

REPORT NO. 1150

**Non-Pathogens In Food
Proficiency Testing Program
Round 26**

August 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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1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the twenty-sixth 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 May / June 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 nine 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 approx. 30 g samples, one containing a whole milk powder matrix (labelled PTA 1), and one containing a skim milk powder matrix (labelled 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	50,000	10,000
Coliforms	900	500
<i>E. coli</i>	900	300
Enterobacteriaceae	900	500
Coagulase-positive <i>Staphylococci</i>	600	0
<i>B. cereus</i>	9,000	0
Yeasts	0	800
Moulds	500	1,000

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	Number of Results	10	10
		Median	4.485	4.095
		Normalised IQR	0.145	0.094
		Uncertainty (Median)	0.057	0.037
Coliforms	Pour Plate / Petrifilm™	Number of Results	8	8
		Median	2.310	2.715
		Normalised IQR	0.165	0.143
		Uncertainty (Median)	0.073	0.063
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other	Number of Results	7	7
		Median	2.320	2.520
		Normalised IQR	0.113	0.180
		Uncertainty (Median)	0.053	0.085
Enterobacteriaceae	Pour Plate / Petrifilm™	Number of Results	7	7
		Median	2.450	2.720
		Normalised IQR	0.059	0.203
		Uncertainty (Median)	0.028	0.096
Coagulase-positive <i>Staphylococci</i>	Spread Plate	Number of Results	10	10
		Median	2.596	n/a
		Normalised IQR	0.109	n/a
		Uncertainty (Median)	0.043	n/a
<i>B. cereus</i>	Spread Plate	Number of Results	10	12
		Median	3.699	n/a
		Normalised IQR	0.133	n/a
		Uncertainty (Median)	0.053	n/a
Yeasts	All Methods Pooled	Number of Results	1	20
		Median	n/a	2.778
		Normalised IQR	n/a	0.173
		Uncertainty (Median)	n/a	0.048
Moulds	All Methods Pooled	Number of Results	7	20
		Median	2.079	2.954
		Normalised IQR	0.179	0.192
		Uncertainty (Median)	0.085	0.054
Total Yeasts and Moulds	All Methods Pooled	Number of Results	11	10
		Median	1.990	3.335
		Normalised IQR	0.093	0.166
		Uncertainty (Median)	0.035	0.066

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	-	3D, 9A, 9B	-	-
Coliforms	Pour Plate / Petrifilm™	1, 9A, 9B	10A, 10B	3A, 3B, 3C, 3D, 8A (x2), 8B (x2)	-
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other	1, 9A, 9B	1	-	-
Enterobacteriaceae	Pour Plate / Petrifilm™	7, 9A, 9B	7	-	-
Coagulase-positive <i>Staphylococci</i>	Spread Plate	-		-	-
<i>B. cereus</i>	Spread Plate	1		-	-
Yeasts	All Methods Pooled		10A, 10B	5	-
Moulds	All Methods Pooled	-	-	2A, 2B, 2C, 2D, 3A, 3B, 3C, 3D, 8A (x2), 8B (x2)	-
Total Yeasts and Moulds	All Methods Pooled	-	-	3A, 3B, 3C, 3D, 8A (x2), 8B (x2)	-

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, for Aerobic Plate Count, Coliforms, *E. coli*, Enterobacteriaceae and Total Yeasts and Moulds.
4. The summary statistics reported (including the number of results) and z-scores were calculated from the pooled participants' results and the Global Proficiency Ltd DairyChek Microbiology program, using the same samples, for *Coagulase-positive Staphylococci* and *B. cereus*.
5. The summary statistics reported (including the number of results) and z-scores were calculated from the participants' results for Yeasts and Moulds.
6. Target CVs were used to calculate the z-scores for sample PTA 1 for Enterobacteriaceae and Total Yeasts and Moulds
7. Sample PTA 2 did not contain *Coagulase-positive Staphylococci* or *B. cereus*.
8. Sample PTA 1 did not contain Yeasts.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 26 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *E. coli* as the Coliform / Enterobacteriaceae organism present in the sample, whereas sample PTA 2 contained *E. coli* and *Cronobacter sakazakii* as the Coliform / Enterobacteriaceae organisms present.

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

Sample PTA 1 contained *Bacillus cereus* and *Staphylococcus aureus* species, whereas sample PTA 2 contained a Coagulase-negative *Staphylococci* 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 nine laboratories that participated in the program submitted results for inclusion in the final report. Of these nine laboratories, one (11%) submitted results where more than one method was used for a specific test, while one laboratory (11%) provided results for all nine tests. The return rate for all tests is as follows:

• Aerobic Plate Count	9 out of 9	100%
• Coliforms	8 out of 9	89%
• <i>E. coli</i>	2 out of 9	22%
• Enterobacteriaceae	6 out of 9	67%
• Coagulase-positive <i>Staphylococci</i>	4 out of 9	44%
• <i>B. cereus</i>	4 out of 9	44%
• Yeasts	9 out of 9	100%
• Moulds	9 out of 9	100%
• Total Yeasts and Moulds	6 out of 9	67%

6.2 Performance Summary

One or more statistical outliers or false results were reported by eight laboratories (89%) for this round of the Non-Pathogens in Food program. For comparison, 57% of the participants in Round 25 of the Non-Pathogens in Food program reported outliers or false results (see Report No. 1121 for more details).

A total of 234 results were analysed in this round of the program. Of these results, 48 (21%) were identified as outliers or false results. For comparison, 9% of the results analysed in Round 25 of the Non-Pathogens in Food program were outliers or false results (see Report No. 1121 for more details).

6.3 Aerobic Plate Count

All of the nine laboratories that undertook testing for Aerobic Plate Count tested using Pour Plate. The results for the Pour Plate method were analysed against the Pour Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CVs of 3.2% and 2.3% for the results for this round compare well with the values of 3.0% and 2.8%, obtained for the results in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

Laboratory codes 3D, 9A and 9B 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	4.485 ± 0.057	4.095 ± 0.037

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

Graphs showing the distribution of results in this round for the Aerobic Plate Count test (including the Global Proficiency data) are included in Figures TA-1 and TA-2 for interest purposes only.

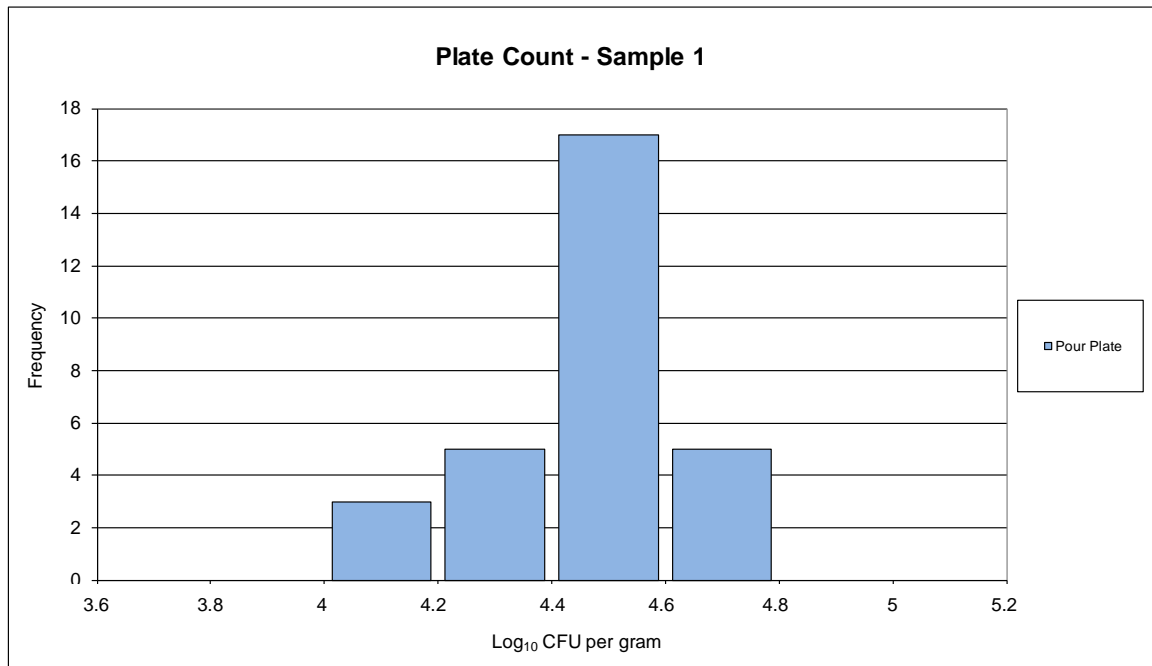


Figure TA-1. APC log₁₀ cfu/g results for sample PTA 1.

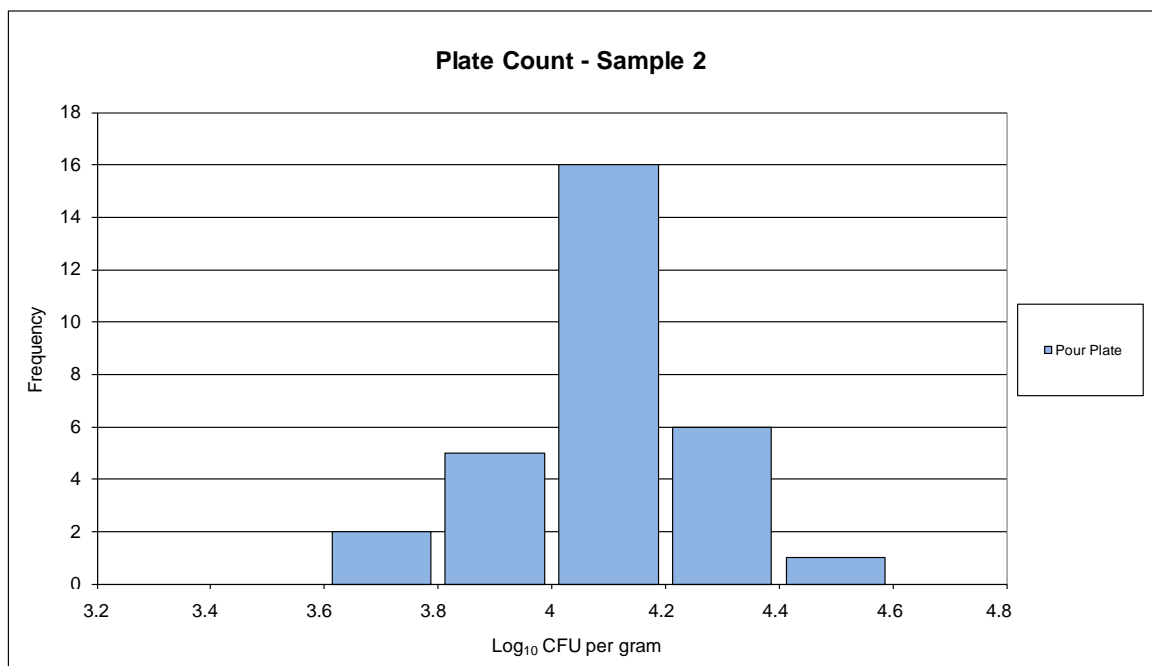


Figure TA-2. APC log₁₀ cfu/g results for sample PTA 2.

6.4 Coliforms

A total of seven laboratories submitted results for Coliforms. Six laboratories tested using Pour Plate. 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 of 7.1% and 5.3% for the results for this round are higher than the values of 4.6% and 3.3%, obtained for the results in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

Laboratory code 1 (using Petrifilm™) and laboratory codes 9A and 9B (using the Pour Plate method) reported outliers for sample PTA 1. Laboratory codes 10A and 10B (using the Pour Plate method) reported outliers for sample PTA 2.

Laboratory codes 3A, 3B, 3C and 3D reported false negative results for sample PTA 1, while laboratory codes 8A and 8B each reported two false negative results for sample PTA 1. All of these laboratories tested using the Pour Plate method.

Sample PTA 1 only had one coliform organism in the sample – the same *E. coli* strain used in sample PTA 2 as the predominant coliform organism.

Consideration of environmental conditions during transit is important for microbiological samples and is the reason specialised packaging is used in this programme. While this packaging keeps samples frozen for up to 8 days, delay outside of this may have an impact on sample condition if the package has not been kept in a chiller or freezer during the period of delay, e.g. while awaiting customs clearance procedures. When PTA is aware of delays in receipt of samples, one of the options considered is to closely monitor performance of the affected laboratory's results to ensure sample integrity has not been compromised. Where a sample may have experienced high temperatures, then a common outcome is to see a decrease in the numbers of the less robust microorganisms contained in the samples, which includes the coliforms group. However, if this has occurred, then we would expect to see it across BOTH samples, not just limited to one, which appears to be the case in this round. Therefore, for those laboratories reporting either false-negative or low-biasing results for sample PTA 1, it is recommended further investigation is undertaken.

