled (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	530	2.72	±0.113	120	2.08	±0.113	5.02 §	0.51	PP	DG-18
3A	100	2.00	-	120	2.08	-	0.59	0.51	SP	RBA
3B	100	2.00	-	120	2.08	-	0.59	0.51	SP	RBC
4A	40	1.60	-	70	1.85	-	-1.84	-0.85	SP	DRBC
4B	30	1.48	-	30	1.48	-	-2.60	-3.00 §	SP	DRBC
5	< 10 †	-	-	60	1.78	-	-	-1.24	PP	PDA
6A	80	1.90	-	130	2.11	-	0.00	0.72	PP	RBCA
6B	130	2.11	-	150	2.18	-	1.29	1.08	SP	RBCA
7	40	1.60	-	80	1.90	-	-1.84	-0.51	PP	DRBC
7	10	1.00	-	20	1.30	-	-5.52 §	-4.03 §	PP	DRBC

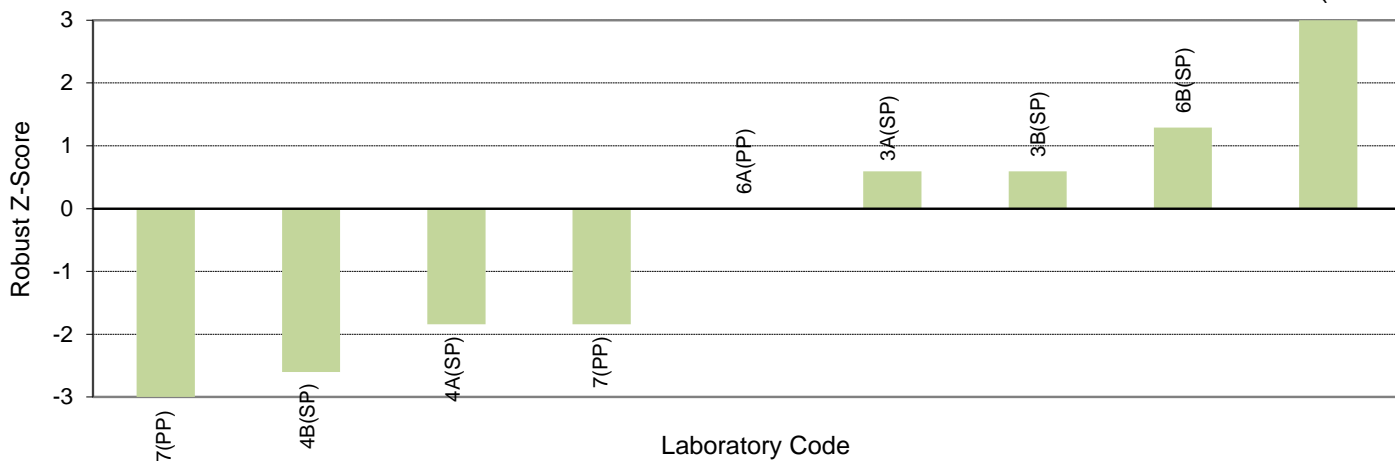
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	10
Median	1.903	1.991
Normalised IQR	0.348	0.243
Uncertainty (Median)	0.145	0.096
Robust CV	18.3%	12.2%
Target SD	0.164	0.171
Target CV	8.6%	8.6%
Minimum	1.00	1.30
Maximum	2.72	2.18
Range	1.72	0.88

Notes:

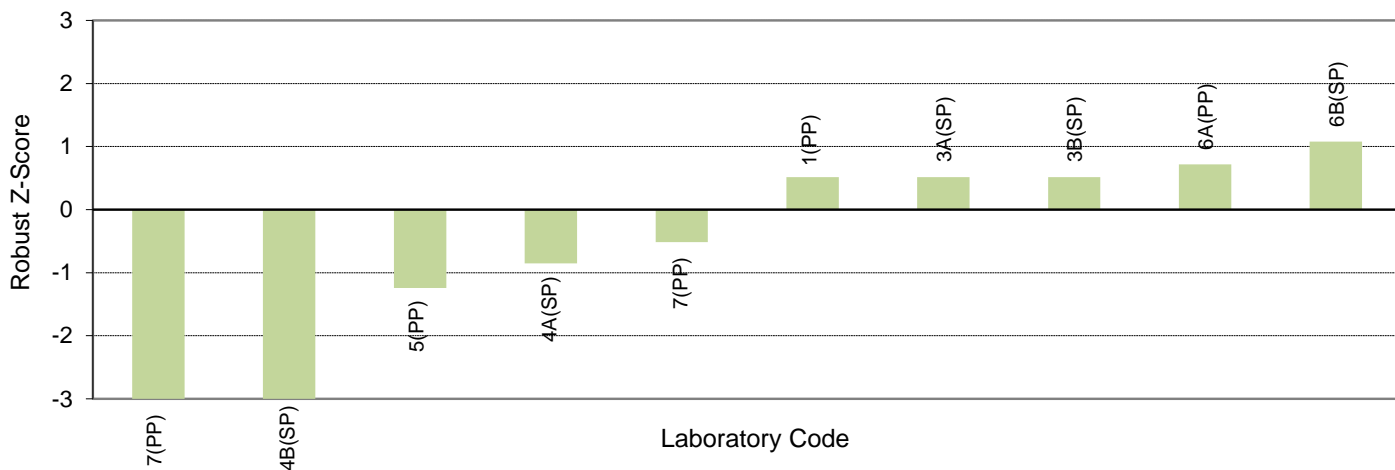
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. † denotes a false negative result.
3. The MU estimates reported by laboratory code 1 are in log₁₀ cfu/g.
4. For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
5. All the methods were pooled when analysing the Moulds results.
6. Target CVs were used to calculate the robust z-scores for both samples. The target CVs chosen were 8.6% and 8.6% for samples PTA 1 and PTA 2, respectively.
7. The target SDs were obtained for each sample by multiplying the target CV by the median. These values were used to calculate the z-scores for each sample. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2019).
8. Z-scores and summary statistics (including the number of results) for Moulds were calculated from the participants' results.
9. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A8.2

Milk Powder - Moulds, All Methods Pooled [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Moulds, All Methods Pooled [$\log_{10}(\text{cfu/g})$] - PTA 2



Section A9

Total Yeasts and Moulds

A9.1

Milk Powder – Total Yeasts and Moulds, All Methods Pooled (cfu/g)

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
3A	610	2.79	-	120	2.08	-	0.93	1.23	SP	RBA
3B	600	2.78	-	120	2.08	-	0.87	1.23	SP	RBC
5	360	2.56	-	60	1.78	-	-1.00	-1.03	PP	PDA
6A	580	2.76	-	130	2.11	-	0.75	1.49	PP	RBCA
6B	630	2.80	-	150	2.18	-	1.05	1.96	SP	RBCA
7	540	2.73	-	80	1.90	-	0.48	-0.09	PP	DRBC
7	610	2.79	-	20	1.30	-	0.93	-4.60 §	PP	DRBC

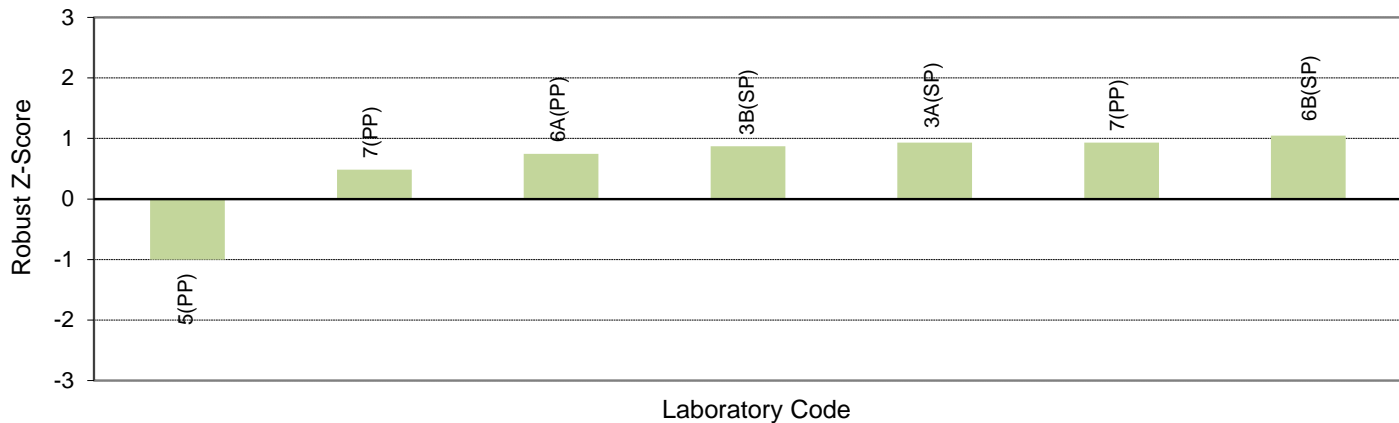
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	12	12
Median	2.675	1.915
Normalised IQR	0.119	0.133
Uncertainty (Median)	0.043	0.048
Robust CV	4.4%	7.0%
Minimum	2.45	1.70
Maximum	2.89	2.11
Range	0.44	0.41

Notes:

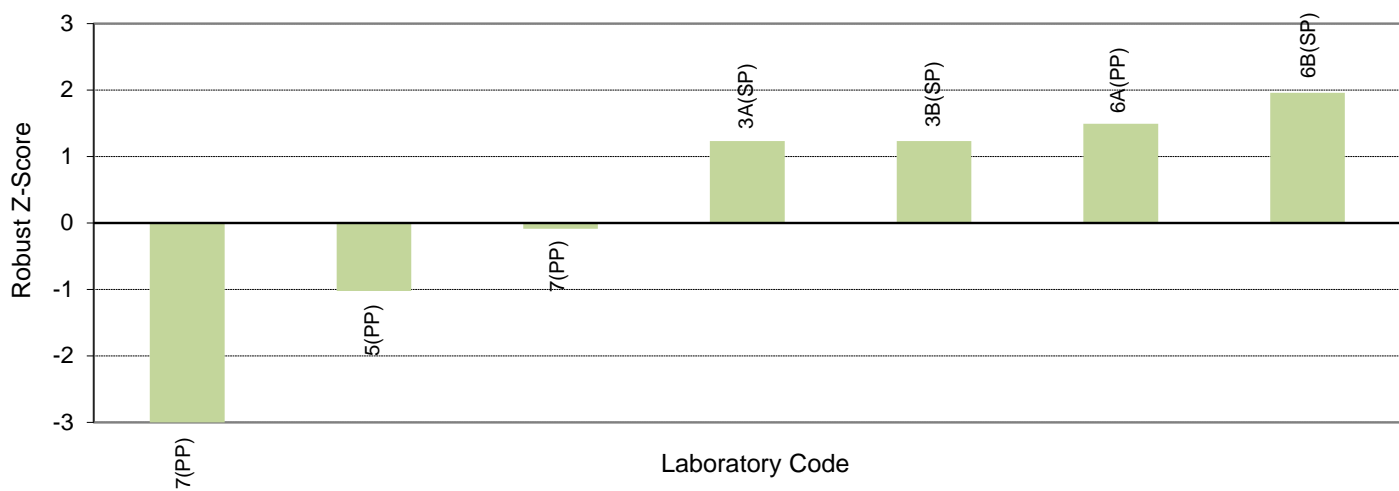
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
3. All the methods were pooled when analysing the Total Yeasts and Moulds results.
4. Z-scores and summary statistics (including the number of results) for Total Yeasts and Moulds were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
5. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A9.2

Milk Powder - Total Yeasts and Moulds,
All Methods Pooled [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Total Yeasts and Moulds,
All Methods Pooled [$\log_{10}(\text{cfu/g})$] - PTA 2



APPENDIX B

Homogeneity and Stability Testing

B1.1

Homogeneity Testing

Samples from PTA 2, chosen at random, were retained for homogeneity testing by Global Proficiency Ltd (New Zealand). These samples were tested for Aerobic Plate Count. The samples were tested in duplicate using 0.1 mL volumes spread-plated onto Plate Count Agar with incubation at 30°C for up to 72 hours. The results of this homogeneity testing appear in the following table.

Aerobic Plate Count (cfu/g)				
PTA 2				
Sample	Result A	Log ₁₀ A	Result B	Log ₁₀ B
2	26000	4.41	33000	4.52
11	31000	4.49	28000	4.45
27	30000	4.48	33000	4.52
33	34000	4.53	31000	4.49
48	33000	4.52	36000	4.56

The analysis of the homogeneity data indicated that the samples were sufficiently homogeneous for use in the program. Therefore, any participant results identified as outliers or false results cannot be attributed to sample variability.

Stability Testing

Samples from PTA 2, chosen at random, were retained for stability testing by Global Proficiency Ltd (New Zealand). These sets of samples were tested for Aerobic Plate Count and were tested after samples had been stored at ambient temperature for three days to simulate conditions which could be experienced in transit. The samples were tested in duplicate using 0.1 mL volumes spread plated onto Plate Count Agar with incubation at 30°C for up to 72 hours. The results of this stability testing appear in the following table.

Aerobic Plate Count (cfu/g)				
PTA 2				
Sample	Result A	Log ₁₀ A	Result B	Log ₁₀ B
10	25000	4.40	35000	4.54
23	27000	4.43	26000	4.41
39	30000	4.48	27000	4.43

Analysis of the results showed minimal loss of viability of the test organisms in the samples in the time period between homogeneity testing and stability testing, in relation to the stability criteria applied. Therefore, the samples were rated as stable.

