

REPORT NO. 1098

**Non-Pathogens In Food
Proficiency Testing Program
Round 24
July 2018**

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.

© COPYRIGHT PROFICIENCY TESTING AUSTRALIA 2018
PO Box 7507 Silverwater NSW 2128 AUSTRALIA

CONTENTS

1. FOREWORD	1
2. FEATURES OF THE PROGRAM	1
3. FORMAT OF THE APPENDICES	2
4. STATISTICAL DESIGN OF THE PROGRAM	2
5. OUTLIER RESULTS	3
Table A: Summary Statistics for All Tests	4
Table B: Summary of Statistical Outliers and False Results	5
6. PTA AND TECHNICAL ADVISER'S COMMENTS	6
7. REFERENCES	21
APPENDICES	
APPENDIX A	
Summary of Results	
Aerobic Plate Count	A1.1
Coliforms	A2.1
<i>E. coli</i>	A3.1
Enterobacteriaceae	A4.1
Coagulase-positive <i>Staphylococci</i>	A5.1
<i>Bacillus cereus</i>	A6.1
Yeasts	A7.1
Moulds	A8.1
Total Yeasts and Moulds	A9.1
APPENDIX B	
Homogeneity and Stability Testing	B1.1
APPENDIX C	
Instructions to Participants	C1.1
Results Sheets	C2.1

1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the twenty-fourth 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 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 ten 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:

<u>Test</u>	<u>Sample PTA 1</u>	<u>Sample PTA 2</u>
Aerobic Plate Count	7,000	10,000
Coliforms	500	800
<i>E. coli</i>	0	600
Enterobacteriaceae	500	800
Coagulase-positive <i>Staphylococci</i>	1,500	0
<i>B. cereus</i>	5,000	0
Yeasts	150	1,000
Moulds	1,000	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 (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	12	12
		Median	3.930	3.980
		Normalised IQR	0.198	0.132
		Uncertainty (Median)	0.072	0.048
Coliforms	Pour Plate / Petrifilm™	Number of Results	11	11
		Median	2.430	2.660
		Normalised IQR	0.145	0.230
		Uncertainty (Median)	0.055	0.087
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other	Number of Results	11	11
		Median	n/a	2.480
		Normalised IQR	n/a	0.189
	MPN	Uncertainty (Median)	n/a	0.071
Number of Results		1	1	
Median		n/a	n/a	
		Normalised IQR	n/a	n/a
		Uncertainty (Median)	n/a	n/a
Enterobacteriaceae	Pour Plate / Petrifilm™	Number of Results	10	10
		Median	2.465	2.620
		Normalised IQR	0.113	0.303
		Uncertainty (Median)	0.045	0.120
Coagulase-positive <i>Staphylococci</i>	Spread Plate / Petrifilm™	Number of Results	9	8
		Median	2.860	n/a
		Normalised IQR	0.122	n/a
	MPN	Uncertainty (Median)	0.051	n/a
Number of Results		1	1	
Median		n/a	n/a	
		Normalised IQR	n/a	n/a
		Uncertainty (Median)	n/a	n/a
<i>B. cereus</i>	Spread Plate	Number of Results	5	5
		Median	n/a	n/a
		Normalised IQR	n/a	n/a
		Uncertainty (Median)	n/a	n/a
Yeasts	All Methods Pooled	Number of Results	15	18
		Median	1.968	2.712
		Normalised IQR	0.248	0.144
		Uncertainty (Median)	0.080	0.043
Moulds	All Methods Pooled	Number of Results	18	18
		Median	2.828	2.908
		Normalised IQR	0.121	0.207
		Uncertainty (Median)	0.036	0.061
Total Yeasts and Moulds	All Methods Pooled	Number of Results	11	11
		Median	2.989	3.104
		Normalised IQR	0.139	0.154
		Uncertainty (Median)	0.053	0.058

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™	10A, 10B, 10C	10A, 10B, 10C	-	-
Coliforms	Pour Plate / Petrifilm™	10C	-	-	-
<i>E. coli</i>	Pour Plate / Petrifilm™ / Other		-	-	-
	MPN			4A	-
Enterobacteriaceae	Pour Plate / Petrifilm™	-	-	-	-
Coagulase-positive <i>Staphylococci</i>	Spread Plate / Petrifilm™	-		-	-
	MPN			-	4A
<i>B. cereus</i>	Spread Plate			-	-
Yeasts	All Methods Pooled	-	1, 7A, 7B, 9	1 (x2 methods)	-
Moulds	All Methods Pooled	3	-	-	-
Total Yeasts and Moulds	All Methods Pooled	-	-	-	-

Notes for Tables A and B:

1. The results reported are for log₁₀ (cfu/g).
2. All the methods used by the participants (other than MPN) 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 *Coagulase-positive Staphylococci*.
4. Summary statistics and z-scores were not calculated for the MPN method results for *E. coli* and *Coagulase-positive Staphylococci*.
5. Summary statistics and z-scores were not calculated for *B. cereus* due to an insufficient number of results reported for either this program or the Global Proficiency Ltd DairyChek Microbiology program.
6. Sample PTA 1 did not contain *E. coli*.
7. Sample PTA 2 did not contain *Coagulase-positive Staphylococci* or *B. cereus*.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 24 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *Klebsiella pneumoniae* as the Coliform / Enterobacteriaceae organism present in the sample, whereas sample PTA 2 contained *E. coli* and *Cronobacter sakazakii*. The *K. pneumoniae* strain used in sample 1 is a thermotolerant coliform organism.