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	2.310 ± 0.073	2.715 ± 0.063

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

Graphs showing the distribution of results in this round for the Coliforms test (including the Global Proficiency data) are included in Figures TA-3 and TA-4 for interest purposes only.

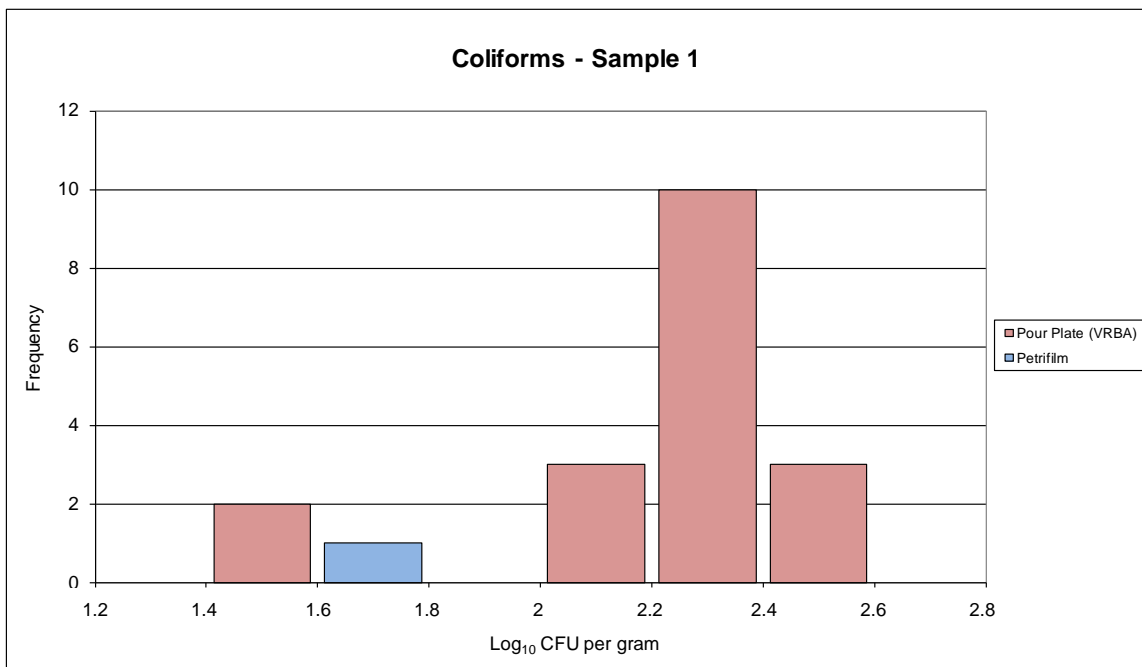


Figure TA-3. Coliform log₁₀ cfu/g results for sample PTA 1.

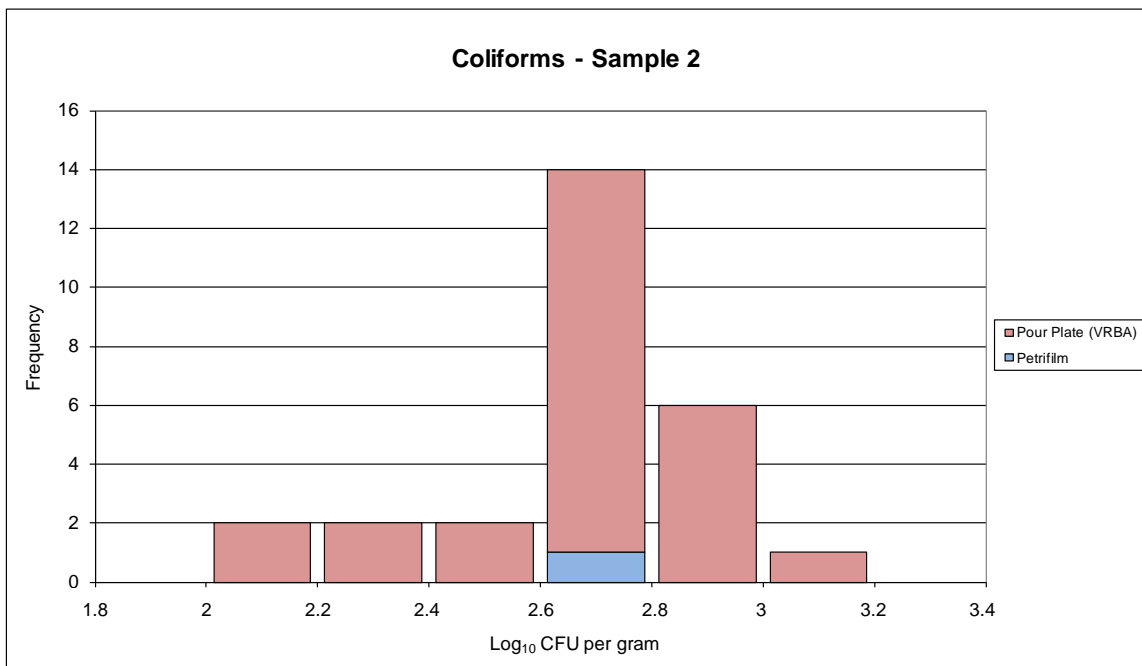


Figure TA-4. Coliform log₁₀ cfu/g results for sample PTA 2.

6.5 *E. coli*

One of the two laboratories that submitted results for *E. coli* tested 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 CVs of 4.9% and 7.2% for the results for this round compare well with the value of 9.0%, obtained for sample PTA 1, and the target CV chosen of 5.0% for sample PTA 2 in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

Laboratory code 1 (using Petrifilm™) reported outliers for both samples. Laboratory codes 9A and 9B (using the Pour Plate method) reported outliers for sample PTA 1 (please refer to the comments included in the Coliforms section relating to false-negative or low-biasing results).

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 ± 0.053	2.520 ± 0.085

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

Graphs showing the distribution of results in this round for the *E. coli* test (including the Global Proficiency data) are included in Figures TA-5 and TA-6 for interest purposes only.

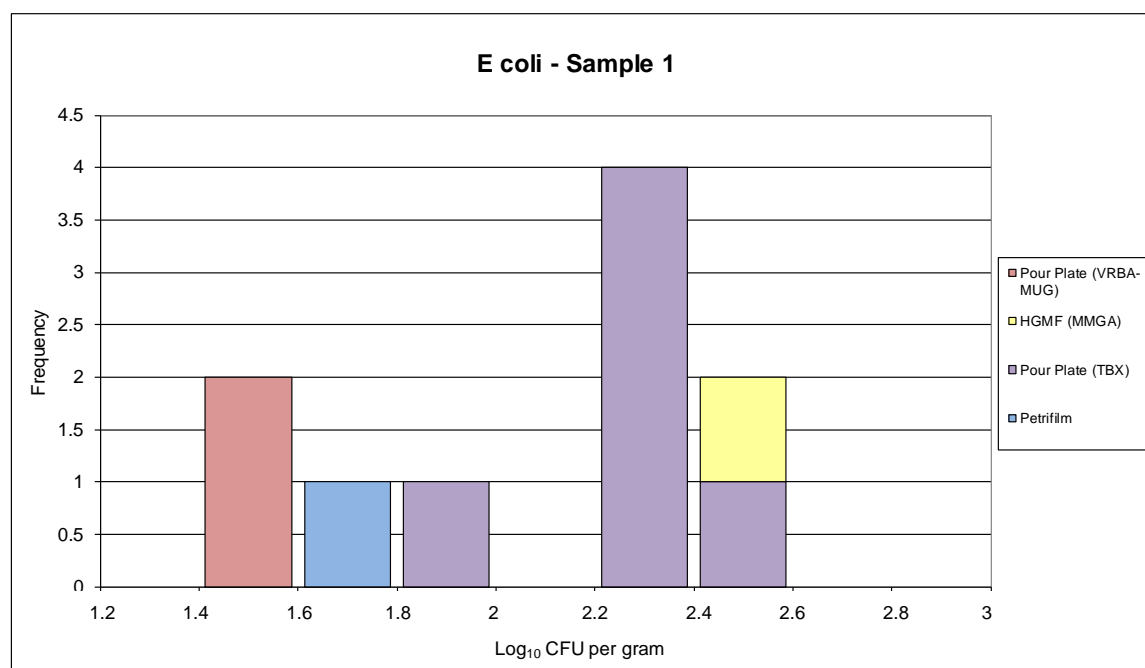


Figure TA-5. *E. coli* log₁₀ cfu/g results for sample PTA 1.

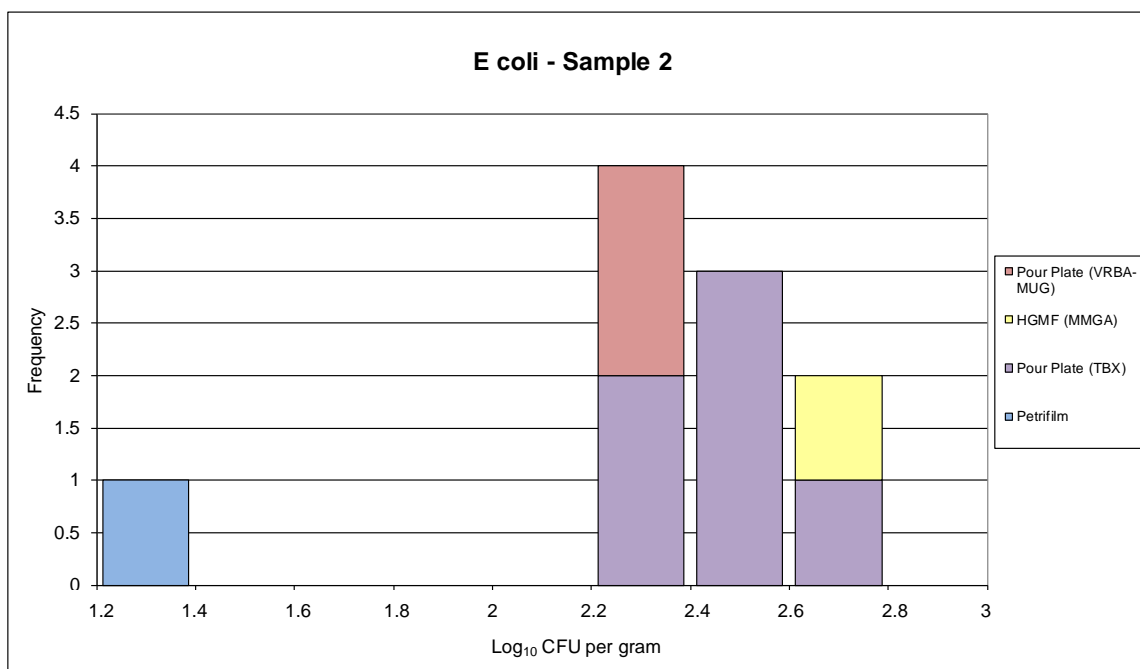


Figure TA-6. *E. coli* log₁₀ cfu/g results for sample PTA 2.

6.6 Enterobacteriaceae

A total of six laboratories submitted results for Enterobacteriaceae. All six of these laboratories tested using Pour Plate. One laboratory also 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 2.4% and 7.5%, for samples PTA 1 and PTA 2, respectively. The robust CV of 2.4% 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 7.5%. This value was chosen because it was the same CV as that obtained for sample PTA 2.

Laboratory code 7 (using the Pour Plate method) reported outliers for both samples. Laboratory codes 9A and 9B (using the Pour Plate method) reported outliers for sample PTA 1 (please refer to the comments included in the Coliforms section relating to false-negative or low-biasing results).

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	2.450 ± 0.028	2.720 ± 0.096

One laboratory reported MUs associated with their test results in this round for Enterobacteriaceae, as \log_{10} values. It is recommended laboratory code 7 re-examines their test results or their MU calculations for both samples as their results were further from the median than their stated uncertainty (taking into consideration the uncertainty associated with the median).

Graphs showing the distribution of results in this round for the Enterobacteriaceae test (including the Global Proficiency data) are included in Figures TA-7 and TA-8 for interest purposes only.

