REPORT NO. 1121

Non-Pathogens In Food

Proficiency Testing Program

Round 25

January 2019

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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CONTENTS

1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the twenty-fifth 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 2018. 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 Mrs K Cividin, PTA Quality Manager.

2. FEATURES OF THE PROGRAM

(a) Participating Laboratories

A total of seven laboratories participated in the program, all of which returned 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:

Test	Sample PTA 1	Sample PTA 2
Aerobic Plate Count	10,000	30,000
Coliforms	800	800
E. coli	600	800
Enterobacteriaceae	800	800
Coagulase-positive *Staphylococci*	0	6,000
B. cereus	0	9,000
Yeasts	1,000	0
Moulds	1,000	600

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 x *Normalised IQR* / *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:

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

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 < |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 (2016)*.

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	14	15
		Median	4.020	4.450
		Normalised IQR	0.119	0.126
		Uncertainty (Median)	0.040	0.041
Coliforms	Pour Plate / Petrifilm™	Number of Results	11	10
		Median	2.750	2.805
		Normalised IQR	0.126	0.092
		Uncertainty (Median)	0.048	0.036
E. coli	Pour Plate / Petrifilm™ / Other	Number of Results	7	6
		Median	2.600	2.775
		Normalised IQR	0.234	0.052
		Uncertainty (Median)	0.111	0.026
Entero-bacteriaceae	Pour Plate / Petrifilm™	Number of Results	8	10
		Median	2.750	2.785
		Normalised IQR	0.145	0.262
		Uncertainty (Median)	0.064	0.104
Coagulase-positive *Staphylococci*	Spread Plate / Petrifilm™	Number of Results	9	10
		Median	n/a	3.695
		Normalised IQR	n/a	0.096
		Uncertainty (Median)	n/a	0.038
B. cereus	Spread Plate	Number of Results	12	12
		Median	n/a	3.765
		Normalised IQR	n/a	0.193
		Uncertainty (Median)	n/a	0.070
Yeasts	All Methods Pooled	Number of Results	6	6
		Median	2.760	n/a
		Normalised IQR	0.418	n/a
		Uncertainty (Median)	0.214	n/a
Moulds	All Methods Pooled	Number of Results	7	7
		Median	2.890	2.640
		Normalised IQR	0.203	0.176
		Uncertainty (Median)	0.096	0.083
Total Yeasts and Moulds	All Methods Pooled	Number of Results	16	16
		Median	3.300	2.750
		Normalised IQR	0.250	0.137
		Uncertainty (Median)	0.078	0.043

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™	-	4A, 4B	-	-
Coliforms	Pour Plate / Petrifilm™	4A, 4B, 7	4A, 4B	-	7
E. coli	Pour Plate / Petrifilm™ / Other	-	-	-	-
Entero-bacteriaceae	Pour Plate / Petrifilm™	-	1	-	-
Coagulase-positive *Staphylococci*	Spread Plate / Petrifilm™		-	7	-
B. cereus	Spread Plate		5	-	-
Yeasts	All Methods Pooled	-		-	7
Moulds	All Methods Pooled	-	-	-	-
Total Yeasts and Moulds	All Methods Pooled	-	-	-	-

Notes for Tables A and B:

1. The results reported are for $\log_{10}$ (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.
4. Sample PTA 1 did not contain *Coagulase-positive Staphylococci* or *B. cereus*.
5. Sample PTA 2 did not contain Yeasts.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 25 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *E. coli* and *Cronobacter sakazakii* as the Coliform / Enterobacteriaceae organisms present in the sample, whereas sample PTA 2 contained *E. coli* only.

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 *Saccharomyces* to contribute to the Yeast count.

Sample PTA 1 contained *a* Coagulase-negative *Staphylococci* species. Sample PTA 2 included *Bacillus cereus* and *Staphylococcus aureus* species. For both samples, 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

All of the seven laboratories that participated in the program submitted results for inclusion in the final report. Of these seven laboratories, one laboratory (14%) provided results for all nine tests. The return rate for all tests is as follows:

• Aerobic Plate Count	7 out of 7	100%
• Coliforms	6 out of 7	86%
• *E. coli*	4 out of 7	57%
• Enterobacteriaceae	6 out of 7	86%
• Coagulase-positive *Staphylococci*	5 out of 7	71%
• *B. cereus*	4 out of 7	57%
• Yeasts	6 out of 7	86%
• Moulds	6 out of 7	86%
• Total Yeasts and Moulds	5 out of 7	71%

6.2 Performance Summary

One or more statistical outliers or false results were reported by four laboratories (57%) for this round of the Non-Pathogens in Food program. For comparison, 60% of the participants in Round 24 of the Non-Pathogens in Food program reported outliers or false results (see Report No. 1098 for more details).

A total of 138 results were analysed in this round of the program. Of these results, 12 (9%) were identified as outliers or false results. For comparison, 7% of the results analysed in Round 24 of the Non-Pathogens in Food program were outliers or false results (see Report No. 1098 for more details).

6.3 Aerobic Plate Count

Of the seven laboratories that undertook testing for Aerobic Plate Count, six laboratories tested using Pour Plate, including three laboratories that submitted two sets of results. One laboratory tested using Petrifilm™. 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 of 3.0% and 2.8% for the results for this round are lower than the values of 5.0% and 3.3%, obtained for the results in Round 24 of this program, for samples containing similar organisms at similar levels (see Report No. 1098).

Laboratory codes 4A and 4B (using the Pour Plate method) reported outliers 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 Aerobic Plate Count test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
APC - Pour Plate / Petrifilm™	4.020 ± 0.040	4.450 ± 0.041
APC - Pour Plate	4.040 ± 0.041	4.465 ± 0.036

Two laboratories reported MUs associated with their test results in this round for Aerobic Plate Count. One reported their MU as a $\log_{10}$ value and the other reported a range in cfu/g.

Of the reported MUs for the Plate Count methods, one did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median), details as follows:

- Laboratory code 7 may need to re-examine their test results or their MU calculations for the Petrifilm™ method, as their results for sample PTA 2 and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

Graphs showing the differentiation of methods used for Aerobic Plate Count testing are included in Figures TA-1 and TA-2. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

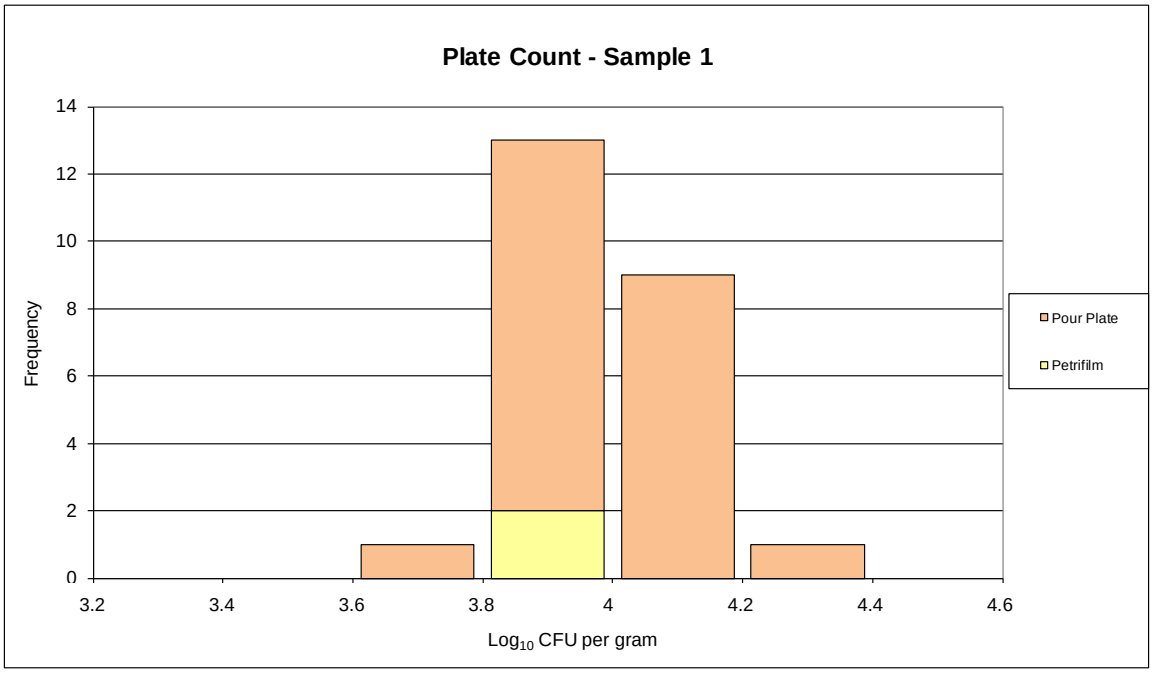


Figure TA-1. APC $\log_{10}$ cfu/g results for sample PTA 1.

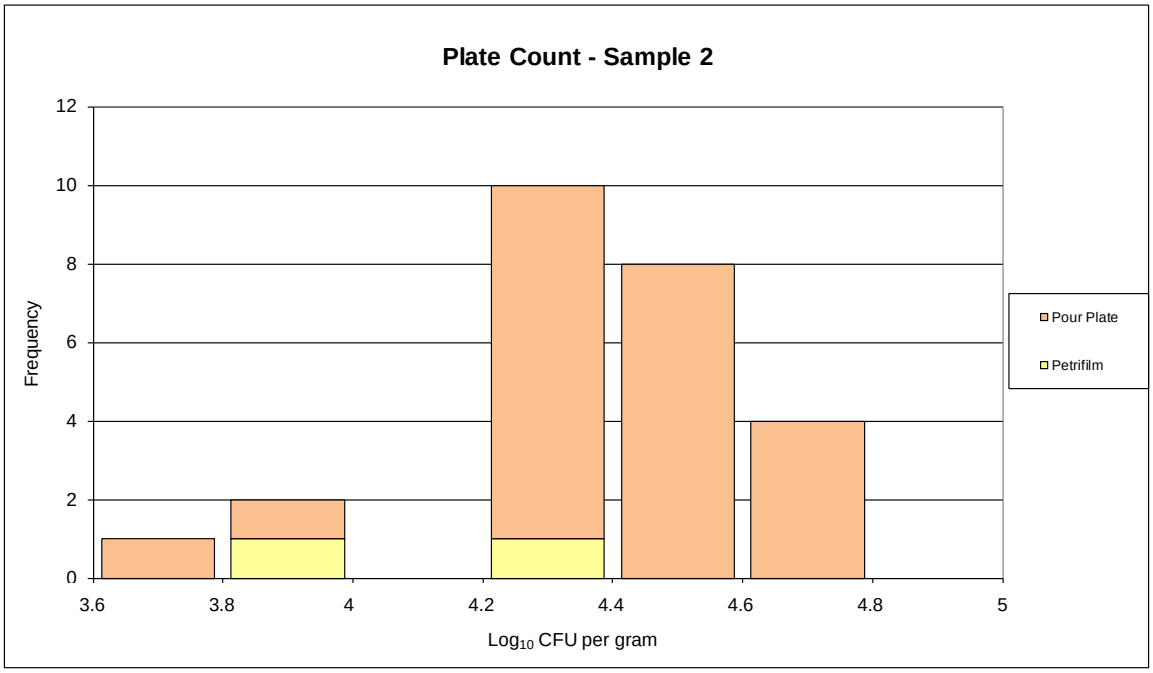


Figure TA-2. APC $\log_{10}$ cfu/g results for sample PTA 2.

6.4 Coliforms

A total of six laboratories submitted results for Coliforms. Three laboratories tested using Pour Plate, all of which submitted two sets of results. Three 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 of 4.6% and 3.3% for the results for this round are lower than the values of 5.9% and 8.6%, obtained for the results in Round 24 of this program, for samples containing similar organisms at similar levels (see Report No. 1098).

Laboratory codes 4A and 4B (using the Pour Plate method) reported outliers for both samples. Laboratory code 7 (using Petrifilm™) reported an outlier for sample PTA 1 and a false negative result for sample PTA 2. Of interest was that this laboratory reported higher results for the *E. coli* and Enterobacteriaceae tests. As the only members of the Enterobacteriaceae group present in the samples were also coliforms, it would be expected that the levels detected for each would be comparable (acknowledging different media would have been used). For sample PTA 2, *E. coli* was the only Coliform/ Enterobacteriaceae organism present and ferments both lactose **and** glucose (dextrose), so again, results should be comparable.