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

Sample PTA 1 also included *Bacillus cereus* and *Staphylococcus aureus* species, while sample PTA 2 contained *Clostridium perfringens* and another non-target species for the *Coagulase-positive Staphylococci* test. 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 ten laboratories that participated in the program submitted results for inclusion in the final report. Of these ten laboratories, five (50%) submitted results where more than one method was used for a specific test, while one laboratory (10%) provided results for all nine tests. The return rate for all tests is as follows:

• Aerobic Plate Count	10 out of 10	100%
• Coliforms	9 out of 10	90%
• <i>E. coli</i>	4 out of 10	40%
• Enterobacteriaceae	7 out of 10	70%
• Coagulase-positive <i>Staphylococci</i>	6 out of 10	60%
• <i>B. cereus</i>	4 out of 10	40%
• Yeasts	10 out of 10	100%
• Moulds	10 out of 10	100%
• Total Yeasts and Moulds	6 out of 10	60%

6.2 Performance Summary

One or more statistical outliers or false results were reported by six laboratories (60%) for this round of the Non-Pathogens in Food program. For comparison, 71% of the participants in Round 23 of the Non-Pathogens in Food program reported outliers or false results (see Report No. 1060 for more details).

A total of 222 results were analysed in this round of the program. Of these results, 16 (7%) were identified as outliers or false results. For comparison, 15% of the results analysed in Round 23 of the Non-Pathogens in Food program were outliers or false results (see Report No. 1060 for more details).

6.3 Aerobic Plate Count

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

Graphs showing the differentiation of methods used for Aerobic Plate Count testing are included in Figures TA-1 and TA-2 on the pages following. 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.

The robust CVs of 5.0% and 3.3% for the results for this round are higher than the values of 2.1% and 2.1%, obtained for the results in Round 23 of this

program, for samples containing similar organisms at similar levels (see Report No. 1060).

Laboratories 10A, 10B and 10C (using the Pour Plate method) reported outliers for both samples with all results being an order of magnitude high.

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™	3.930 ± 0.072	3.980 ± 0.048
APC - Pour Plate	3.910 ± 0.069	3.980 ± 0.043

Three laboratories reported MUs associated with their test results in this round for Aerobic Plate Count. It is recommended that laboratory 3 re-examines their test results or their MU calculations for sample PTA 2, as their results were further from the median than their stated uncertainty (taking into consideration the uncertainty associated with the median). Laboratory 7 submitted two sets of results with very low uncertainties; while most results and their associated uncertainties were within the median and its uncertainty, the result for sample PTA 2 for result set A was further from the median than their stated uncertainty, and is recommended to be examined.