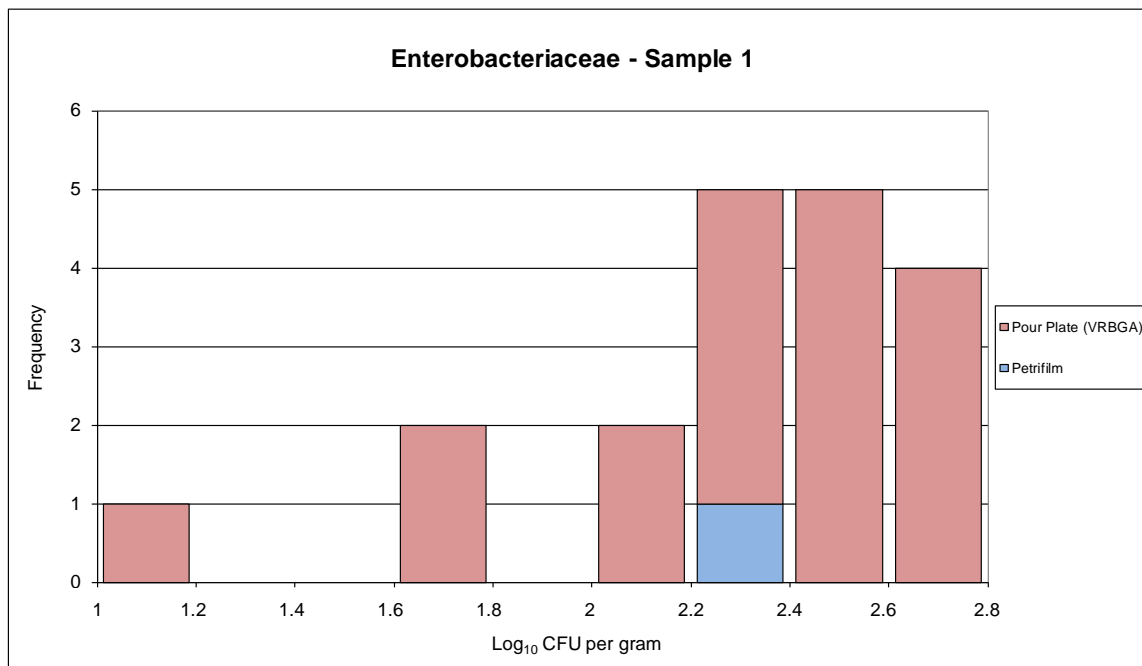


Figure TA-7. Enterobacteriaceae \log_{10} cfu/g results for sample PTA 1.

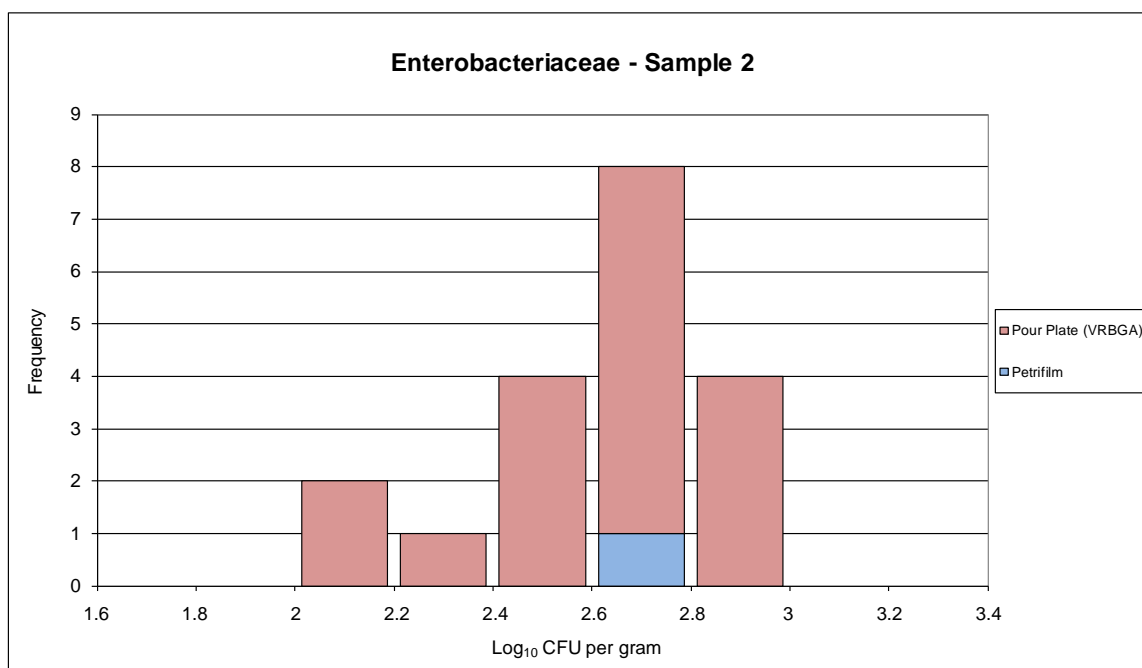


Figure TA-8. Enterobacteriaceae \log_{10} cfu/g results for sample PTA 2.

6.7 Coagulase-positive *Staphylococci*

All four of the laboratories that submitted results for Coagulase-positive *Staphylococci* tested using Spread Plate. The Spread Plate results were pooled with the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples, for analysis.

The robust CV of 4.2% for sample PTA 1 for this round is higher than the value of 2.6%, obtained for the results in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

There were no outliers reported for sample PTA 1. Sample PTA 2 did not contain Coagulase-positive *Staphylococci*.

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	2.596 ± 0.043	-

One laboratory reported MUs associated with their test results in this round for Coagulase-positive *Staphylococci*, as log₁₀ values, which overlapped the median and associated standard error (se) for each sample.

A graph showing the distribution of results for sample PTA 1 in this round for the Coagulase-positive *Staphylococci* test (including the Global Proficiency data) is included in Figure TA-9 for interest purposes only.

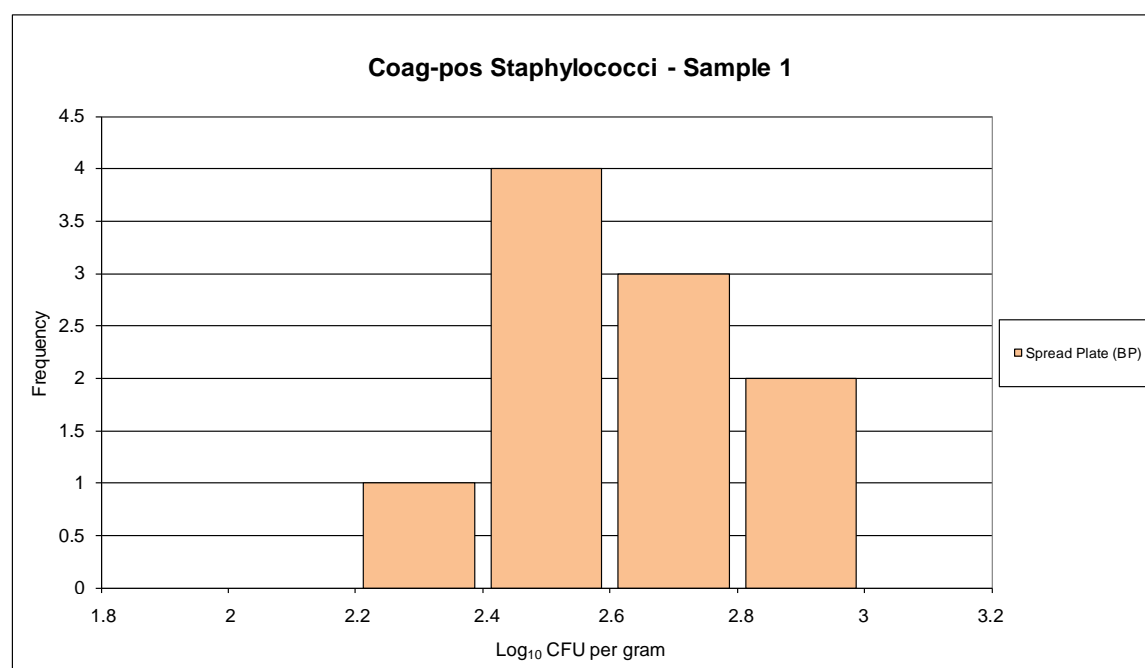


Figure TA-9. Coagulase-positive *Staphylococci* log₁₀ cfu/g results for sample PTA 1.

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. The Spread Plate results were pooled with the Spread Plate results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples, for analysis.

The robust CV of 3.6% for sample PTA 1 for this round is lower than the value of 5.1%, obtained for the results in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

Laboratory code 1 (using the Spread Plate method) reported an outlier for sample PTA 1. Sample PTA 2 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 log₁₀ cfu/g) was as follows:

	PTA 1	PTA 2
<i>B. cereus</i> - Spread Plate	3.699 ± 0.053	-

One laboratory reported MUs associated with their test results in this round for *B. cereus*, as log₁₀ values, which overlapped the median and associated standard error (se) for each sample.

A graph showing the distribution of results for sample PTA 1 in this round for the *Bacillus cereus* test (including the Global Proficiency data) is included in Figure TA-10 for interest purposes only.

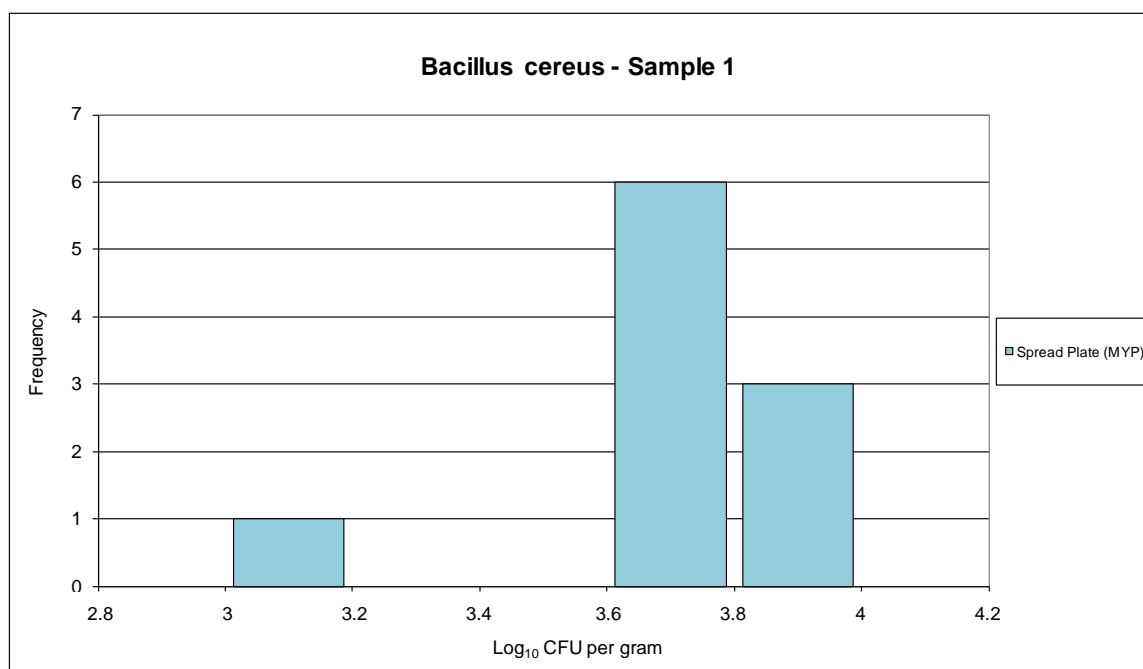


Figure TA-10. *Bacillus cereus* log₁₀ cfu/g results for sample PTA 1.

6.9 Yeasts

A total of nine laboratories submitted results for Yeasts. Five laboratories tested using Pour Plate. Four laboratories tested using Spread Plate. All the methods were pooled for analysis.

The robust CV of 6.2% for sample PTA 2 for this round is lower than the value of 15.2%, obtained for the results in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

Laboratory codes 10A and 10B (using Spread Plate) reported outliers for sample PTA 2. Sample PTA 1 did not contain Yeasts. Laboratory code 5 (using Pour Plate) reported a false positive 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 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	-	2.778 ± 0.048

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

A graph showing the distribution of results for sample PTA 2 in this round for the Yeasts test (including the Global Proficiency data) is included in Figure TA-11 for interest purposes only.