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Coliforms - Pour Plate	2.750 ± 0.048	2.805 ± 0.036

One laboratory reported MUs associated with their test results in this round for Coliforms as a range in cfu/g. It is recommended this laboratory (code 7) may need to re-examine their test results or their MU calculations for the Petrifilm™ method, as their results for sample PTA 1 and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

Graphs showing the differentiation of methods used for Coliform testing are included in Figures TA-3 and TA-4. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

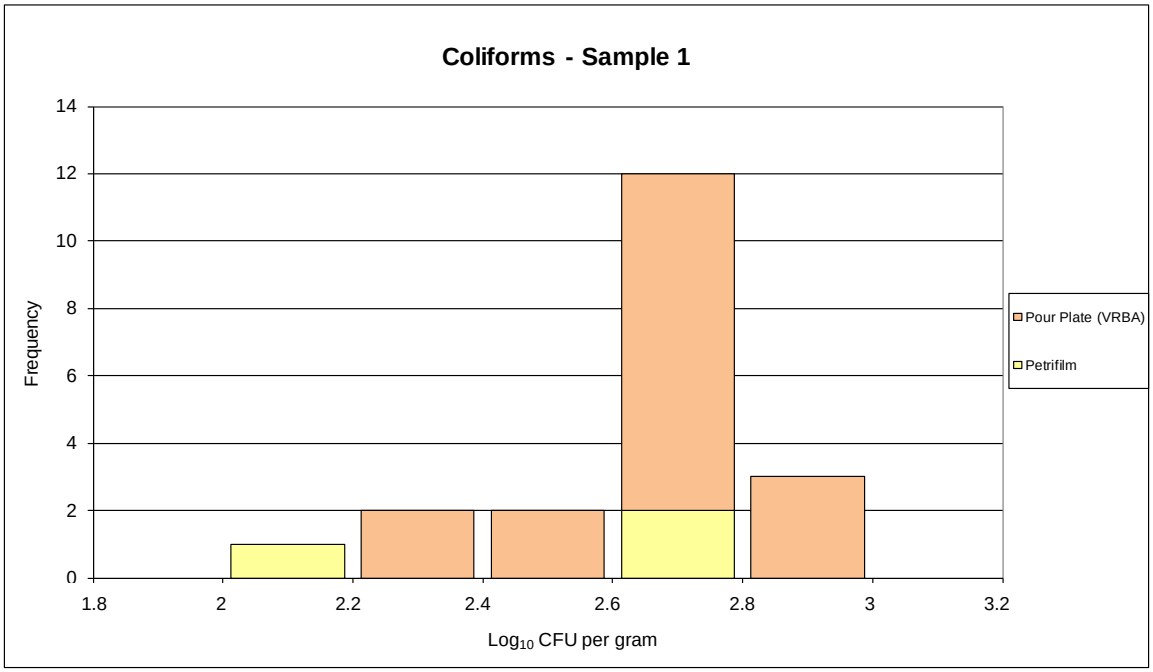


Figure TA-3. Coliforms $\log_{10}$ cfu/g results for sample PTA 1.

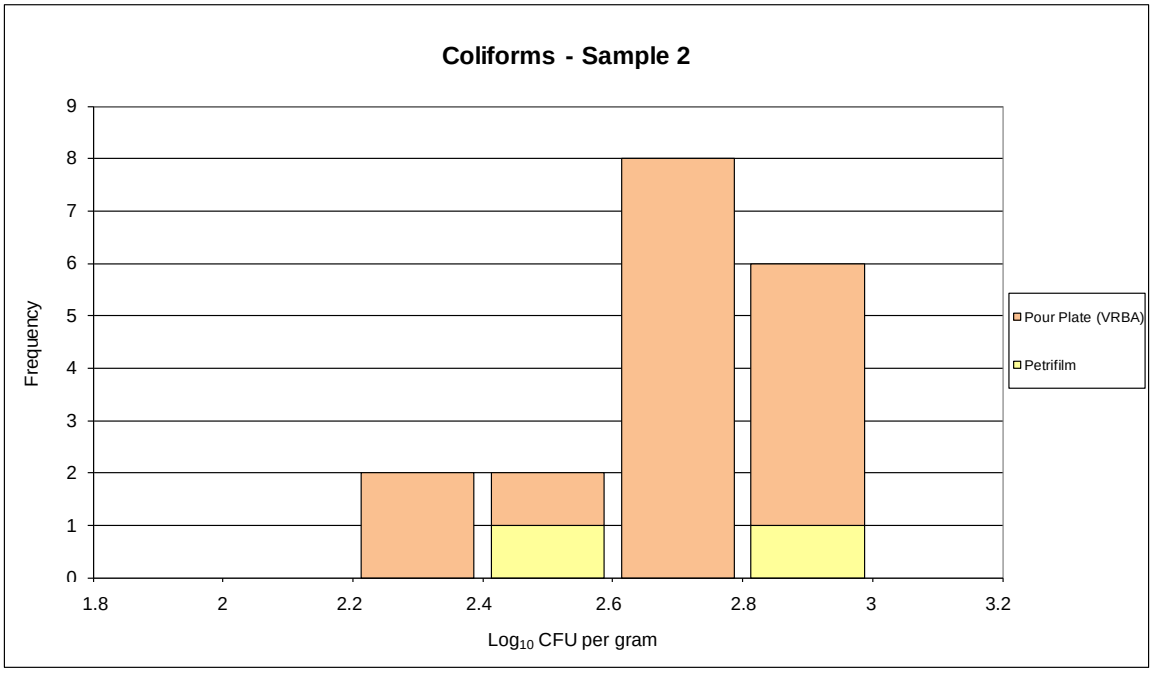


Figure TA-4. Coliforms $\log_{10}$ cfu/g results for sample PTA 2.

6.5 *E. coli*

Of the four laboratories that submitted results for *E. coli,* three laboratories tested using Petrifilm™. One laboratory submitted two sets of results for the Pour Plate method. 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 CVs for the results for this round were 9.0% and 1.9%, for samples PTA 1 and PTA 2, respectively. The robust CV of 1.9% 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 5.0%, based on performance across the last two rounds.

There were no outliers reported for either sample.

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
E. coli - Pour Plate / HGMF	2.600 ± 0.111	2.775 ± 0.026

One laboratory reported MUs associated with their test results in this round for *E. coli* as a range in cfu/g, which overlapped the median and associated standard error (se) for each sample.

Graphs showing the differentiation of methods used for *E. coli* testing is included in Figures TA-5 and TA-6. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

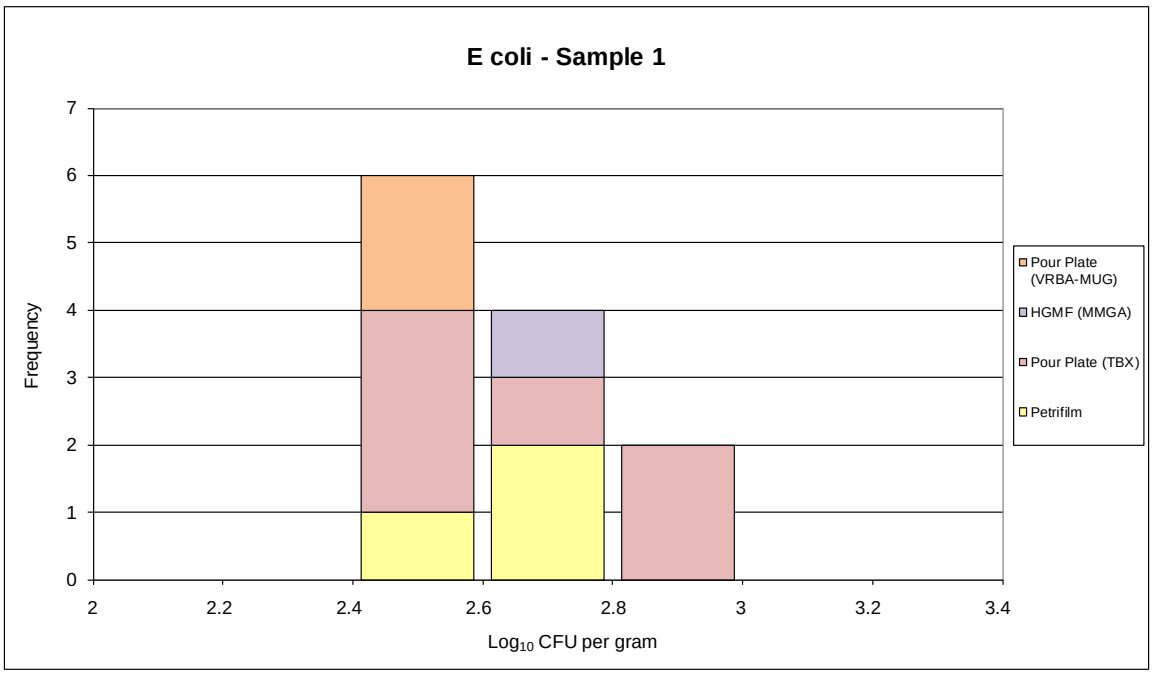


Figure TA-5. *E. coli* $\log_{10}$ cfu results for sample PTA 1.

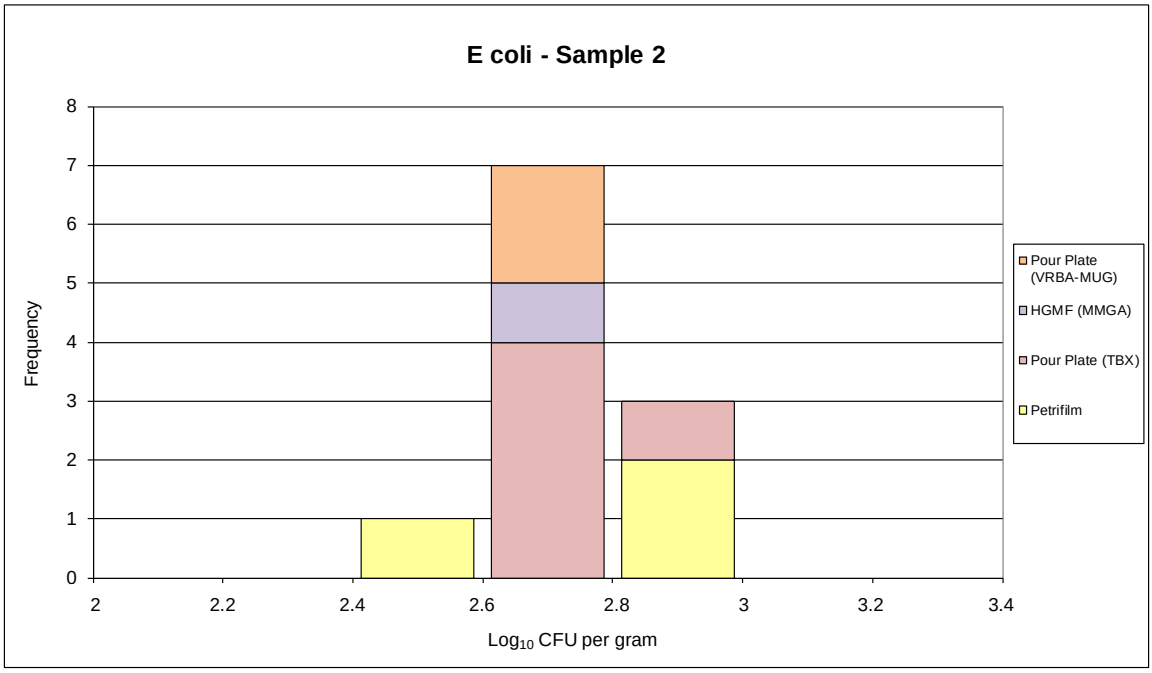


Figure TA-6. *E. coli* $\log_{10}$ cfu results for sample PTA 2.

6.6 Enterobacteriaceae

A total of six laboratories submitted results for Enterobacteriaceae. Five of these laboratories tested using Pour Plate, including three laboratories that submitted two sets of results. One laboratory 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 5.3% and 9.4%, for samples PTA 1 and PTA 2, respectively. The robust CV of 5.3% for sample PTA 1 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 1. The target CV chosen was 10.0% and is based on performance of the same batch used in a previous round.

Laboratory code 1 (using the Pour Plate method) reported an outlier 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 Enterobacteriaceae test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Enterobacteriaceae - Pour Plate	2.750 ± 0.064	2.785 ± 0.104

Two laboratories reported MUs associated with their test results in this round for Enterobacteriaceae. One reported their MU as a $\log_{10}$ value and the other reported a range in cfu/g.
Of the reported MUs for the Enterobacteriaceae methods, two did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median), details as follows:

- Laboratory code 2 may need to re-examine their test results or their MU calculations for the VRBA method as their results for both samples and the stated uncertainty was outside the expected range of the medians and their associated uncertainties.
- Laboratory code 7 reported a MU range for sample PTA 1 that appeared to have been transcribed incorrectly; the result was 650 cfu/g and the MU was 62 – 72 cfu/g – it is assumed this should read 620 – 720 cfu/g.