APPENDIX C

Instructions to Participants and Results Sheets

PROFICIENCY TESTING AUSTRALIA
Non-Pathogens in Food
Proficiency Testing Program
Round 27, October 2019



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:

- ❖ The samples available for testing in this program are as follows:

Two approx. 30 g whole milk powder samples, labelled PTA 1 and PTA 2, with two accompanying freeze-dried vials are provided for microbiological analysis. The powder samples are provided in sealed foil laminate sachets and the vials are glass – both should be stored at 2–5 °C prior to testing. These samples may be tested for some or all of the following tests, according to each laboratory's requirements:

- | | |
|---|-------------------------------|
| • Aerobic Plate Count | • <i>Bacillus cereus</i> |
| • Coliforms | • Yeasts |
| • <i>E. coli</i> | • Moulds |
| • Enterobacteriaceae | • Total Yeast and Mould Count |
| • Coagulase-positive <i>Staphylococci</i> | |

- ❖ It is strongly recommended that testing is initiated within 48 hours of receipt of the samples.
- ❖ In order for results to be analysed, laboratories are requested to report quantitative results, so **please ensure adequate dilutions are prepared**. Samples may contain up to 1,000 cfu/g coliforms, 1,000 cfu/g *E. coli*, 1,000 cfu/g Enterobacteriaceae, 1,000 cfu/g Coagulase-positive *Staphylococci*, 10,000 cfu/g *Bacillus cereus*, 1,000 cfu/g yeasts and moulds, and 50,000 cfu/g aerobic mesophilic organisms per gram. **Results should not be reported as “greater than” as such data cannot be statistically analysed.**
- ❖ For each of the tests being performed, the laboratory may report results for up to two different methods. If a Pour Plate or Spread Plate technique is used, please record the medium type used in the testing process, e.g. Coliforms: “VRBA”, Moulds: “DRBCA”.
- ❖ For results using other methods than those listed, the method used should be clearly written in the **Method** column of the **Results Sheet**.
- ❖ **Please note:** For the Coliforms, *E. coli*, Enterobacteriaceae, *Bacillus cereus* and Coagulase-positive *Staphylococci* tests, we request that participants use plating methods and do not submit results via Most Probable Number (MPN).

C1.2

- ❖ Laboratories are also requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of measurement uncertainty must be given as a 95% confidence interval (coverage factor $k \approx 2$). You may provide MU as a \pm value in log format (preferred), or a range if reported in standard form, e.g. 7.5×10^3 cfu/g.

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 vials by adding 3.0 mL of sterile diluent (e.g. 0.1% (w/v) peptone and 0.85% (w/v) NaCl (ISO 6887-1)) at room temperature.
2. Allow standing at room temperature for 10 minutes.
3. Mix the vial contents using a vortex mixer for 15 seconds.
4. Aseptically open the sachets. Weigh out 10 g for each sample. Add 90 mL diluent. Mix to dissolve the milk powder. Add 1 mL of the rehydrated vial contents and homogenize/mix. This is now your prepared **homogenate**, i.e. simulated sample, and should be referred to as 10^{-1} . Continue as per your Standard methods.
5. Report results on the attached **Results Sheet** to the specified number of significant figures. Laboratories should report their results in the row corresponding to the method used for each particular test.
6. Return Results Sheets, either by mail, facsimile or email 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
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All results should arrive at the above address by no later than **Tuesday 5 November 2019**. Results reported later than this date may not be analysed in the final report.

Participants are advised that there may be instances where a particular test, using a particular method, may not be assessed due to insufficient participant numbers.

PROFICIENCY TESTING AUSTRALIA
Non-Pathogens in Food Proficiency Testing Program
Round 27, October 2019
RESULTS SHEET 1

Laboratory Code:

Date Samples Received: _____

Temperature of samples: _____ °C

Determination	Report results to nearest	Sample 1		Sample 2		Test Date	Method (see Note)
		Result	MU	Result	MU		
Aerobic Plate Count	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
Coliforms	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
<i>E. coli</i>	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
Enterobacteriaceae	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
Coagulase-positive <i>Staphylococci</i>	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:

PROFICIENCY TESTING AUSTRALIA
Non-Pathogens in Food Proficiency Testing Program
Round 27, October 2019
RESULTS SHEET 2

Laboratory Code:

Determination	Report results to nearest	Sample 1		Sample 2		Test Date	Method (see Note)
		Result	MU	Result	MU		
<i>Bacillus cereus</i>	2 sig. figures (cfu/g)						<input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Other:
Yeasts	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
Moulds	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:
Total Yeasts & Moulds	2 sig. figures (cfu/g)						<input type="checkbox"/> Pour plate <input type="checkbox"/> Spread plate Medium used: <input type="checkbox"/> Petrifilm™ <input type="checkbox"/> Other:

Note₁: For each of the tests being performed, the laboratory may report results for up to two different methods. If a Pour Plate or Spread Plate technique is used, please record the medium type used in the testing process, e.g. Coliforms: "VRBA", Moulds: "DRBCA".

Note₂: For results using other methods than those listed, the method used should be clearly written in the Method column.

Print Name: _____

Signature & Date: _____

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