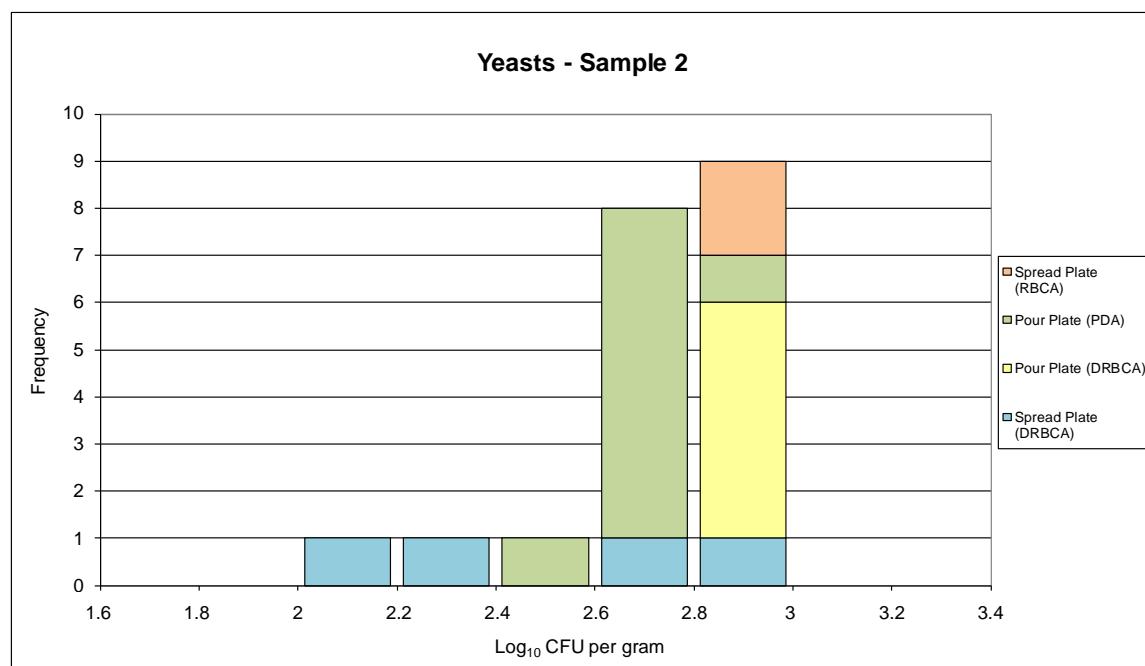


Figure TA-11. Yeasts log₁₀ cfu/g results for sample PTA 2.

6.10 Moulds

A total of nine laboratories submitted results for Moulds. Five laboratories tested using Pour Plate. Four laboratories tested using Spread Plate. All the methods were pooled for analysis.

The robust CVs of 8.6% and 6.5% for this round compare well with the values of 7.0% and 6.7%, obtained in Round 25 of this program, for samples containing similar organisms at similar levels (see Report No. 1121).

There were no outliers reported for either sample.

Laboratory codes 2A, 2B, 2C, 2D, 3A, 3B, 3C and 3D reported false negative results for sample PTA 1, while laboratory codes 8A and 8B each reported two false negative results for sample PTA 1. All of these laboratories tested using the Pour Plate method.

Laboratory code 1 (using Spread Plate) reported a result of < 100 cfu/g for sample PTA 1. This was not considered to be a false negative result, as their inoculum size (spread plate) combined with the dilution tested was likely insufficient to detect the lower levels of Moulds present in 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	2.079 ± 0.085	2.954 ± 0.054

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

Graphs showing the distribution of results in this round for the Moulds test (including the Global Proficiency data) are included in Figures TA-12 and TA-13 for interest purposes only.

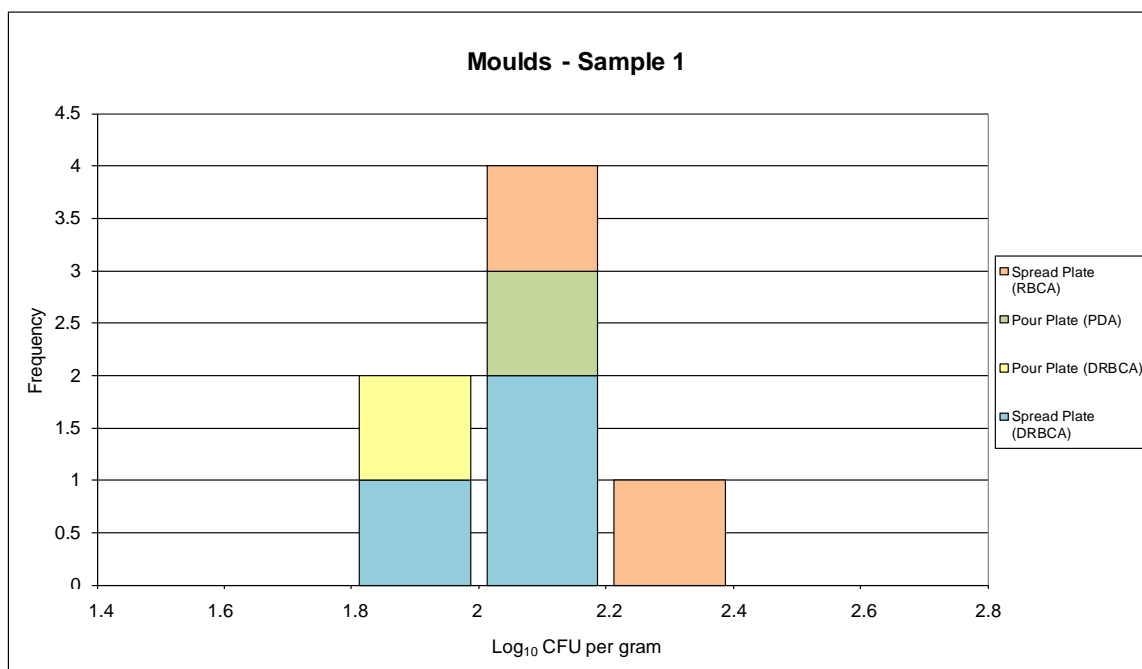


Figure TA-12. Moulds log₁₀ cfu/g results for sample PTA 1.

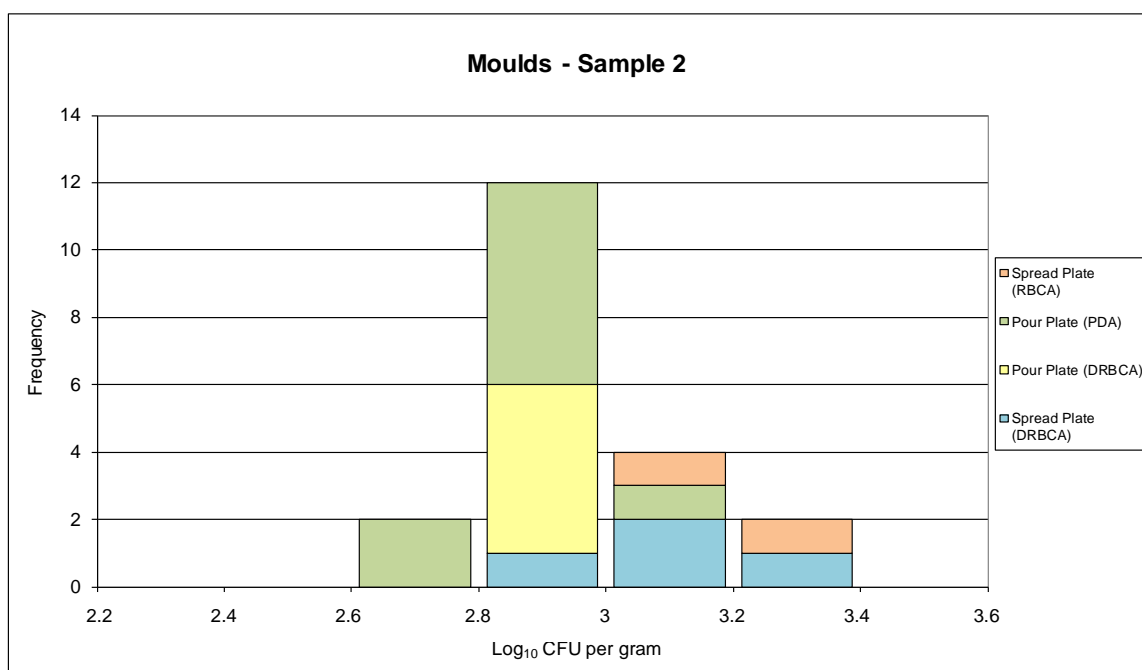


Figure TA-13. Moulds log₁₀ cfu/g results for sample PTA 2.

6.11 Total Yeasts and Moulds

A total of six laboratories submitted results for Total Yeasts and Moulds. Four laboratories tested using Pour Plate. Two laboratories tested using Spread Plate. 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 4.7% and 5.0%, for samples PTA 1 and PTA 2, respectively. The robust CV of 4.7% 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 8.6%. This value was chosen because the sample did not contain Yeasts and, so, it seemed reasonable to match the CV for the Moulds test, of 8.6%. A CV of 8.6% is also consistent with CV values obtained for Total Yeasts and Moulds in previous rounds of this program.

There were no outliers reported for either sample.

Laboratory codes 3A, 3B, 3C and 3D reported false negative results for sample PTA 1, while laboratory codes 8A and 8B each reported two false negative results for sample PTA 1. All of these laboratories tested using the Pour Plate method.

Sample PTA 1 had the identical Mould strain present as sample PTA 2, so it would be expected performance between the samples should be comparable.

Consideration of environmental conditions during transit is important for microbiological samples and is the reason specialised packaging is used in this programme. While this packaging keeps samples frozen for up to 8 days, delay outside of this may have an impact on sample condition if the package has not been kept in a chiller or freezer during the period of delay, e.g. while awaiting customs clearance procedures. When PTA is aware of delays in receipt of samples, one of the options considered is to closely monitor performance of the affected laboratory's results to ensure sample integrity has not been compromised. Where a sample may have experienced high temperatures, then a common outcome is to see a decrease in the numbers of the less robust microorganisms contained in the samples, which includes the fungi used in these samples. However, if this has occurred, then we would expect to see it across BOTH samples, not just limited to one, which appears to be the case in this round. Therefore, for those laboratories reporting either false-negative or low-biasing results for sample PTA 1, it is recommended further investigation is undertaken.

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	1.990 ± 0.035	3.335 ± 0.066

One laboratory reported MUs associated with their test results in this round for Total Yeasts and Moulds, as \log_{10} values, which overlapped the median and associated standard error (se) for each sample.

Graphs showing the distribution of results in this round for the Total Yeasts and Moulds test (including the Global Proficiency data) are included in Figures TA-14 and TA-15 for interest purposes only.

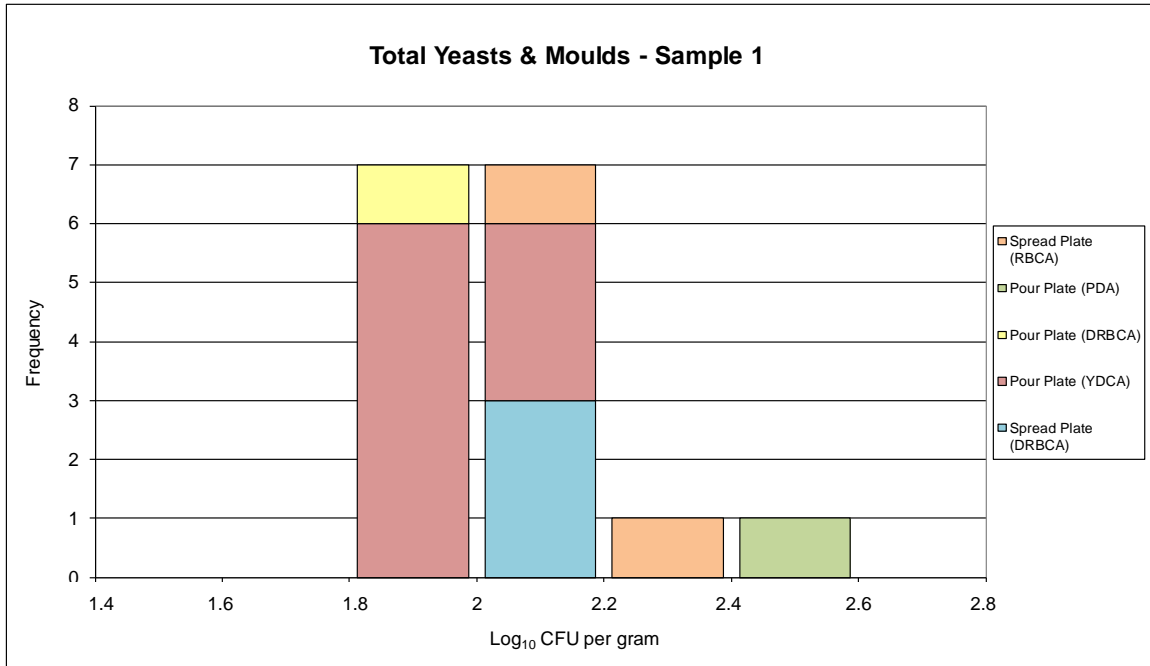


Figure TA-14. Total Yeasts and Moulds log₁₀ cfu/g results for sample PTA 1.