Graphs showing the differentiation of methods used for Enterobacteriaceae testing are included in Figures TA-7 and TA-8. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

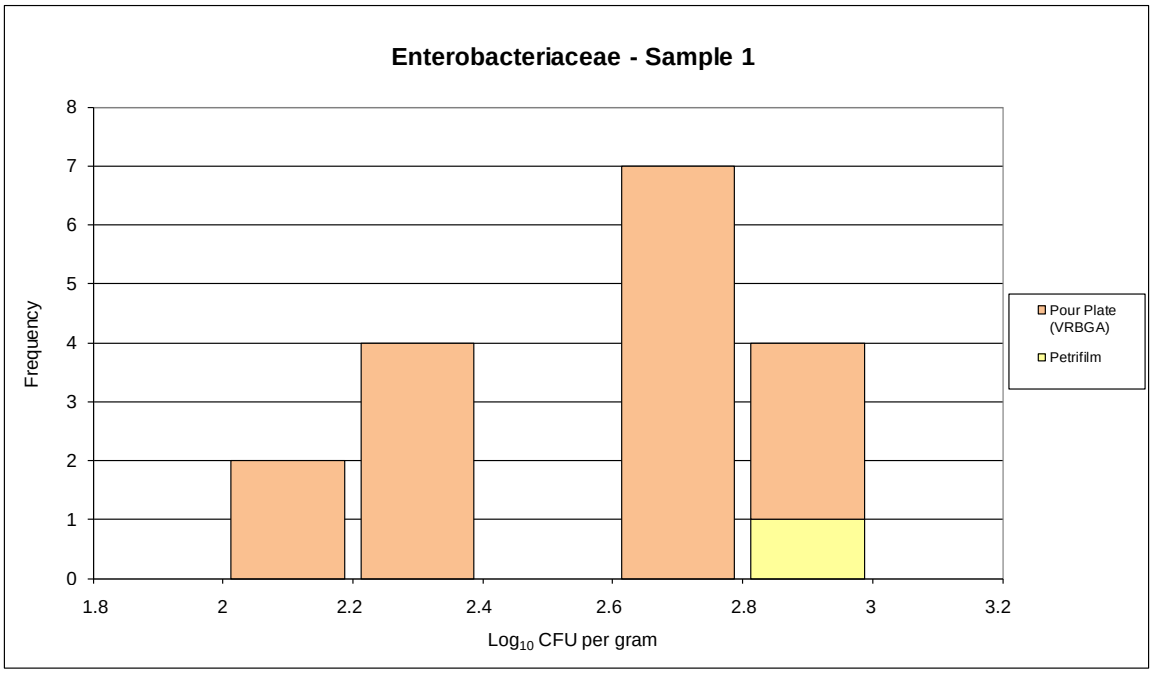


Figure TA-7. Enterobacteriaceae $\log_{10}$ cfu/g results for sample PTA 1.

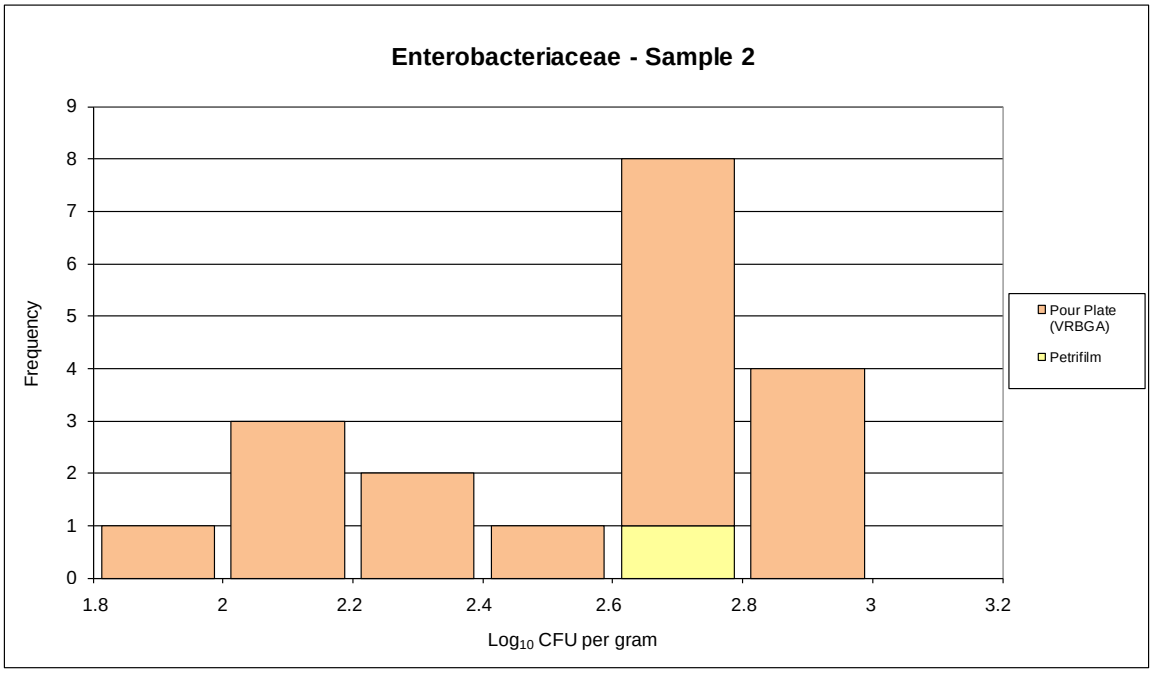


Figure TA-8. Enterobacteriaceae $\log_{10}$ cfu/g results for sample PTA 2.

6.7 Coagulase-positive *Staphylococci*

A total of five laboratories submitted results for Coagulase-positive *Staphylococci*. Four laboratories tested using Spread Plate, including two laboratories that submitted two sets of results. One laboratory tested 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 CV of 2.6% for sample PTA 2 for this round is lower than the value of 4.3%, obtained for the results in Round 24 of this program, for samples containing similar organisms at similar levels (see Report No. 1098).

There were no outliers reported for sample PTA 2. Sample PTA 1 did not contain Coagulase-positive *Staphylococci.* Laboratory code 7 (using Petrifilm™) reported a false positive result for sample PTA 1. This sample contained a Coagulase-negative *Staphylococcus* species.

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Coagulase-positive *Staphylococci* - Spread Plate	-	3.695 ± 0.038

Two laboratories reported MUs associated with their test results in this round for Coagulase-positive *Staphylococci*. One reported their MU as a $\log_{10}$ value and the other reported a range in cfu/g; both overlapped the median and associated standard error (se) for each sample.

A graph showing the distribution of results for Coagulase-positive *Staphylococci* testing is included in Figure TA-9. This graph shows the distribution of results from the methods used in this round including the Global Proficiency data and is included for interest purposes only.

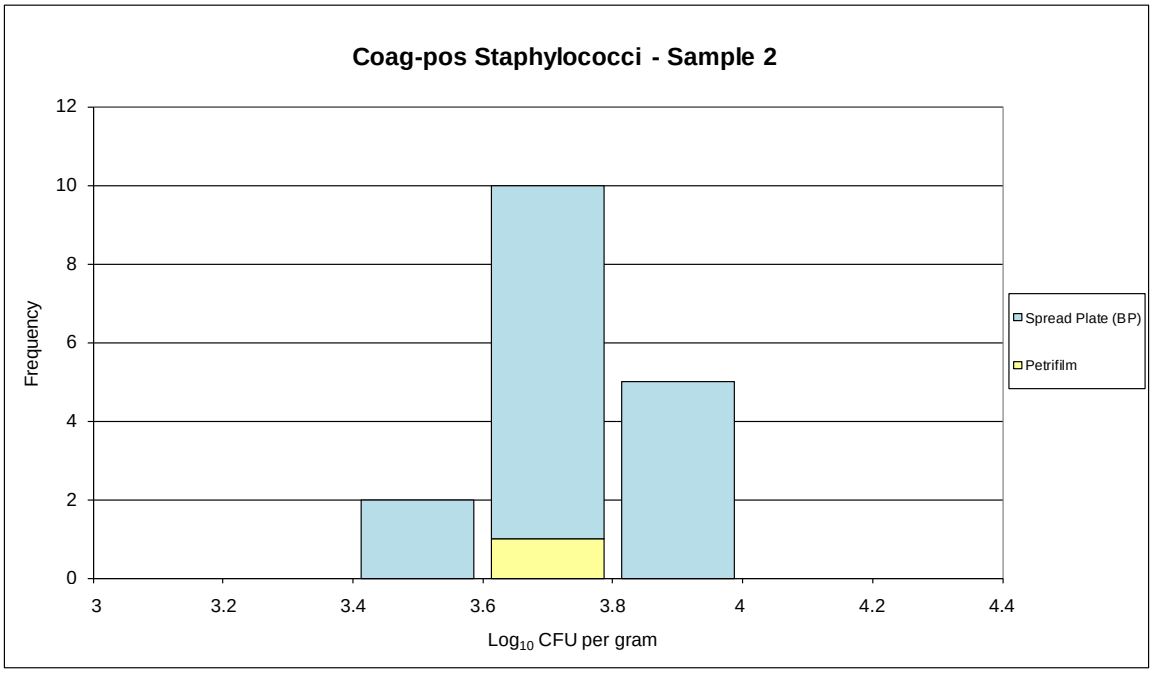


Figure TA-9. Coagulase-positive *Staphylococci* $\log_{10}$ cfu/g results for sample PTA 2.

6.8 *B. cereus*

A total of four laboratories tested the samples for *B. cereus*. All of these laboratories tested using the Spread Plate method, including two laboratories that submitted two sets of results.

The results were analysed against the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The *B. cereus* results were not analysed in Round 24 of this program. The robust CV of 5.1% for the results for sample PTA 2 for this round is higher than the value of 2.1%, obtained for the results in Round 23 of this program, for samples containing similar organisms at similar levels (see Report No. 1060).

Laboratory code 5 (using the Spread Plate method) reported an outlier for sample PTA 2. Sample PTA 1 did not contain *B. cereus.*

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
B. cereus - Spread Plate	-	3.765 ± 0.070

One laboratory reported MUs associated with their test results in this round for *B. cereus* as a $\log_{10}$ value; the value for sample PTA 2 overlapped the median and associated standard error (se).

A graph showing the distribution of results for *B. cereus* testing for sample PTA 2 is included in Figure TA-10. This graph shows the distribution of results from the methods used in this round including the Global Proficiency data and is included for interest purposes only.

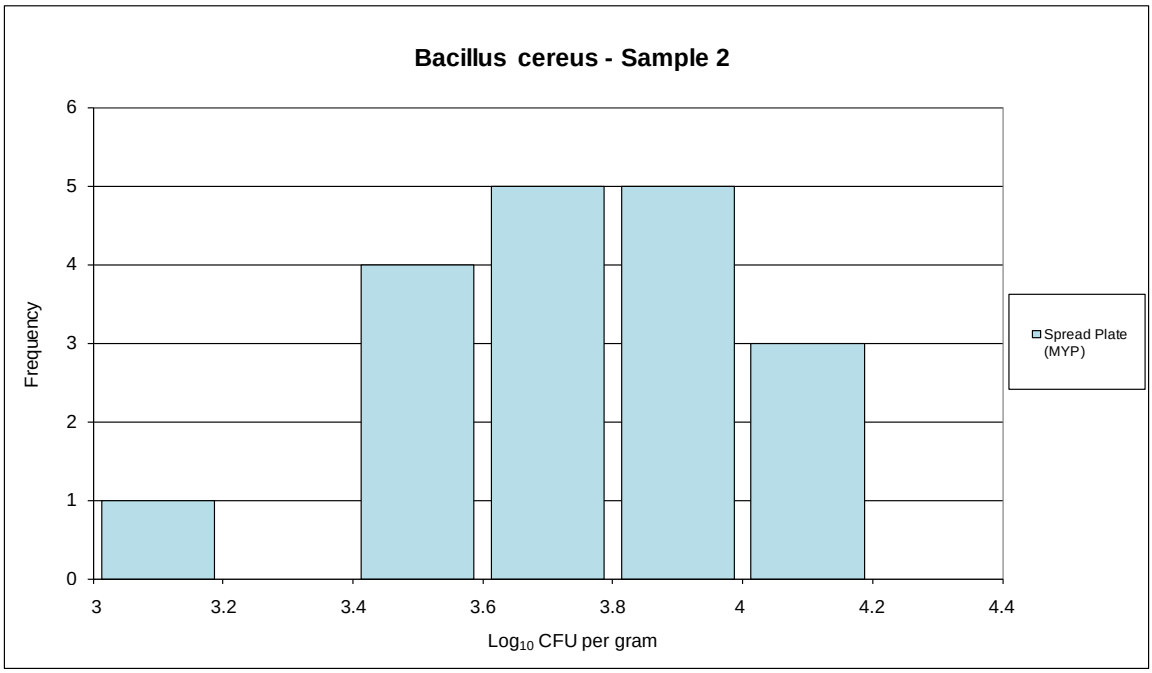


Figure TA-10. *B. cereus* $\log_{10}$ cfu/g results for sample PTA 2.

6.9 Yeasts

A total of six laboratories submitted results for Yeasts. Four laboratories tested using Spread Plate, including two laboratories that submitted two sets of results. One laboratory tested using Pour Plate. One laboratory tested using Petrifilm™. 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 CV for the results for sample PTA 1 was 15.2%. This robust CV 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 1. The target CV chosen was 5.3% and is based on performance of the same batch used in a previous round.

There were no outliers reported for sample PTA 1. Sample PTA 2 did not contain Yeasts. Laboratory code 7 (using Petrifilm™) reported a false positive result 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 Yeasts test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Yeasts - Pour Plate / Spread Plate	2.760 ± 0.214	-

One laboratory reported MUs associated with their test results in this round for Yeasts as a range in cfu/g. It is recommended laboratory code 7 may need to re-examine their test results or their MU calculations for the Petrifilm™ method as their results for sample PTA 1 and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

A graph showing the distribution of results for Yeasts testing for sample PTA 1 is included in Figure TA-11. This graph shows the distribution of results from the methods used in this round including the Global Proficiency data and is included for interest purposes only.