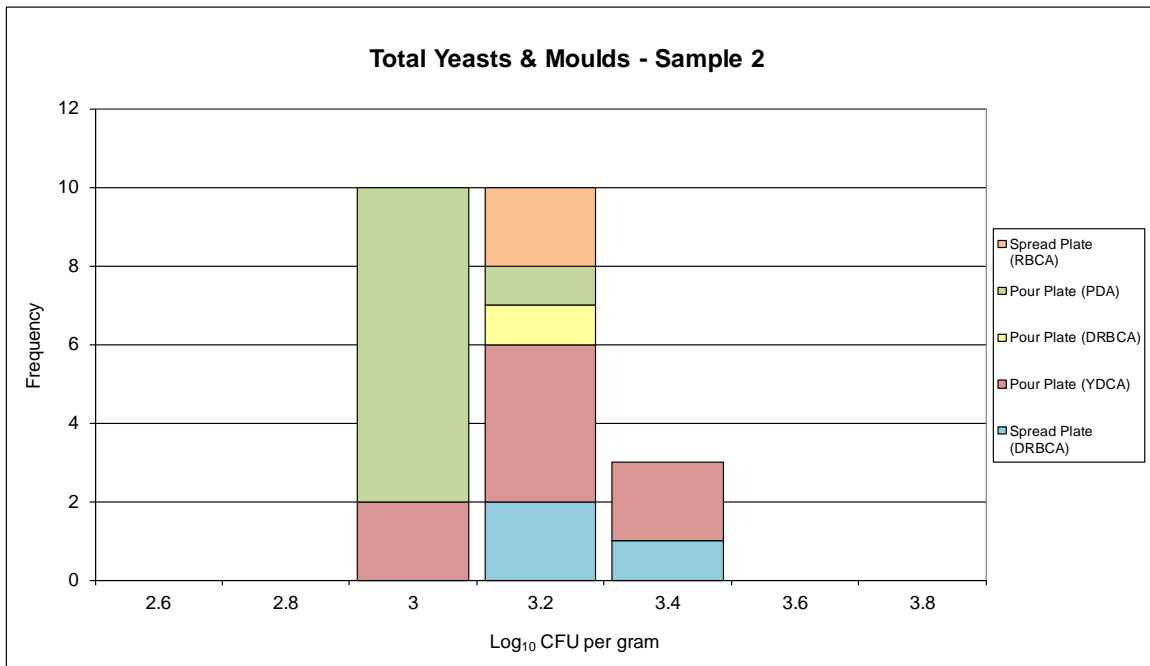


Figure TA-15. Total Yeasts and Moulds log₁₀ cfu/g results for sample PTA 2.

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 (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	48300	4.68	-	8400	3.92	-	1.37	-1.82	PP	-
2A	30000	4.48	-	18000	4.26	-	-0.05	1.71	PP	PCA
2B	32000	4.51	-	16500	4.22	-	0.14	1.31	PP	PCA
2C	26000	4.41	-	17900	4.25	-	-0.48	1.68	PP	PCA
2D	27000	4.43	-	14800	4.17	-	-0.37	0.80	PP	PCA
3A	21100	4.32	-	10700	4.03	-	-1.11	-0.70	PP	PCA
3B	23350	4.37	-	16250	4.21	-	-0.80	1.23	PP	PCA
3C	29400	4.47	-	10000	4.00	-	-0.11	-1.01	PP	PCA
3D	12150	4.08	-	26700	4.43	-	-2.76	3.53 §	PP	PCA
4	42000	4.62	-	10500	4.02	-	0.95	-0.79	PP	PCA
5	46000	4.66	-	10400	4.02	-	1.23	-0.83	PP	PCA
7	35000	4.54	0.15	11000	4.04	0.15	0.41	-0.57	PP	PCA
8A	22700	4.36	-	17650	4.25	-	-0.89	1.62	PP	PCA
8A	25600	4.41	-	16250	4.21	-	-0.53	1.23	PP	PCA
8B	23800	4.38	-	14700	4.17	-	-0.75	0.77	PP	PCA
8B	25300	4.40	-	15200	4.18	-	-0.56	0.93	PP	PCA
9A	29000	4.46	-	6100	3.79	-	-0.16	-3.30 §	PP	PCA
9B	28000	4.45	-	6000	3.78	-	-0.26	-3.38 §	PP	PCA
10A	37000	4.57	-	8400	3.92	-	0.57	-1.82	PP	PCA
10B	48000	4.68	-	9000	3.95	-	1.35	-1.50	PP	PCA

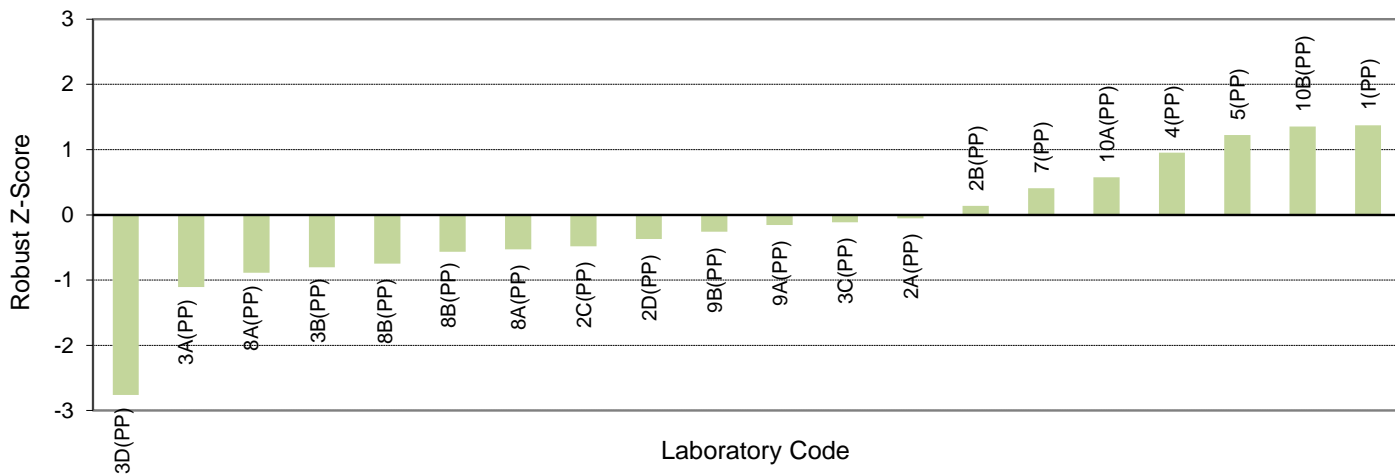
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	10	10
Median	4.485	4.095
Normalised IQR	0.145	0.094
Uncertainty (Median)	0.057	0.037
Robust CV	3.2%	2.3%
Minimum	4.08	3.83
Maximum	4.76	4.15
Range	0.68	0.32

Notes:

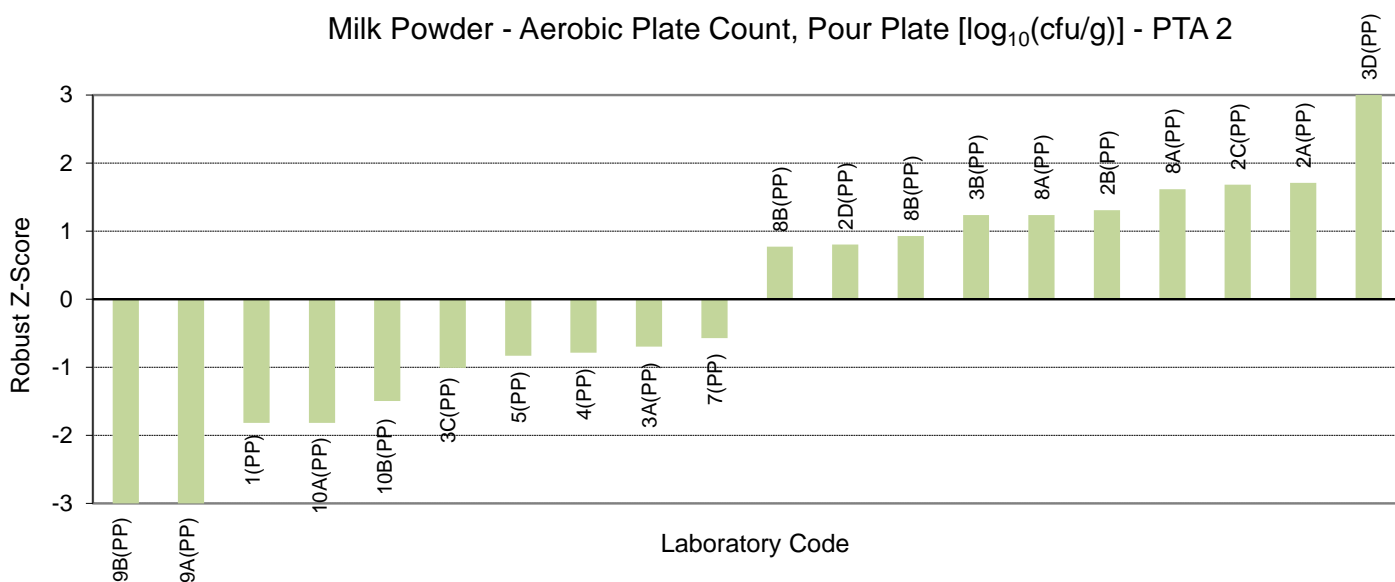
1. § denotes an outlier (i.e. $|z\text{-score}| \geq 3.0$).
2. For the method abbreviations in the table above, PP = Pour Plate.
3. Z-scores and summary statistics (including the number of results) for Aerobic Plate Count were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
4. 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 [$\log_{10}(\text{cfu/g})$] - PTA 1



Milk Powder - Aerobic Plate Count, Pour Plate [$\log_{10}(\text{cfu/g})$] - 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		
1	50	1.70	-	460	2.66	-	-3.70 §	-0.37	Pfm	-
2A	180	2.26	-	610	2.79	-	-0.33	0.49	PP	VRBA
2B	210	2.32	-	1000	3.00	-	0.07	1.99	PP	VRBA
2C	250	2.40	-	600	2.78	-	0.53	0.44	PP	VRBA
2D	260	2.41	-	600	2.78	-	0.64	0.44	PP	VRBA
3A	0 †	-	-	590	2.77	-	-	0.39	PP	VRBA
3B	0 †	-	-	910	2.96	-	-	1.71	PP	VRBA
3C	0 †	-	-	650	2.81	-	-	0.68	PP	VRBA
3D	0 †	-	-	800	2.90	-	-	1.31	PP	VRBA
4	170	2.23	-	400	2.60	-	-0.48	-0.79	PP	VRBA
5	210	2.32	-	420	2.62	-	0.07	-0.64	PP	VRBA
8A	< 10 †	-	-	570	2.76	-	-	0.29	PP	VRBA
8A	< 10 †	-	-	610	2.79	-	-	0.49	PP	VRBA
8B	< 10 †	-	-	710	2.85	-	-	0.95	PP	VRBA
8B	< 10 †	-	-	680	2.83	-	-	0.82	PP	VRBA
9A	30	1.48	-	250	2.40	-	-5.04 §	-2.22	PP	VRBA
9B	30	1.48	-	230	2.36	-	-5.04 §	-2.47	PP	VRBA
10A	130	2.11	-	120	2.08	-	-1.19	-4.44 §	PP	VRBA
10B	100	2.00	-	130	2.11	-	-1.88	-4.20 §	PP	VRBA

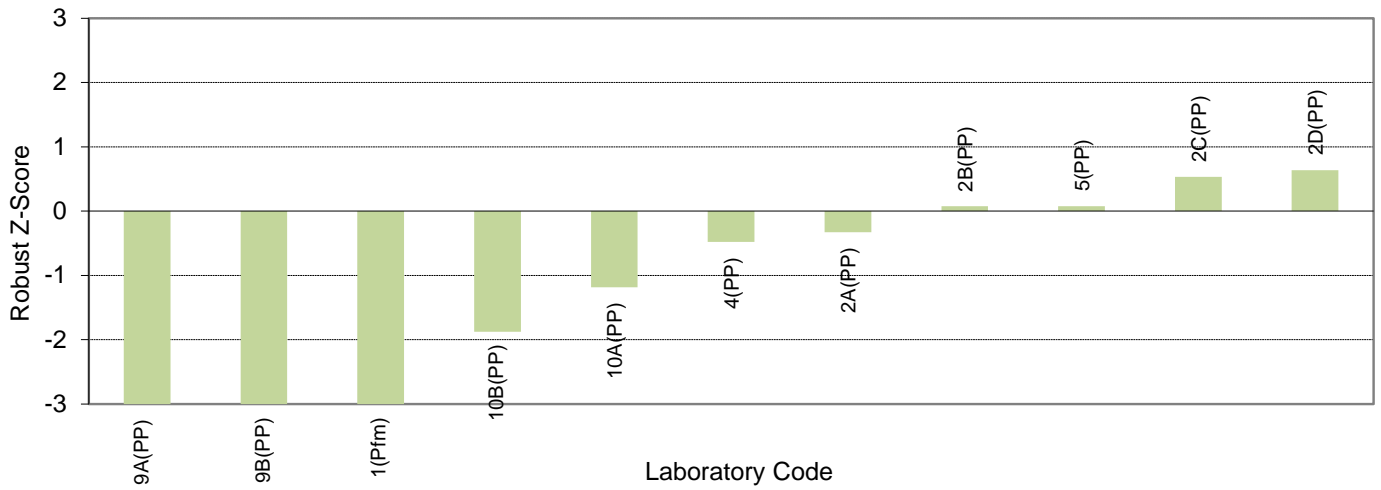
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	8	8
Median	2.310	2.715
Normalised IQR	0.165	0.143
Uncertainty (Median)	0.073	0.063
Robust CV	7.1%	5.3%
Minimum	2.15	2.40
Maximum	2.53	2.82
Range	0.38	0.42

Notes:

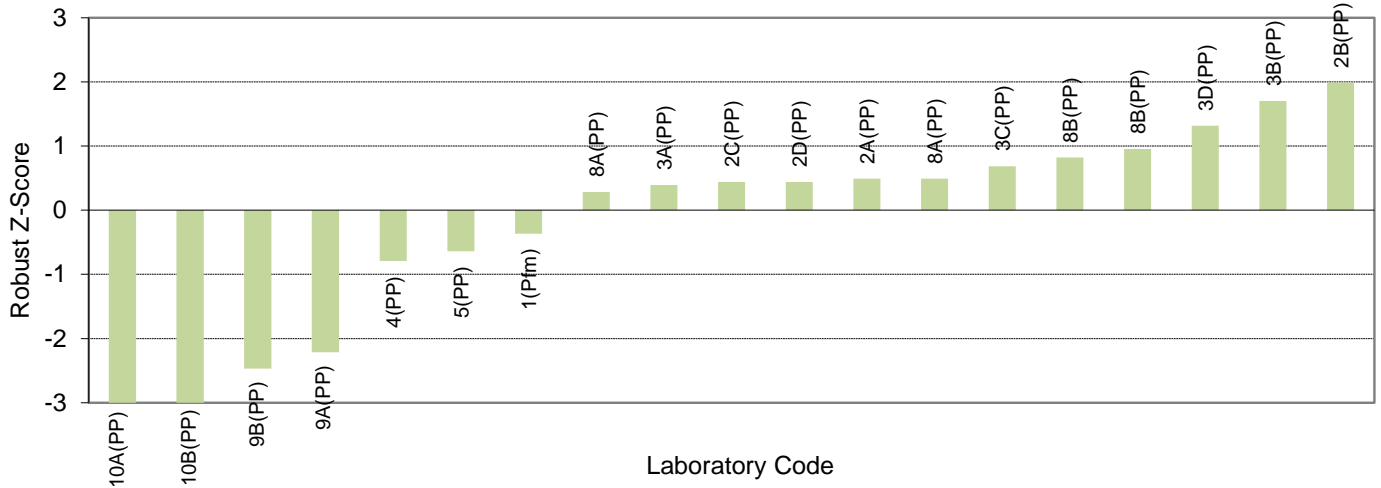
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- † denotes a false negative result.
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
- Z-scores and summary statistics (including 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		
1	50	1.70	-	20	1.30	-	-5.51 §	-6.77 §	Pfm	-
9A	30	1.48	-	230	2.36	-	-7.48 §	-0.88	PP	VRBA MUG
9B	30	1.48	-	220	2.34	-	-7.48 §	-0.99	PP	VRBA MUG

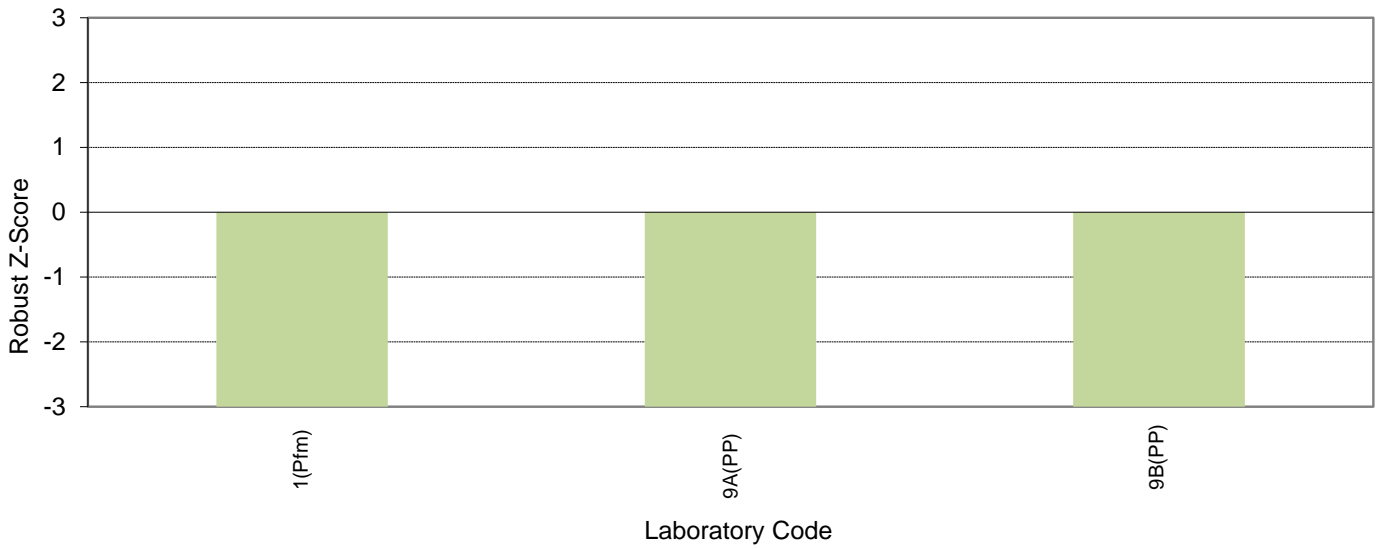
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	7	7
Median	2.320	2.520
Normalised IQR	0.113	0.180
Uncertainty (Median)	0.053	0.085
Robust CV	4.9%	7.2%
Minimum	1.95	2.28
Maximum	2.43	2.65
Range	0.48	0.37

Notes:

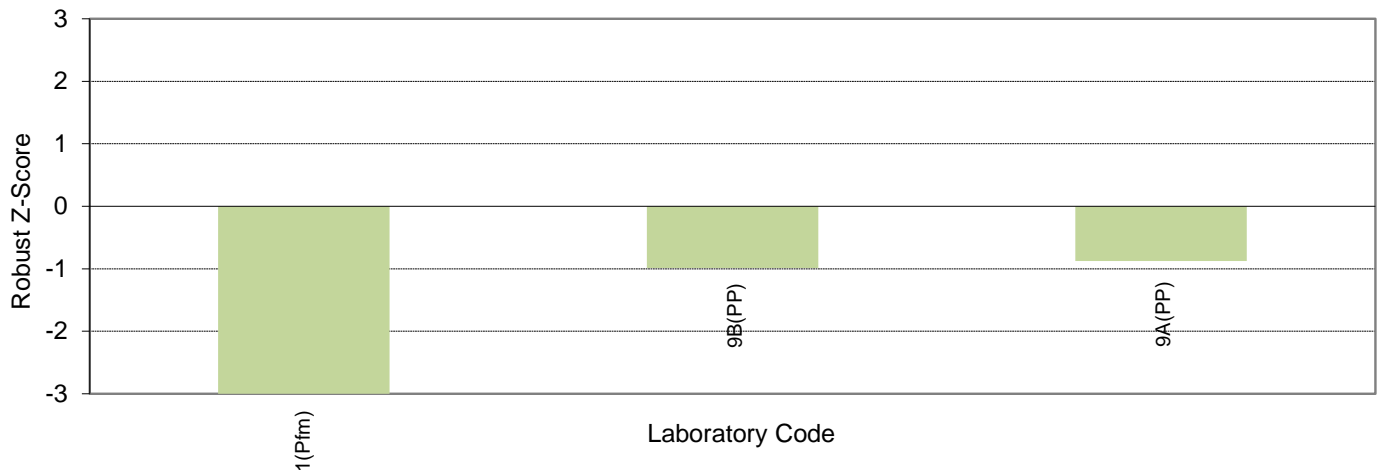
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the *E. coli* results.
4. Z-scores and summary statistics (including the number of results) for *E. coli* were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
5. 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™ [$\log_{10}(\text{cfu/g})$] - PTA 1



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		
2A	620	2.79	-	590	2.77	-	1.88	0.25	PP	VRBGA
2B	620	2.79	-	880	2.94	-	1.88	1.11	PP	VRBGA
2C	550	2.74	-	600	2.78	-	1.59	0.29	PP	VRBGA
2D	570	2.76	-	550	2.74	-	1.68	0.10	PP	VRBGA
4	150	2.18	-	580	2.76	-	-1.50	0.21	PP	VRBGA
5	220	2.34	-	460	2.66	-	-0.59	-0.28	PP	VRBGA
5	200	2.30	-	430	2.63	-	-0.82	-0.43	Pfm	-
7	10	1.00	0.22	110	2.04	0.22	-7.94 §	-3.35 §	PP	VRBA
9A	40	1.60	-	310	2.49	-	-4.64 §	-1.13	PP	VRBGA
9B	40	1.60	-	310	2.49	-	-4.64 §	-1.13	PP	VRBGA
10A	170	2.23	-	140	2.15	-	-1.20	-2.83	PP	VRBD
10B	140	2.15	-	160	2.20	-	-1.66	-2.55	PP	VRBD

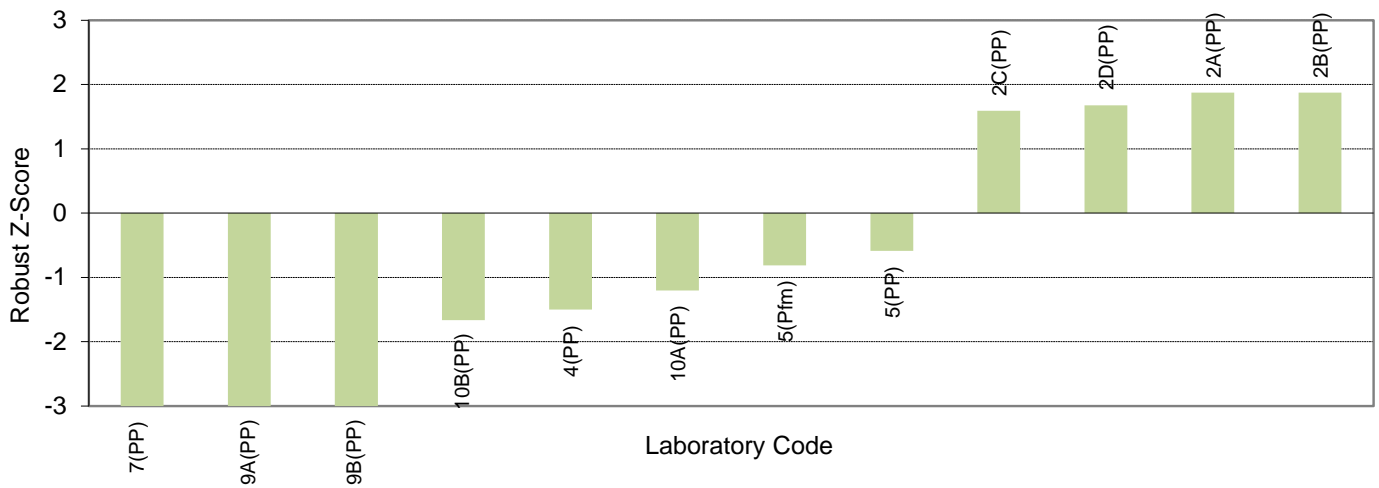
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	7	7
Median	2.450	2.720
Normalised IQR	0.059	0.203
Uncertainty (Median)	0.028	0.096
Robust CV	2.4%	7.5%
Target SD	0.183	-
Target CV	7.5%	-
Minimum	2.28	2.51
Maximum	2.52	2.81
Range	0.24	0.30

Notes:

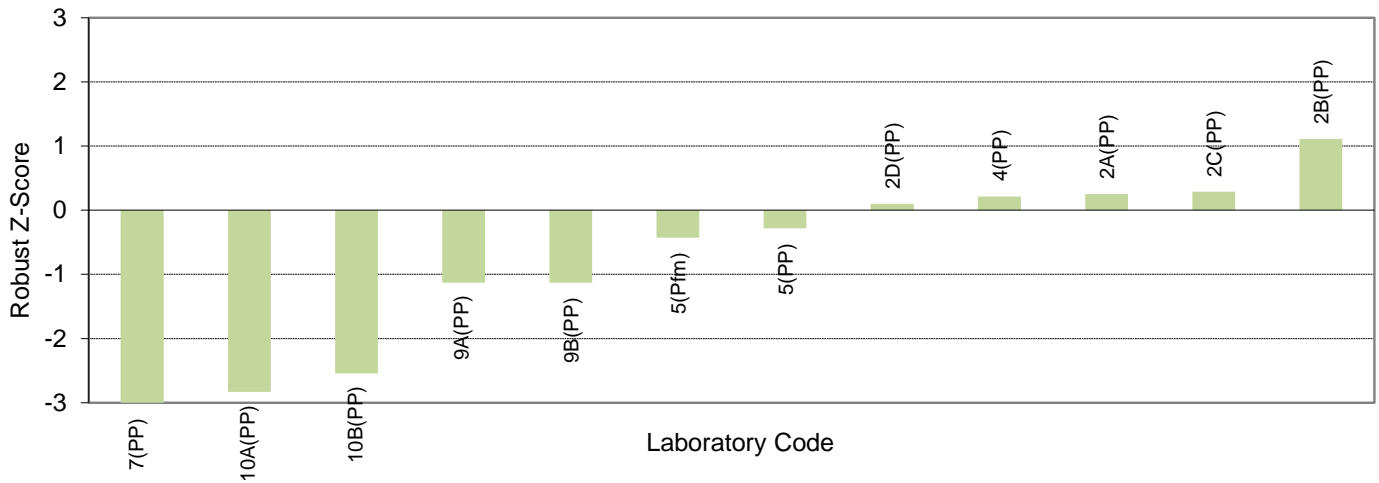
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- For the method abbreviations in the table above, PP = Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Enterobacteriaceae results.
- A target CV was used to calculate the robust z-scores for sample PTA 1. The target CV chosen was 7.5%.
- 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 (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 (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	300	2.48	-	< 100	-	-	-1.09	-	SP	BPA
5	530	2.72	-	< 10	-	-	1.17	-	SP	BPA
7	700	2.85	0.17	< 100	-	0.17	2.28	-	SP	BPA
9A	400	2.60	-	< 10	-	-	0.06	-	SP	BPA
9B	380	2.58	-	< 10	-	-	-0.15	-	SP	BPA

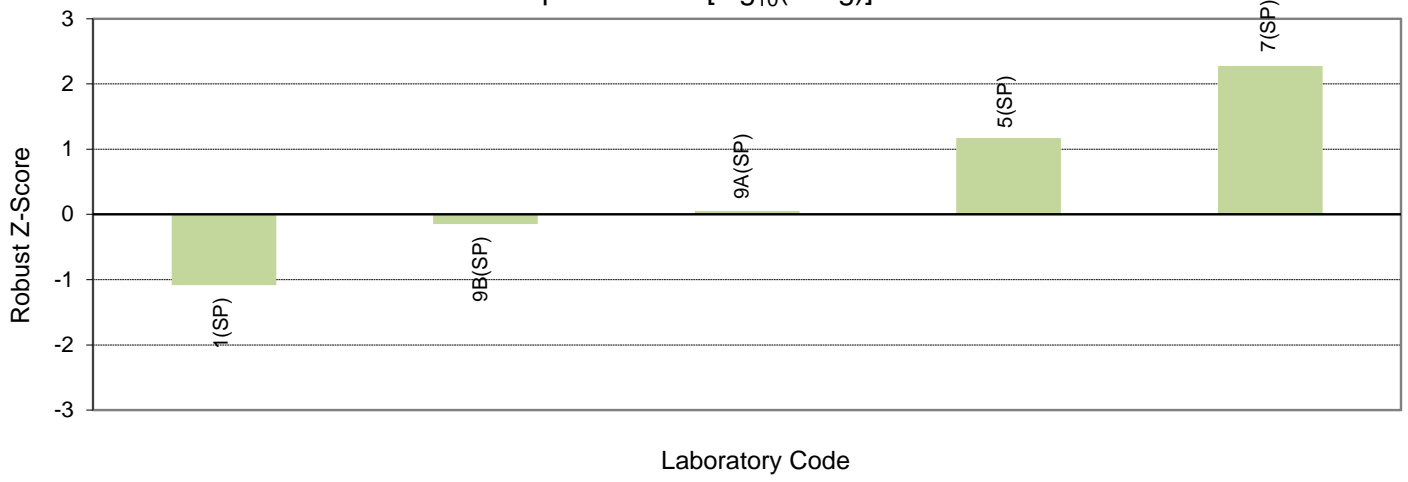
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	10	10
Median	2.596	n/a
Normalised IQR	0.109	n/a
Uncertainty (Median)	0.043	n/a
Robust CV	4.2%	n/a
Minimum	2.34	n/a
Maximum	2.85	n/a
Range	0.51	n/a

Notes:

1. For the method abbreviations in the table above, SP = Spread Plate.
2. Z-scores and summary statistics (including the number of results) for Coagulase-positive *Staphylococci* were calculated from the pooled participants' results and the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
3. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
4. Sample PTA 2 did not contain Coagulase-positive *Staphylococci*.

A5.2

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



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	1200	3.08	-	< 100	-	-	-4.68 §	-	SP	MYP
5	7350	3.87	-	< 100	-	-	1.26	-	SP	MYP
7	4600	3.66	0.15	< 100	-	0.15	-0.28	-	SP	MYP
9A	5000	3.70	-	< 10	-	-	0.00	-	SP	MYP
9B	4500	3.65	-	< 10	-	-	-0.35	-	SP	MYP

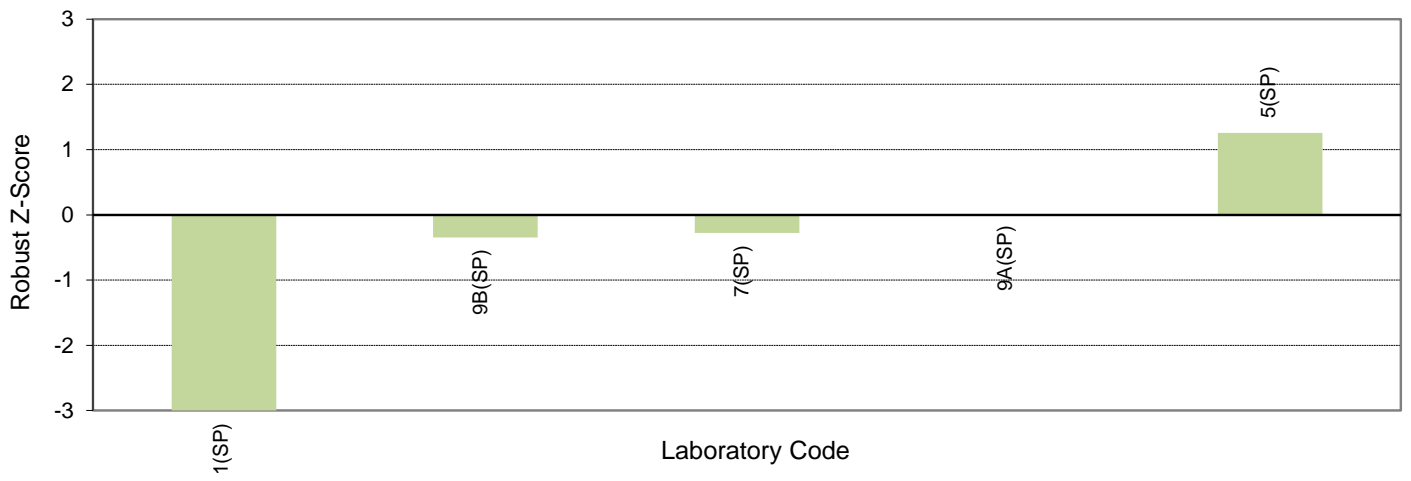
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	10	12
Median	3.699	n/a
Normalised IQR	0.133	n/a
Uncertainty (Median)	0.053	n/a
Robust CV	3.6%	n/a
Minimum	3.08	n/a
Maximum	3.88	n/a
Range	0.80	n/a

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 pooled participants' results and 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 2 did not contain *Bacillus cereus*.

A6.2

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



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	< 100	-	-	700	2.85	-	-	0.39	SP	DRBCA
2A	< 10	-	-	950	2.98	-	-	1.16	PP	DRBCA
2B	< 10	-	-	940	2.97	-	-	1.13	PP	DRBCA
2C	< 10	-	-	650	2.81	-	-	0.20	PP	DRBCA
2D	< 10	-	-	650	2.81	-	-	0.20	PP	DRBCA
3A	0	-	-	555	2.74	-	-	-0.20	PP	PDA
3B	0	-	-	410	2.61	-	-	-0.96	PP	PDA
3C	0	-	-	350	2.54	-	-	-1.36	PP	PDA
3D	0	-	-	600	2.78	-	-	0.00	PP	PDA
4	0	-	-	830	2.92	-	-	0.82	PP	DRBCA
5	105 ‡	2.02	-	810	2.91	-	-	0.75	PP	PDA
7	< 100	-	-	600	2.78	-	-	0.00	SP	DRBCA
8A	< 10	-	-	467	2.67	-	-	-0.63	PP	PDA
8A	< 10	-	-	550	2.74	-	-	-0.22	PP	PDA
8B	< 10	-	-	540	2.73	-	-	-0.27	PP	PDA
8B	< 10	-	-	480	2.68	-	-	-0.56	PP	PDA
9A	< 10	-	-	860	2.93	-	-	0.91	SP	RBCA
9B	< 10	-	-	850	2.93	-	-	0.88	SP	RBCA
10A	< 10	-	-	150	2.18	-	-	-3.49 §	SP	DRBCA
10B	< 10	-	-	160	2.20	-	-	-3.33 §	SP	DRBCA

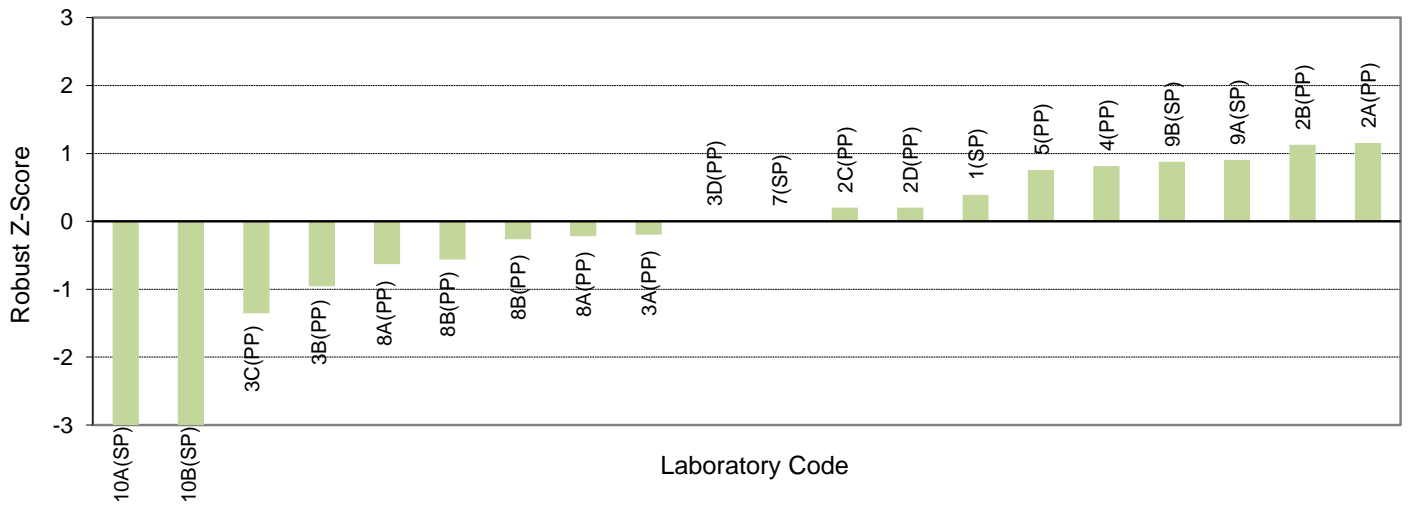
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	1	20
Median	n/a	2.778
Normalised IQR	n/a	0.173
Uncertainty (Median)	n/a	0.048
Robust CV	n/a	6.2%
Minimum	n/a	2.18
Maximum	n/a	2.98
Range	n/a	0.80

Notes:

- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- ‡ denotes a false positive result.
- For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
- All the methods were pooled when analysing the Yeasts results.
- Z-scores and summary statistics (including the number of results) for Yeasts were calculated from the participants' results.
- 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 Yeasts.