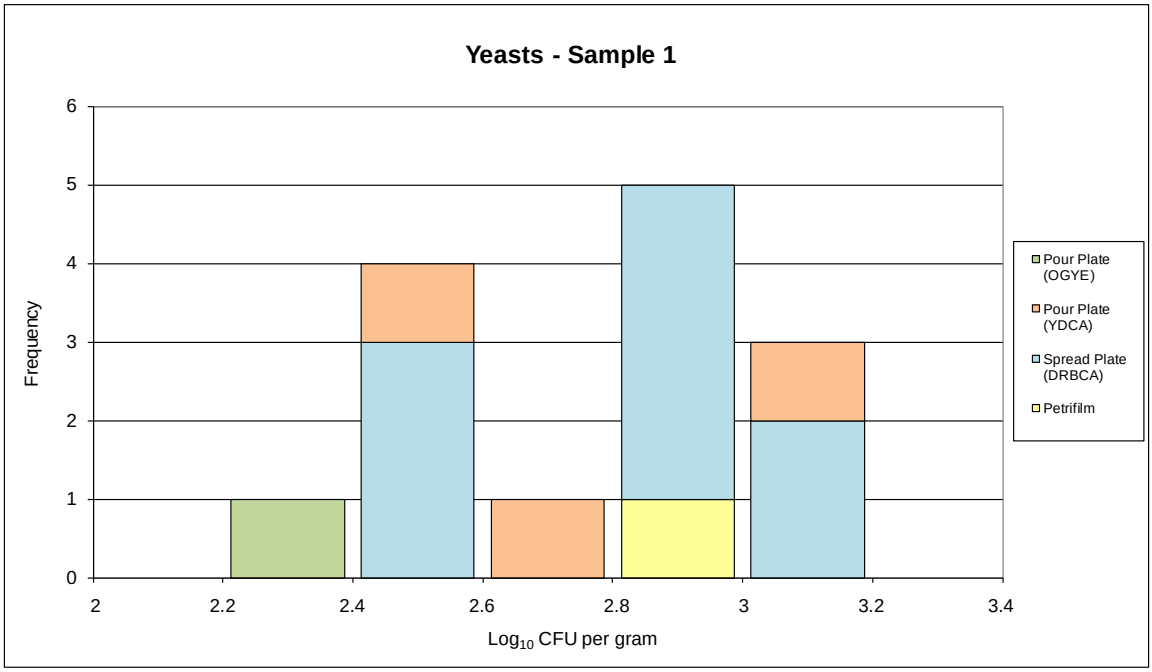


Figure TA-11. Yeasts $\log_{10}$ cfu/g results for sample PTA 1.

6.10 Moulds

A total of six laboratories submitted results for Moulds. Four laboratories tested using Spread Plate, including two laboratories that submitted two sets of results. One laboratory tested using Pour Plate. One laboratory tested using Petrifilm™. 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 of 7.0% and 6.7% for this round compare well with the values of 4.3% and 7.1%, obtained in Round 24 of this program, for samples containing the same organisms at similar levels (see Report No. 1098).

There were no outliers reported for either sample.

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Moulds - Pour Plate / Spread Plate	2.890 ± 0.096	2.640 ± 0.083

One laboratory reported MUs associated with their test results in this round for Moulds as a range in cfu/g. It is recommended laboratory code 7 may need to re-examine their test results or their MU calculations for the Petrifilm™ method, as their results and the stated uncertainty for both samples was outside the expected range of the medians and their associated uncertainties.

Graphs showing the distribution of results for Mould testing for sample PTA 1 and PTA 2 are included in Figures TA-12 and TA-13. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

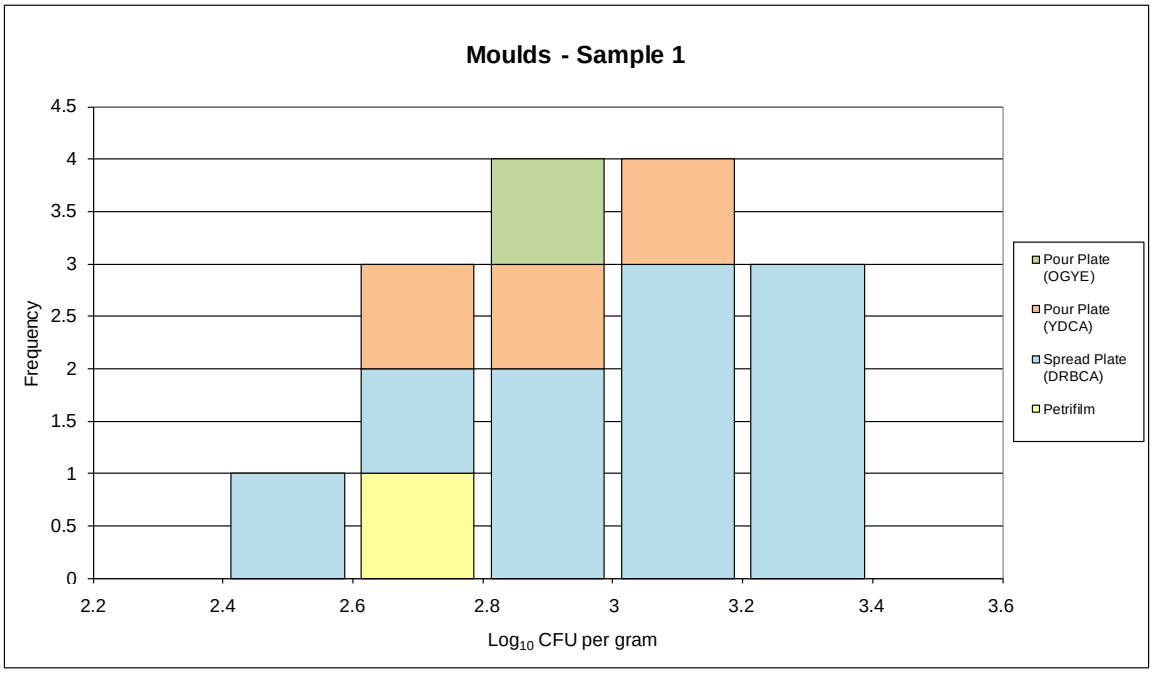


Figure TA-12. Moulds $\log_{10}$ cfu/g results for sample PTA 1.

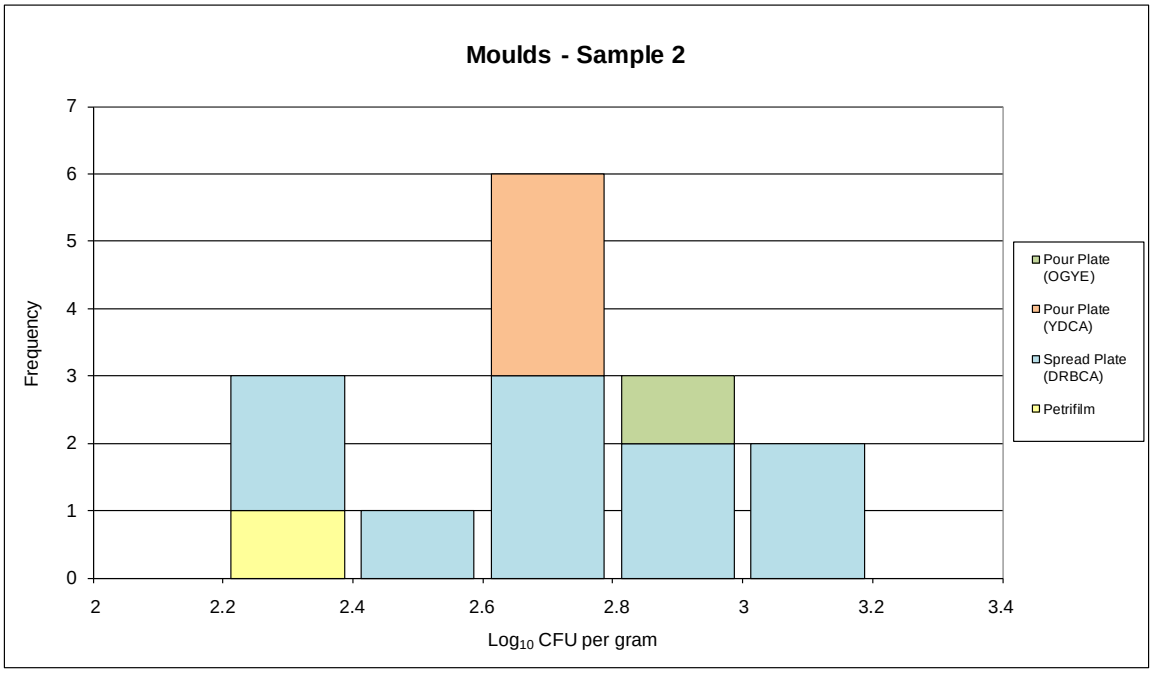


Figure TA-13. Moulds $\log_{10}$ cfu/g results for sample PTA 2.

6.11 Total Yeasts and Moulds

A total of five laboratories submitted results for Total Yeasts and Moulds. Two 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. One laboratory tested using Petrifilm™. 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 of 7.6% and 5.0% for this round compare well with the values of 4.7% and 5.0%, obtained in Round 24 of this program, for samples containing the same organisms at similar levels (see Report No. 1098).

There were no outliers reported for either sample.

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 $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Total Yeasts and Moulds - Pour Plate / Spread Plate	3.300 ± 0.078	2.750 ± 0.043
Total Yeasts and Moulds - Pour Plate	3.330 ± 0.058	2.740 ± 0.041

Two laboratories reported MUs associated with their test results in this round for Total Yeasts and Moulds. One reported their MU as a $\log_{10}$ value and the other reported a range in cfu/g.

Of the reported MUs for the Yeasts and Moulds methods, one did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median), details as follows:

- Laboratory code 7 may need to re-examine their test results or their MU calculations for the Petrifilm™ method as their results for sample PTA 1 and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

Graphs showing the distribution of results for Total Yeasts and Moulds testing for sample PTA 1 and PTA 2 are included in Figures TA-14 and TA-15. These graphs show the distribution of results from the methods used in this round including the Global Proficiency data and are included for interest purposes only.

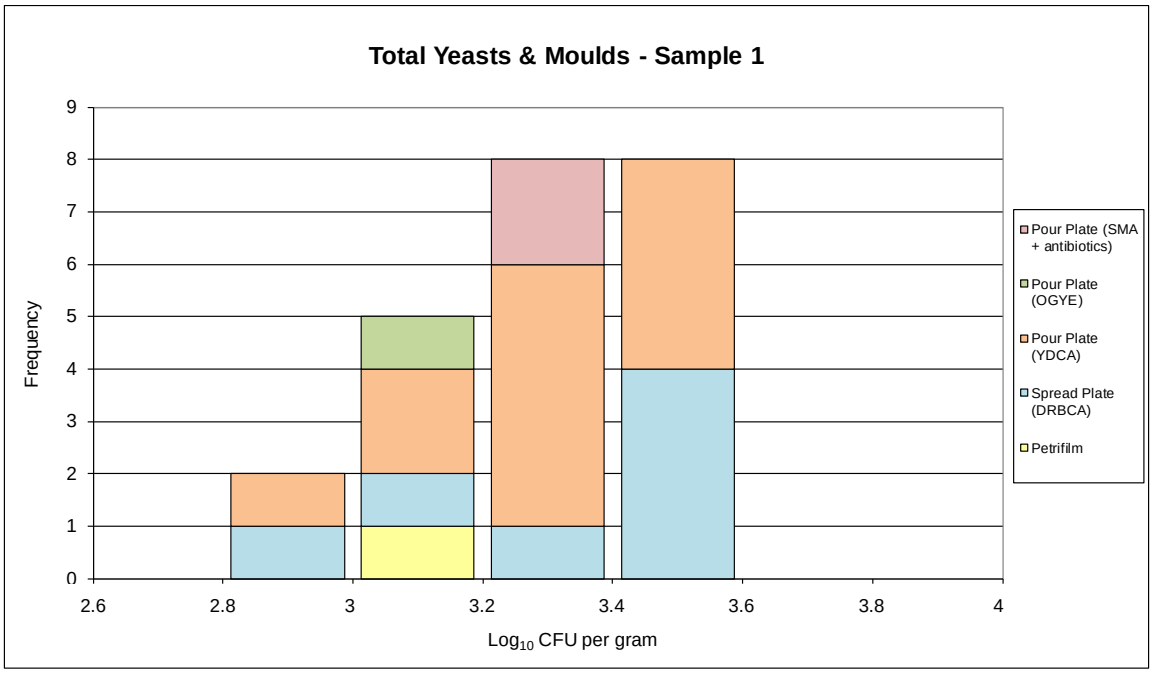


Figure TA-14. Total Yeasts & Moulds $\log_{10}$ cfu/g results for sample PTA 1.

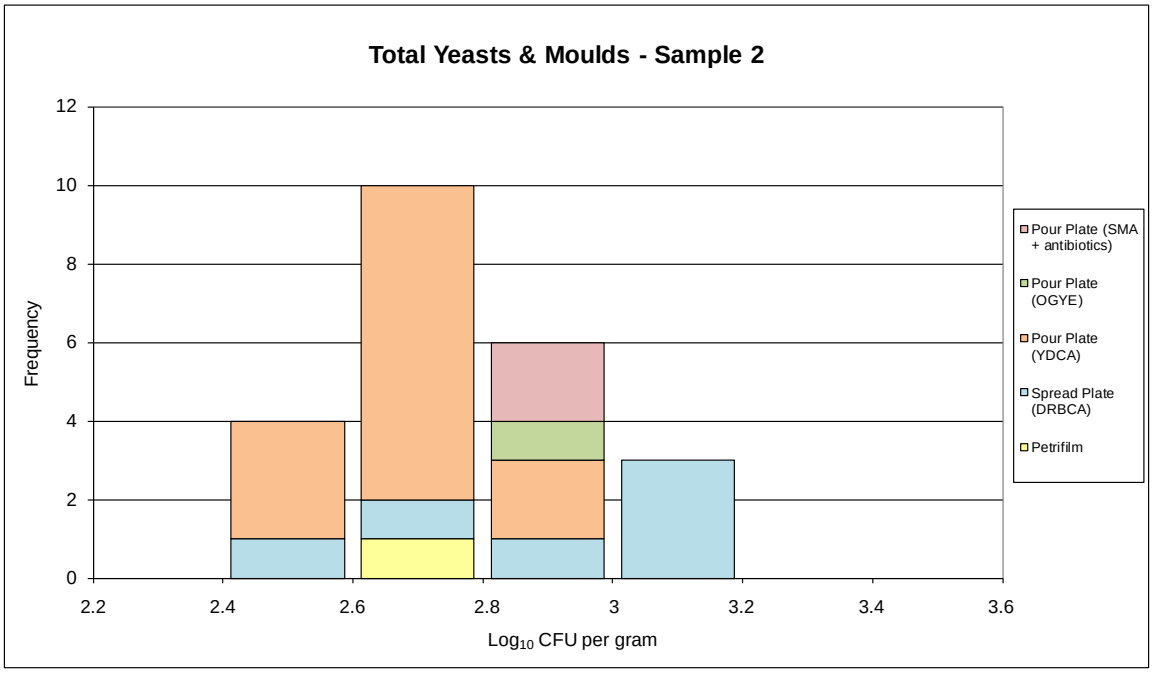


Figure TA-15. Total Yeasts & Moulds $\log_{10}$ cfu/g results for sample PTA 2.

7. 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 5013.2 (2007) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Bacillus cereus - Colony-count technique at 30ºC (ISO 7932: 2004, MOD).*
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 30ºC 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)

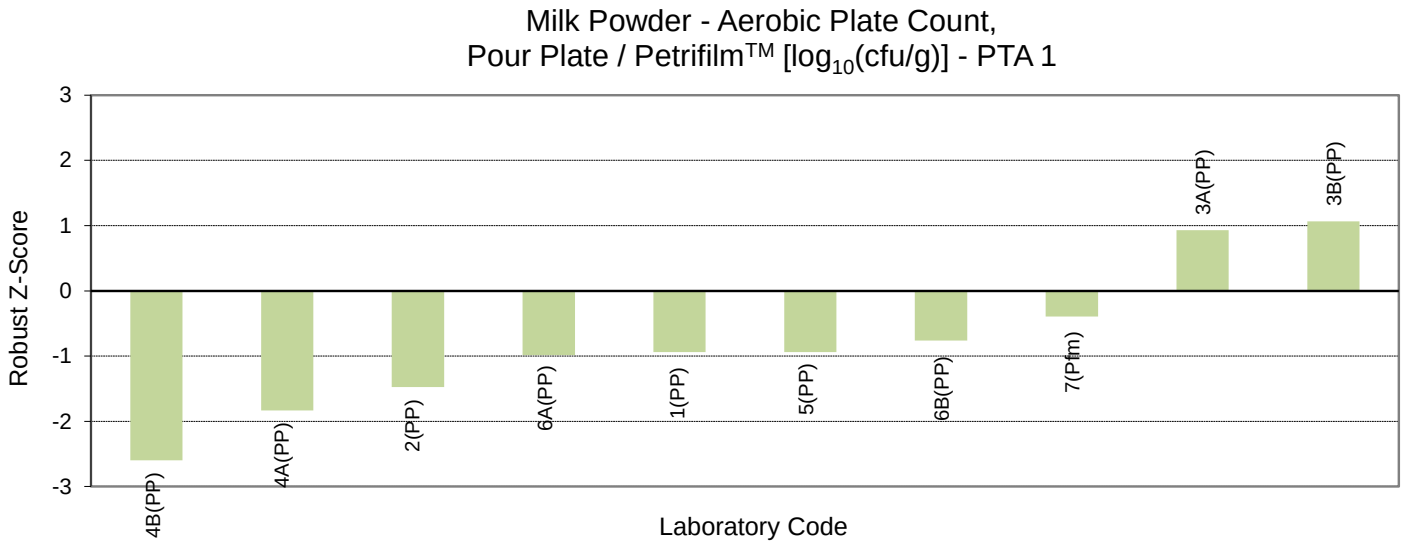
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
1	8100	3.91	-	26000	4.41	-	-0.94	-0.28	PP	PCA
2	7000	3.85	0.15	20000	4.30	0.15	-1.47	-1.18	PP	PCA
3A	13500	4.13	-	42000	4.62	-	0.93	1.37	PP	SMA
3B	14000	4.15	-	41500	4.62	-	1.06	1.33	PP	SMA
4A	6350	3.80	-	5350	3.73	-	-1.83	-5.73 **§**	PP	PCA
4B	5150	3.71	-	7500	3.88	-	-2.60	-4.56 **§**	PP	PCA
5	8100	3.91	-	20700	4.32	-	-0.94	-1.06	PP	-
6A	8000	3.90	-	20000	4.30	-	-0.99	-1.18	PP	PCA
6B	8500	3.93	-	21000	4.32	-	-0.76	-1.01	PP	PCA
7	9400	3.97	8600 - 11400	23500	4.37	22800 - 25300	-0.40	-0.63	Pfm	-

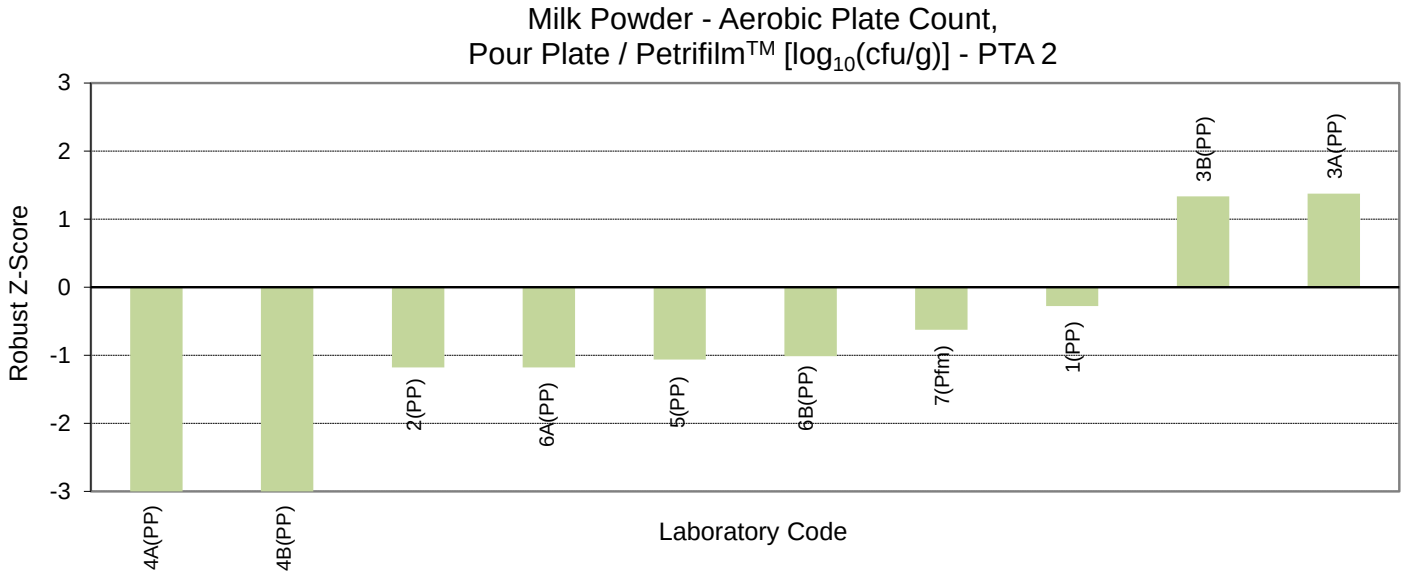
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	14	15
Median	4.020	4.450
Normalised IQR	0.119	0.126
Uncertainty (Median)	0.040	0.041
Robust CV	3.0%	2.8%
Minimum	3.85	3.95
Maximum	4.20	4.71
Range	0.35	0.76

Notes:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Aerobic Plate Count results.
4. 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.
5. 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™ [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
4B(PP)
4A(PP)
2(PP)
6A(PP)
1(PP)
5(PP)
6B(PP)
7(Pfm)
3A(PP)
3B(PP)
Laboratory Code


Milk Powder - Aerobic Plate Count,
Pour Plate / Petrifilm™ [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
4A(PP)
4B(PP)
2(PP)
6A(PP)
5(PP)
6B(PP)
7(Pfm)
1(PP)
3B(PP)
3A(PP)
Laboratory Code

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		
1	540	2.73	-	360	2.56	-	-0.14	-2.71	Pfm	-
3A	400	2.60	-	420	2.62	-	-1.17	-1.98	PP	VRBA
3B	410	2.61	-	390	2.59	-	-1.09	-2.33	PP	VRBA
4A	200	2.30	-	170	2.23	-	-3.56 **§**	-6.26 **§**	PP	VRBA
4B	140	2.15	-	180	2.26	-	-4.79 **§**	-5.99 **§**	PP	VRBA
5	640	2.81	-	900	2.95	-	0.45	1.63	Pfm	-
6A	380	2.58	-	450	2.65	-	-1.35	-1.65	PP	VRBA
6B	410	2.61	-	450	2.65	-	-1.09	-1.65	PP	VRBA
7	160	2.20	140 - 190	< 10 **†**	-	-	-4.33 **§**	-	Pfm	-

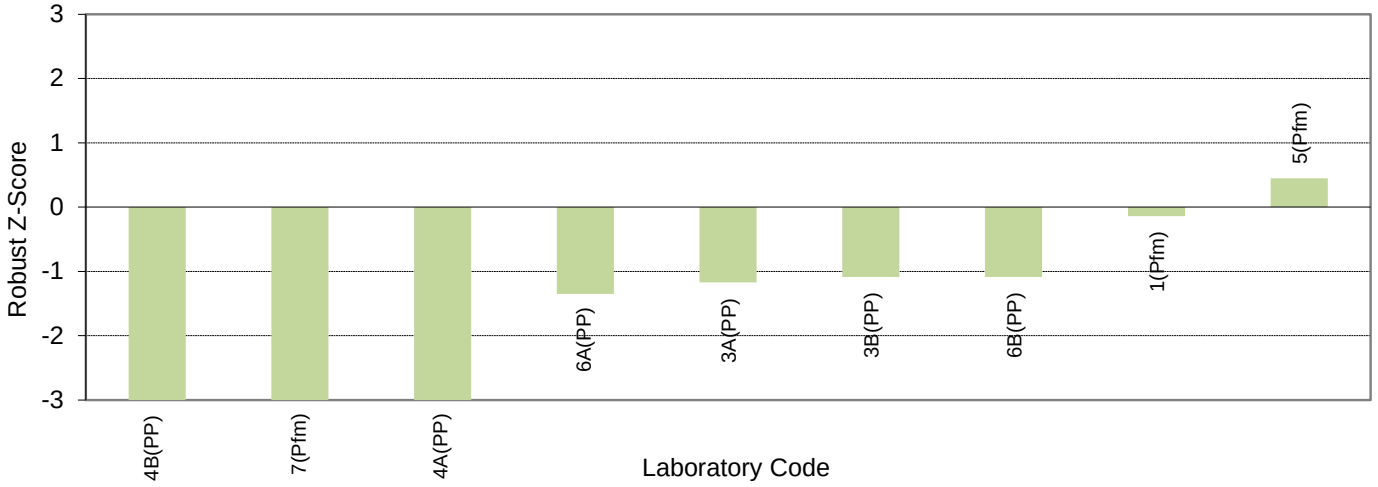
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	11	10
Median	2.750	2.805
Normalised IQR	0.126	0.092
Uncertainty (Median)	0.048	0.036
Robust CV	4.6%	3.3%
Minimum	2.53	2.63
Maximum	2.82	2.88
Range	0.29	0.25

Notes:

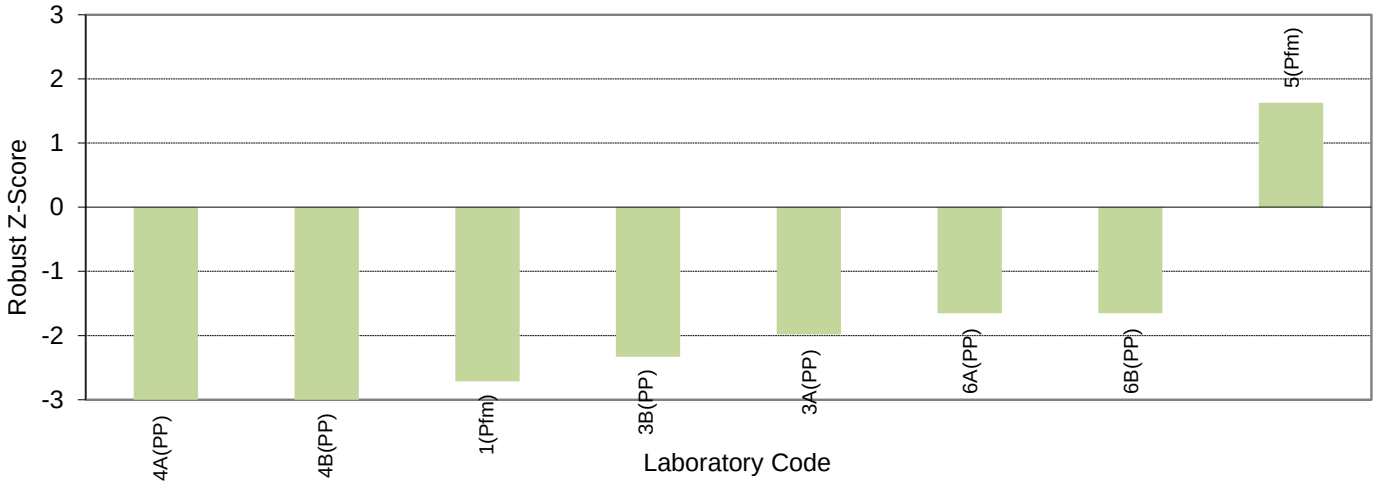
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. **†** denotes a false negative result.
3. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
4. The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
5. Z-scores and summary statistics (including the number of results) for Coliforms were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
6. 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}(cfu/g)] - PTA 1



Milk Powder - Coliforms, Pour Plate / Petrifilm™ [log_{10}(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		
1	360	2.56	-	360	2.56	-	-0.19	-1.58	Pfm	-
5	460	2.66	-	900	2.95	-	0.27	1.29	Pfm	-
6A	320	2.51	-	400	2.60	-	-0.40	-1.25	PP	VRBA MUG
6B	380	2.58	-	410	2.61	-	-0.09	-1.17	PP	VRBA MUG
7	400	2.60	360 - 460	670	2.83	630 - 720	0.01	0.37	Pfm	-

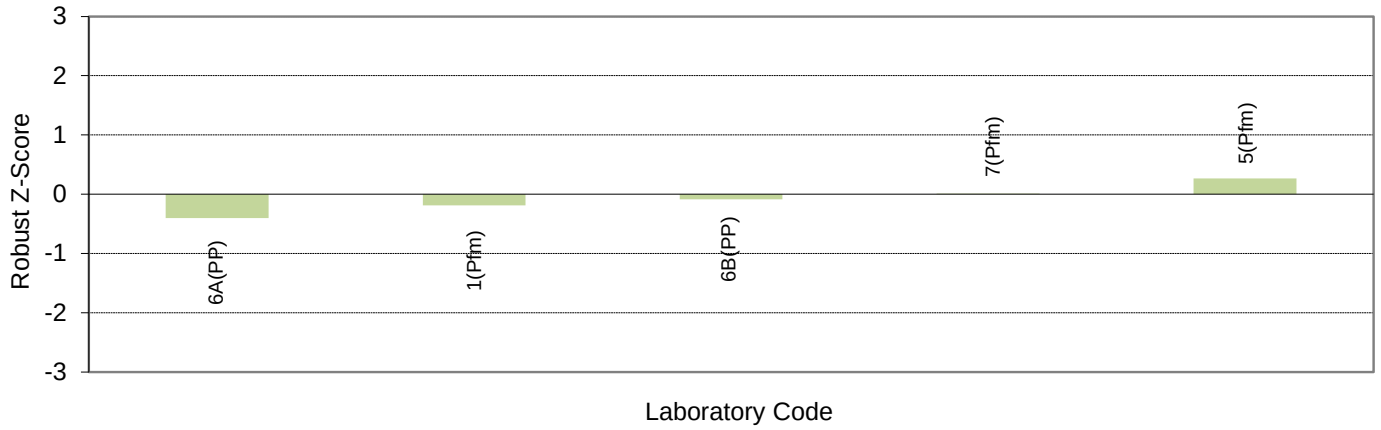
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	7	6
Median	2.600	2.775
Normalised IQR	0.234	0.052
Uncertainty (Median)	0.111	0.026
Robust CV	9.0%	1.9%
Target SD	n/a	0.139
Target CV	n/a	5.0%
Minimum	2.40	2.61
Maximum	2.96	2.90
Range	0.56	0.29

Notes:

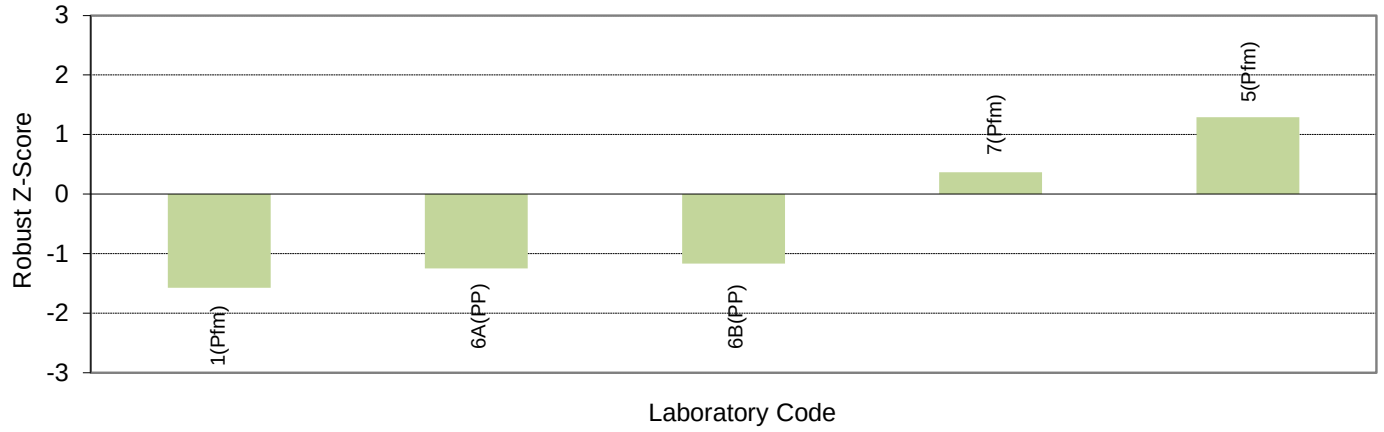
1. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
2. The Pour Plate and Petrifilm™ methods were pooled when analysing the *E. coli* results.
3. A target CV was used to calculate the robust z-scores for sample PTA 2. The target CV chosen was 5.0%.
4. The target SD 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 (2016).
5. 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.
6. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A3.2

Milk Powder - *E.coli*, Pour Plate / Petrifilm™ [log_{10}(cfu/g)] - PTA 1



Milk Powder - *E.coli*, Pour Plate / Petrifilm™ [log_{10}(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		
1	180	2.26	-	70	1.85	-	-1.80	-3.58 **§**	PP	VRBGA
2	120	2.08	0.22	150	2.18	0.22	-2.44	-2.32	PP	VRBA
3A	200	2.30	-	450	2.65	-	-1.63	-0.50	PP	VRBGA
3B	230	2.36	-	400	2.60	-	-1.41	-0.70	PP	VRBGA
4A	150	2.18	-	130	2.11	-	-2.09	-2.56	PP	VRBD
4B	200	2.30	-	160	2.20	-	-1.63	-2.21	PP	VRBD
6A	400	2.60	-	460	2.66	-	-0.54	-0.47	PP	VRBGA
6B	420	2.62	-	500	2.70	-	-0.46	-0.33	PP	VRBGA
7	650	2.81	62 - 72	550	2.74	510 - 630	0.23	-0.17	Pfm	-

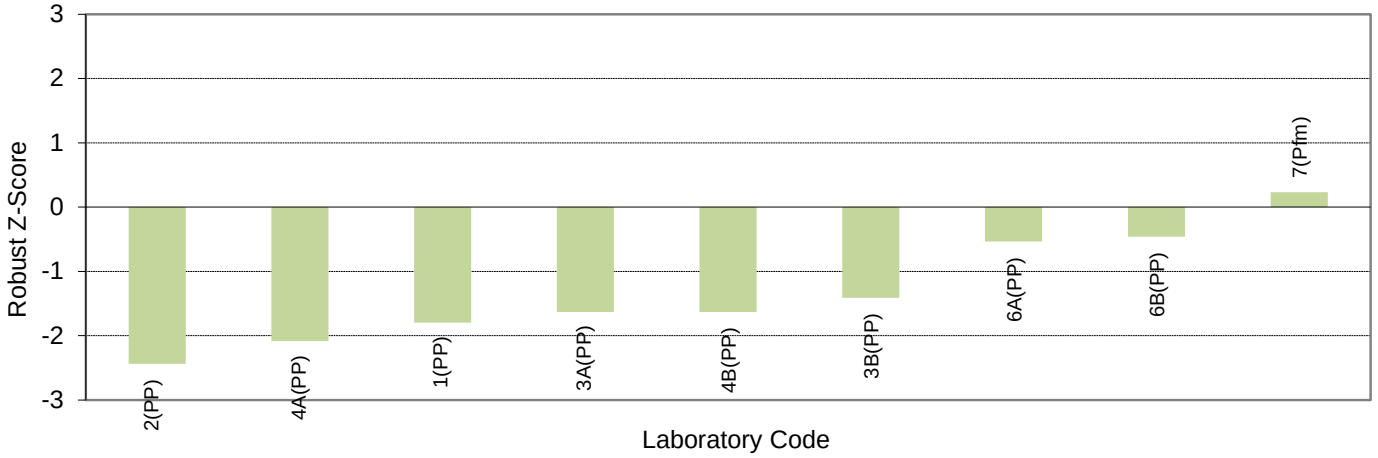
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	8	10
Median	2.750	2.785
Normalised IQR	0.145	0.262
Uncertainty (Median)	0.064	0.104
Robust CV	5.3%	9.4%
Target SD	0.275	n/a
Target CV	10.0%	n/a
Minimum	2.60	2.18
Maximum	2.87	2.86
Range	0.27	0.68

Notes:

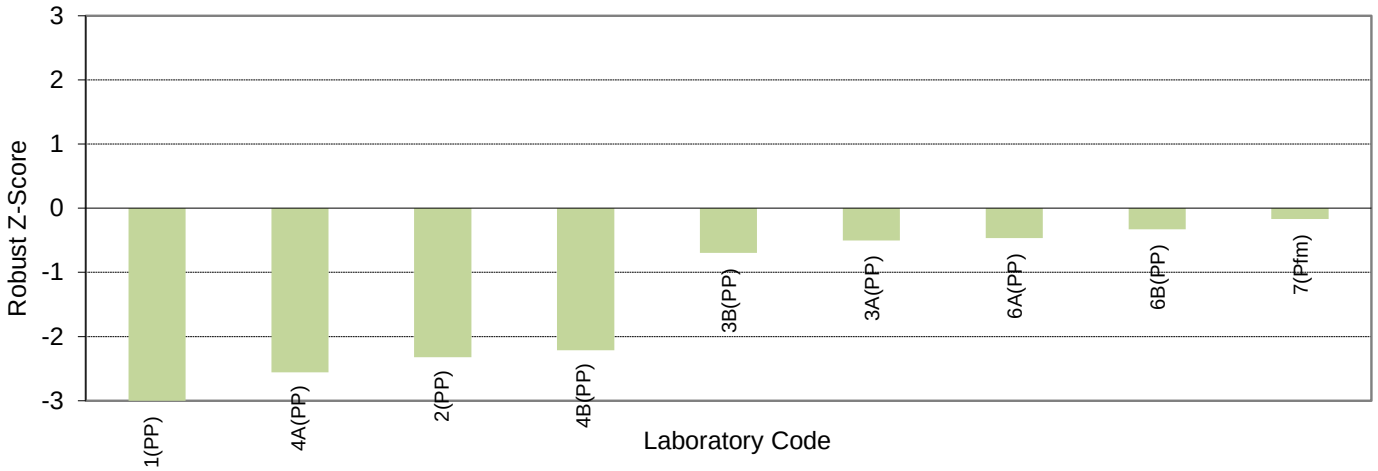
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Enterobacteriaceae results.
4. A target CV was used to calculate the robust z-scores for sample PTA 1. The target CV chosen was 10.0%.
5. The target SD was obtained for sample PTA 1 by multiplying the target CV by the median. This value was used to calculate the z-scores for sample PTA 1. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2016).
6. 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.
7. 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}(cfu/g)] - PTA 1