A7.2

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



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	< 100 *	-	-	900	2.95	-	-	0.00	SP	DRBCA
2A	< 10 †	-	-	980	2.99	-	-	0.19	PP	DRBCA
2B	< 10 †	-	-	950	2.98	-	-	0.12	PP	DRBCA
2C	< 10 †	-	-	820	2.91	-	-	-0.21	PP	DRBCA
2D	< 10 †	-	-	900	2.95	-	-	0.00	PP	DRBCA
3A	0 †	-	-	825	2.92	-	-	-0.20	PP	PDA
3B	0 †	-	-	635	2.80	-	-	-0.79	PP	PDA
3C	0 †	-	-	950	2.98	-	-	0.12	PP	PDA
3D	0 †	-	-	1500	3.18	-	-	1.16	PP	PDA
4	90	1.95	-	900	2.95	-	-0.70	0.00	PP	DRBCA
5	150	2.18	-	660	2.82	-	0.54	-0.70	PP	PDA
7	100	2.00	-	1600	3.20	-	-0.44	1.30	SP	DRBCA
8A	< 10 †	-	-	625	2.80	-	-	-0.83	PP	PDA
8A	< 10 †	-	-	770	2.89	-	-	-0.35	PP	PDA
8B	< 10 †	-	-	790	2.90	-	-	-0.30	PP	PDA
8B	< 10 †	-	-	610	2.79	-	-	-0.88	PP	PDA
9A	180	2.26	-	1600	3.20	-	0.98	1.30	SP	RBCA
9B	150	2.18	-	1500	3.18	-	0.54	1.16	SP	RBCA
10A	70	1.85	-	1400	3.15	-	-1.31	1.00	SP	DRBCA
10B	120	2.08	-	1500	3.18	-	0.00	1.16	SP	DRBCA

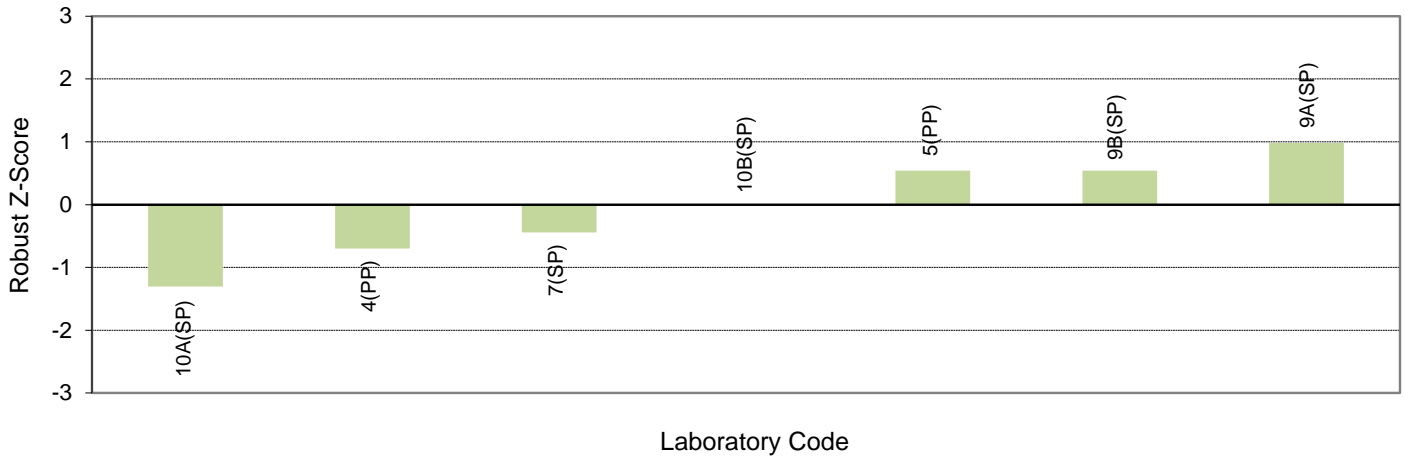
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	7	20
Median	2.079	2.954
Normalised IQR	0.179	0.192
Uncertainty (Median)	0.085	0.054
Robust CV	8.6%	6.5%
Minimum	1.85	2.79
Maximum	2.26	3.20
Range	0.41	0.42

Notes:

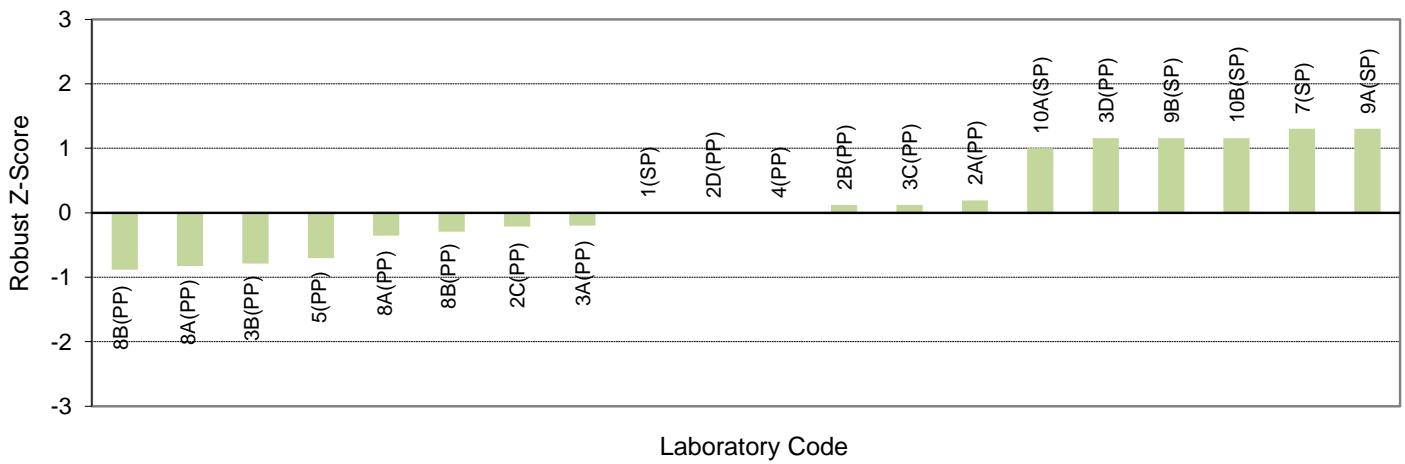
- † denotes a false negative result.
- * The result reported by laboratory code 1 for sample PTA 1 was not considered to be a false negative result.
- For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
- All the methods were pooled when analysing the Moulds results.
- Z-scores and summary statistics (including the number of results) for Moulds were calculated from the participants' results.
- 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	0 †	-	-	1380	3.14	-	-	-1.17	PP	PDA
3B	0 †	-	-	1045	3.02	-	-	-1.90	PP	PDA
3C	0 †	-	-	1300	3.11	-	-	-1.33	PP	PDA
3D	0 †	-	-	2100	3.32	-	-	-0.08	PP	PDA
4	90	1.95	-	1730	3.24	-	-0.21	-0.58	PP	DRBCA
5	255	2.41	-	1470	3.17	-	2.43	-1.01	PP	PDA
7	100	2.00	0.17	2200	3.34	0.17	0.06	0.04	SP	DRBCA
8A	< 10 †	-	-	1092	3.04	-	-	-1.78	PP	PDA
8A	< 10 †	-	-	1320	3.12	-	-	-1.29	PP	PDA
8B	< 10 †	-	-	1330	3.12	-	-	-1.27	PP	PDA
8B	< 10 †	-	-	1090	3.04	-	-	-1.79	PP	PDA
9A	180	2.26	-	2460	3.39	-	1.55	0.34	SP	RBCA
9B	150	2.18	-	2350	3.37	-	1.08	0.22	SP	RBCA

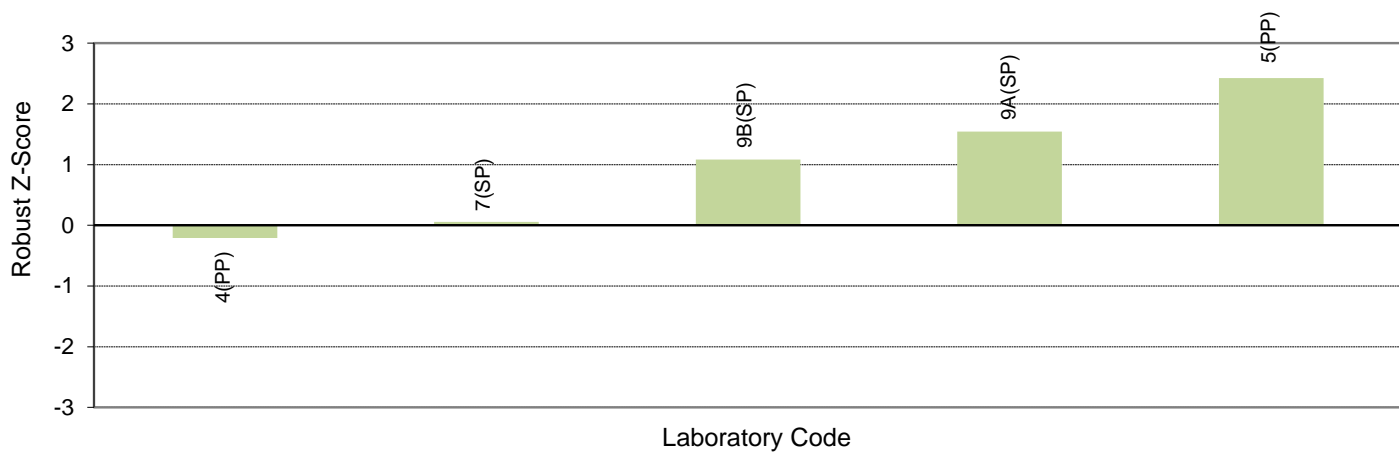
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	11	10
Median	1.990	3.335
Normalised IQR	0.093	0.166
Uncertainty (Median)	0.035	0.066
Robust CV	4.7%	5.0%
Target SD	0.172	-
Target CV	8.6%	-
Minimum	1.82	3.08
Maximum	2.08	3.57
Range	0.26	0.49

Notes:

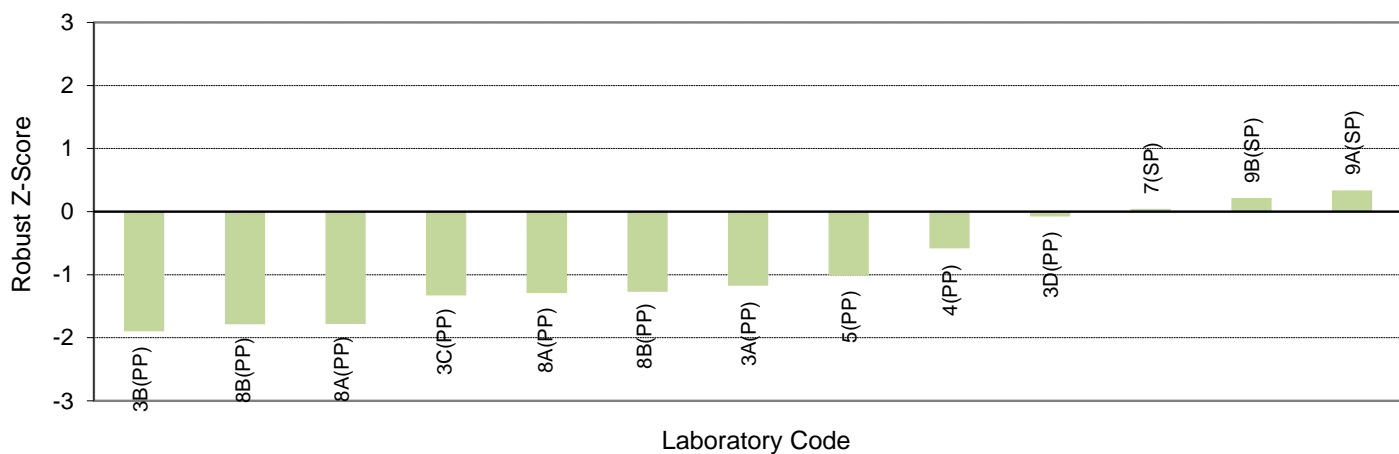
- † denotes a false negative result.
- For the method abbreviations in the table above, PP = Pour Plate and SP = Spread Plate.
- All the methods were pooled when analysing the Total Yeasts and Moulds results.
- A target CV was used to calculate the robust z-scores for sample PTA 1. The target CV chosen was 8.6%.
- 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 (2019).
- 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.
- 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 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 up to 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
5	39000	4.59	37000	4.57
20	33000	4.52	35000	4.54
39	36000	4.56	40000	4.60
40	35000	4.54	32000	4.51
49	29000	4.46	30000	4.48

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 up to 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
4	34000	4.53	34000	4.53
37	34000	4.53	37000	4.57
42	30000	4.48	30000	4.48

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 26, May 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 samples, one containing a whole milk powder matrix (labelled PTA 1), and one containing a skim milk powder matrix (labelled 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*, 2,000 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 **Monday 10 June 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 26, May 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 26, May 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-----