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



Section A5

Coagulase-positive *Staphylococci*

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

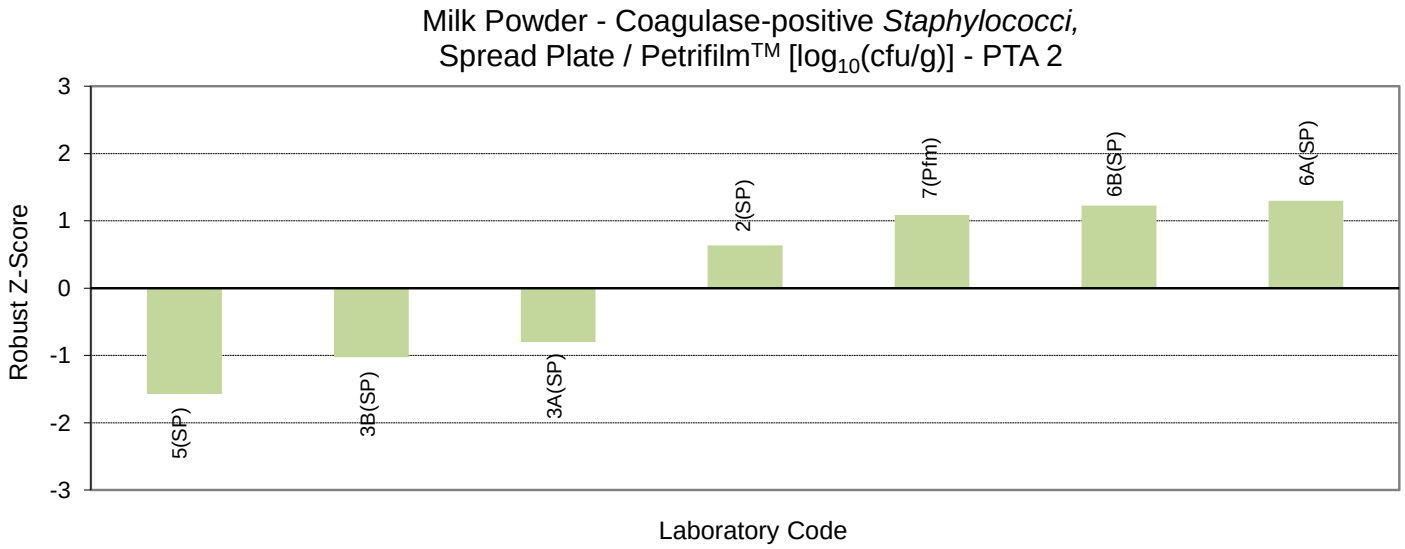
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
2	< 100	-	0.17	5700	3.76	0.17	-	0.63	SP	BP
3A	0	-	-	4150	3.62	-	-	-0.80	SP	BPA
3B	0	-	-	3950	3.60	-	-	-1.03	SP	BPA
5	< 100	-	-	3500	3.54	-	-	-1.57	SP	BP
6A	< 10	-	-	6600	3.82	-	-	1.30	SP	BPA
6B	< 10	-	-	6500	3.81	-	-	1.23	SP	BPA
7	410 ‡	2.61	370 - 500	6300	3.80	5400 - 7800	-	1.09	Pfm	-

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	9	10
Median	n/a	3.695
Normalised IQR	n/a	0.096
Uncertainty (Median)	n/a	0.038
Robust CV	n/a	2.6%
Minimum	n/a	3.64
Maximum	n/a	3.90
Range	n/a	0.26

Notes:

1. ‡ denotes a false positive result.
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. 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.
5. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
6. Sample PTA 1 did not contain Coagulase-positive *Staphylococci.*

A5.2


Milk Powder - Coagulase-positive *Staphylococci*,
Spread Plate / Petrifilm™ [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
5(SP)
3B(SP)
3A(SP)
2(SP)
7(Pfm)
6B(SP)
6A(SP)
Laboratory Code

Section A6

Bacillus cereus

A6.1

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

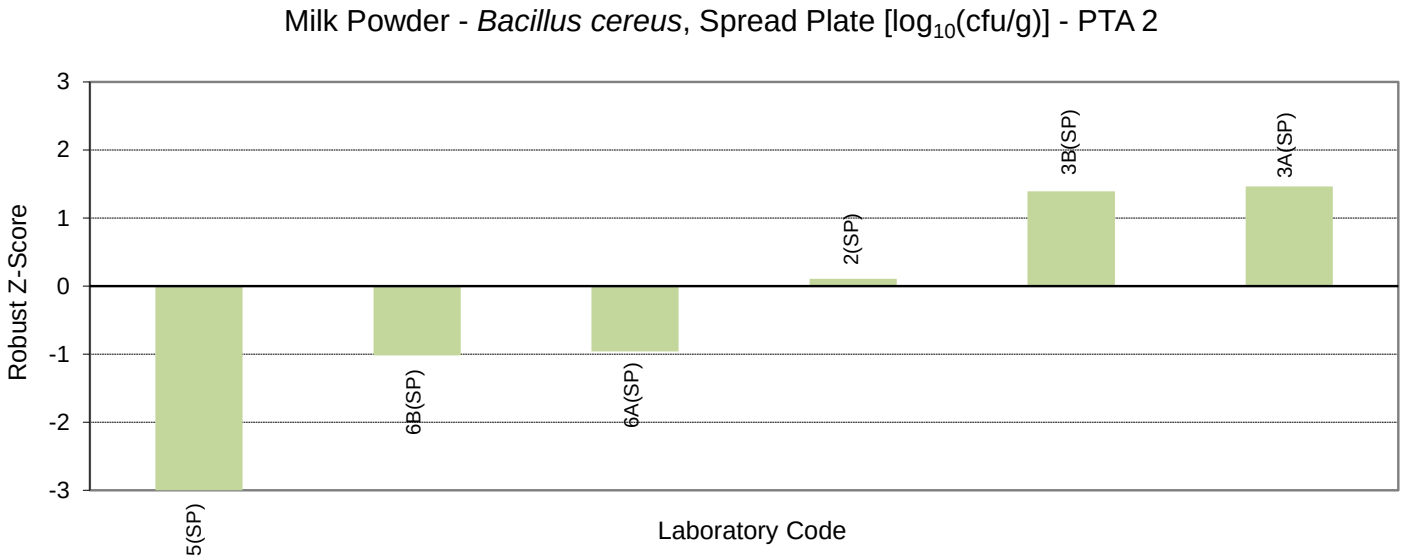
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
2	< 100	-	0.15	6100	3.79	0.15	-	0.11	SP	MYP
3A	0	-	-	11150	4.05	-	-	1.46	SP	MYPA
3B	0	-	-	10800	4.03	-	-	1.39	SP	MYPA
5	< 100	-	-	1300	3.11	-	-	-3.38 **§**	SP	MYP
6A	< 10	-	-	3800	3.58	-	-	-0.96	SP	MYP
6B	< 10	-	-	3700	3.57	-	-	-1.02	SP	MYB

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	12	12
Median	n/a	3.765
Normalised IQR	n/a	0.193
Uncertainty (Median)	n/a	0.070
Robust CV	n/a	5.1%
Minimum	n/a	3.49
Maximum	n/a	4.00
Range	n/a	0.51

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 chart on the following page.
5. Sample PTA 1 did not contain *Bacillus cereus.*

A6.2


Milk Powder - *Bacillus cereus*, Spread Plate [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
5(SP)
6B(SP)
6A(SP)
2(SP)
3B(SP)
3A(SP)
Laboratory Code

Section A7

Yeasts

A7.1

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

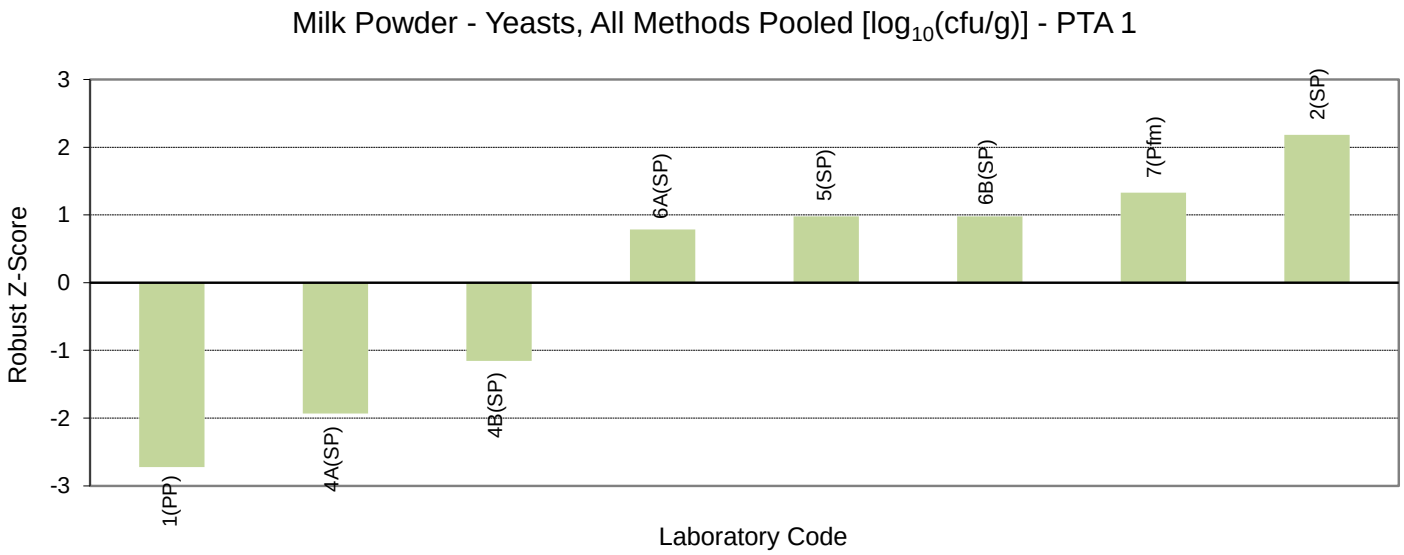
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
1	230	2.36	-	< 10	-	-	-2.72	-	PP	OGYE
2	1200	3.08	-	< 100	-	-	2.18	-	SP	DRBCA
4A	300	2.48	-	0	-	-	-1.93	-	SP	DRBC
4B	390	2.59	-	0	-	-	-1.15	-	SP	DRBC
5	800	2.90	-	< 100	-	-	0.98	-	SP	DRBC
6A	750	2.88	-	< 10	-	-	0.79	-	SP	*
6B	800	2.90	-	< 10	-	-	0.98	-	SP	DRBCA
7	900	2.95	840 - 1020	340 ‡	2.53	290 - 390	1.33	-	Pfm	-

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	6	6
Median	2.760	n/a
Normalised IQR	0.418	n/a
Uncertainty (Median)	0.214	n/a
Robust CV	15.2%	n/a
Target SD	0.146	n/a
Target CV	5.3%	n/a
Minimum	2.49	n/a
Maximum	3.15	n/a
Range	0.66	n/a

Notes:

1. ‡ denotes a false positive result.
2. * denotes DRBCA agar and SDA agar.
3. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
4. All the methods were pooled when analysing the Yeasts results.
5. A target CV was used to calculate the robust z-scores for sample PTA 1. The target CV chosen was 5.3%.
6. The target SD was obtained for sample PTA 1 by multiplying the target CV by the median. This value was used to calculate the z-scores for sample PTA 1. For more information on the use of target CVs to calculate z-scores, please see the Guide to Proficiency Testing Australia (2016).
7. Z-scores and summary statistics (including the number of results) for Yeasts were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
8. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
9. Sample PTA 2 did not contain Yeasts.

A7.2


Milk Powder - Yeasts, All Methods Pooled [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
1(PP)
4A(SP)
4B(SP)
6A(SP)
5(SP)
6B(SP)
7(Pfm)
2(SP)
Laboratory Code

Section A8

Moulds

A8.1

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

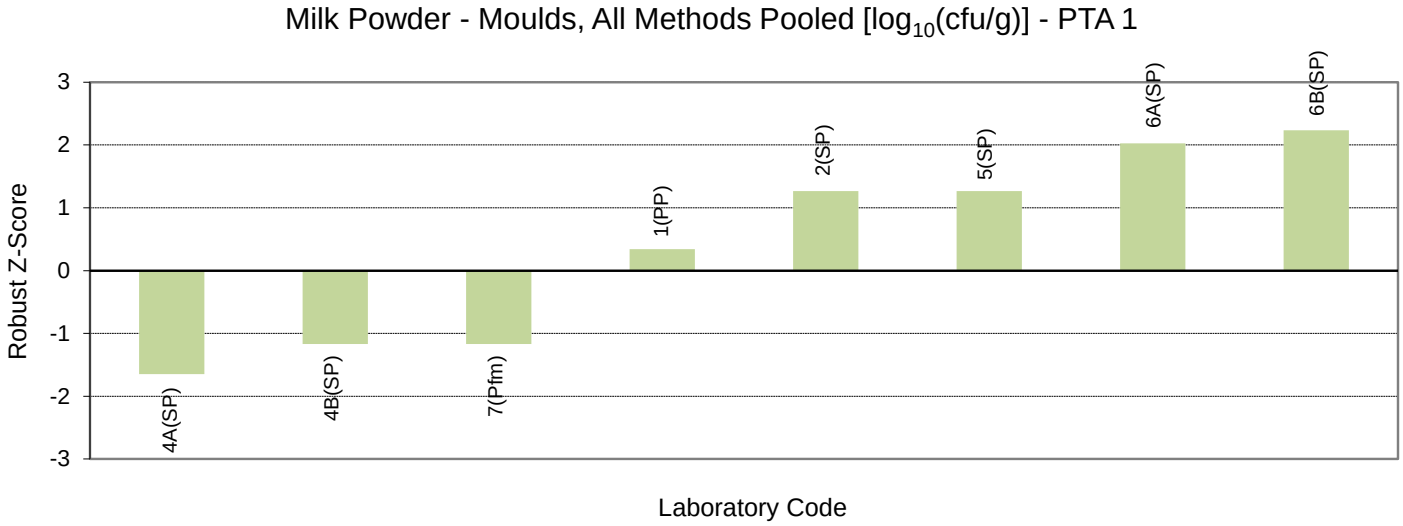
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
1	910	2.96	-	640	2.81	-	0.34	0.95	PP	OGYE
2	1400	3.15	-	400	2.60	-	1.26	-0.22	SP	DRBCA
4A	360	2.56	-	250	2.40	-	-1.65	-1.38	SP	DRBC
4B	450	2.65	-	220	2.34	-	-1.17	-1.69	SP	DRBC
5	1400	3.15	-	600	2.78	-	1.26	0.79	SP	DRBC
6A	2000	3.30	-	1200	3.08	-	2.03	2.50	SP	*
6B	2200	3.34	-	1100	3.04	-	2.23	2.28	SP	DRBCA
7	450	2.65	410 - 470	160	2.20	110 - 210	-1.17	-2.48	Pfm	-

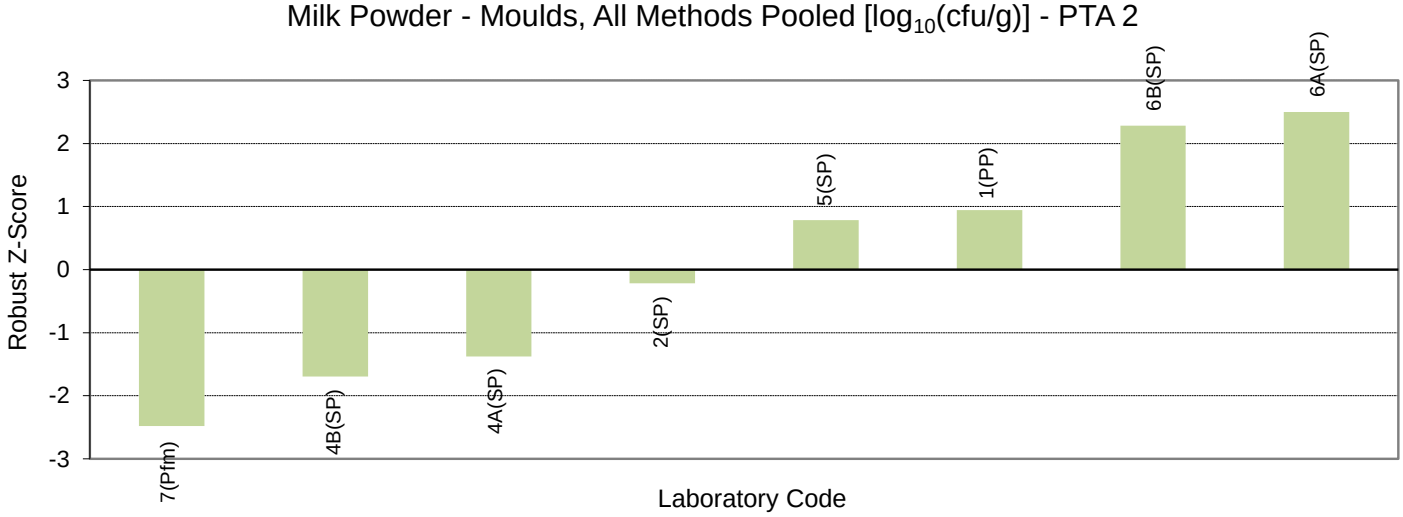
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	7	7
Median	2.890	2.640
Normalised IQR	0.203	0.176
Uncertainty (Median)	0.096	0.083
Robust CV	7.0%	6.7%
Minimum	2.79	2.40
Maximum	3.20	2.98
Range	0.41	0.58

Notes:

1. ***** denotes DRBCA agar and SDA agar.
2. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
3. All the methods were pooled when analysing the Moulds results.
4. Z-scores and summary statistics (including the number of results) for 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.

A8.2


Milk Powder - Moulds, All Methods Pooled [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
4A(SP)
4B(SP)
7(Pfm)
1(PP)
2(SP)
5(SP)
6A(SP)
6B(SP)
Laboratory Code


Milk Powder - Moulds, All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
7(Pfm)
4B(SP)
4A(SP)
2(SP)
5(SP)
1(PP)
6B(SP)
6A(SP)
Laboratory Code

Section A9

Total Yeasts and Moulds

A9.1

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

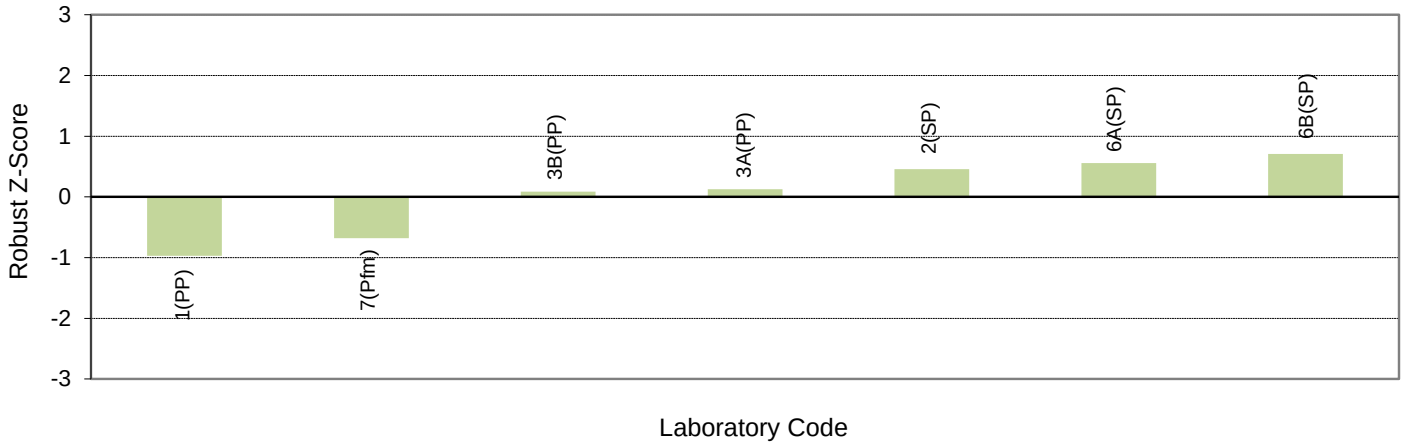
Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
1	1140	3.06	-	640	2.81	-	-0.97	0.41	PP	OGYE
2	2600	3.41	0.17	400	2.60	0.17	0.46	-1.08	SP	DRBCA
3A	2150	3.33	-	900	2.95	-	0.13	1.49	PP	^
3B	2100	3.32	-	800	2.90	-	0.09	1.12	PP	^
6A	2750	3.44	-	1200	3.08	-	0.56	2.40	SP	*
6B	3000	3.48	-	1100	3.04	-	0.71	2.12	SP	DRBCA
7	1350	3.13	1300 - 1450	500	2.70	450 - 600	-0.68	-0.37	Pfm	-

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	16	16
Median	3.300	2.750
Normalised IQR	0.250	0.137
Uncertainty (Median)	0.078	0.043
Robust CV	7.6%	5.0%
Minimum	2.91	2.40
Maximum	3.59	3.07
Range	0.68	0.67

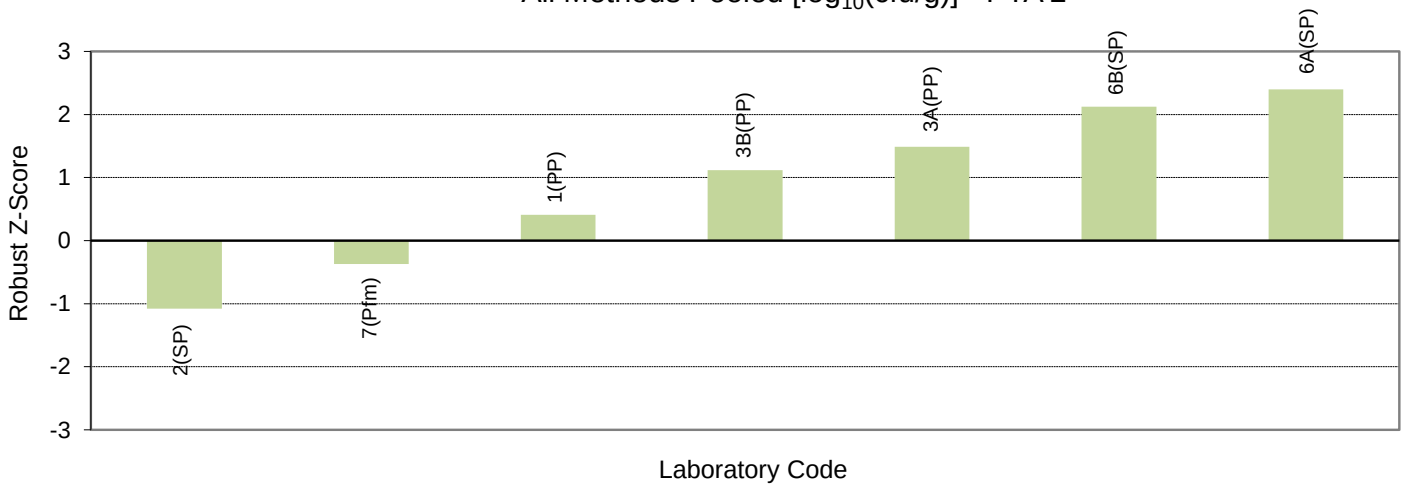
Notes:

1. ***** denotes DRBCA agar and SDA agar.
2. **^** denotes SMA and antibiotics.
3. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
4. All the methods were pooled when analysing the Total Yeasts and Moulds results.
5. 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.
6. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

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



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



APPENDIX B

Homogeneity and Stability Testing

Homogeneity Testing

Samples from PTA 1, 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 72 hours. The results of this homogeneity testing appear in the following table.

Aerobic Plate Count (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
13	10000	4.00	11000	4.04
65	9300	3.97	11000	4.04
111	6600	3.82	9600	3.98
212	9500	3.98	9900	4.00
239	8400	3.92	8600	3.93

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 1, 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 72 hours. The results of this stability testing appear in the following table.

Aerobic Plate Count (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
26	7600	3.88	7100	3.85
27	9000	3.95	8300	3.92
39	8800	3.94	8900	3.95

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 25, October 2018

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
- Coliforms
- *E. coli*
- Enterobacteriaceae
- Coagulase-positive *Staphylococci*
- *Bacillus cereus*
- Yeasts
- Moulds
- Total Yeast and Mould Count

❖ 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, 8,000 cfu/g Coagulase-positive *Staphylococci*, 10,000 *Bacillus cereus,* 2,000 cfu/g yeasts and moulds, and 30,000 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).

- 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 ≈ 2). You may provide MU as a ± 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

All results should arrive at the above address by no later than **Friday 16 November 2018**. 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.

C2.1

PROFICIENCY TESTING AUSTRALIA

Non-Pathogens in Food Proficiency Testing Program
Round 25, October 2018
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)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
Coliforms	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
E. coli	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
Enterobacteriaceae	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
Coagulase-positive *Staphylococci*	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:

C2.2

PROFICIENCY TESTING AUSTRALIA

Non-Pathogens in Food Proficiency Testing Program
Round 25, October 2018
RESULTS SHEET 2

Laboratory Code: ☐

Determination	Report results to nearest	Sample 1		Sample 2		Test Date	Method (see Note)
		Result	MU	Result	MU		
Bacillus cereus	*2 sig. figures* **(cfu/g)**						❑ Spread plate Medium used: ❑ Other:
Yeasts	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
Moulds	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:
Total Yeasts & Moulds	*2 sig. figures* **(cfu/g)**						❑ Pour plate ❑ Spread plate Medium used: ❑ Petrifilm™ ❑ Other:

Note$_1$: 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".

Note$_2$: 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-----