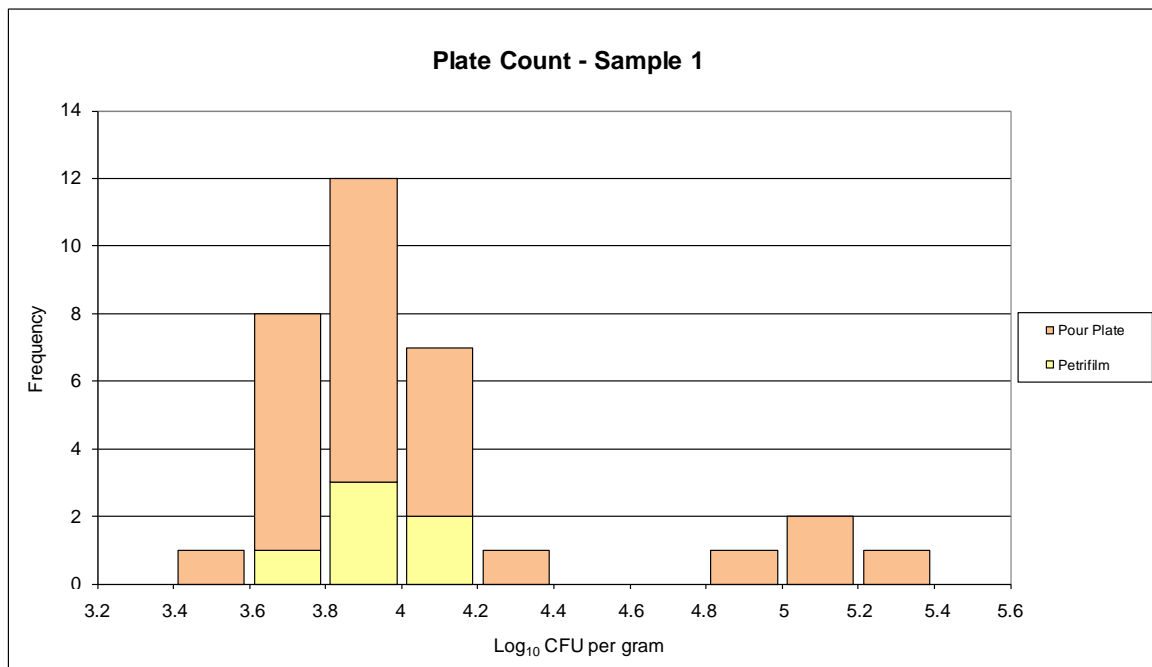


Figure TA-1. APC \log_{10} cfu/g results for sample PTA 1.

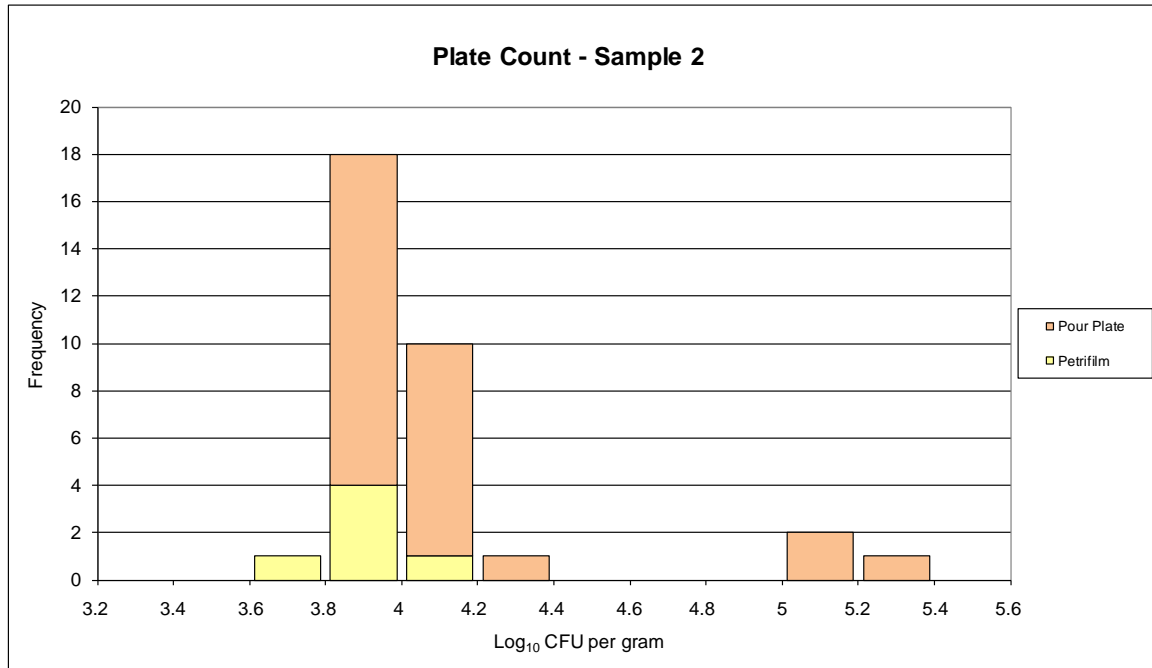


Figure TA-2. APC log₁₀ cfu/g results for sample PTA 1.

6.4 Coliforms

A total of nine laboratories submitted results for Coliforms. One of these laboratories used more than one method. Six laboratories tested using Pour Plate, including two laboratories that submitted three sets of results and two laboratories that submitted two sets of results. Four laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results. The Pour Plate and Petrifilm™ results 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 5.9% and 8.6% for the results for this round are higher than the values of 3.4% and 5.1%, obtained for the results in Round 23 of this program, for samples containing similar organisms at similar levels (see Report No. 1060).

Laboratory 10C (using the Pour Plate method) reported an outlier for sample PTA 1. There were no outliers reported for sample PTA 2.

Graphs showing the differentiation of methods used for Coliform testing are included in Figures TA-3 and TA-4 below. 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.

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 / Petrifilm™	2.430 ± 0.055	2.660 ± 0.087
Coliforms - Pour Plate	2.410 ± 0.058	2.640 ± 0.157

Two laboratories reported MUs associated with their test results in this round for Coliforms. Laboratory 7 submitted two sets of results with very low uncertainties; while results for sample PTA 1 and their associated uncertainties were within the median and its uncertainty, both results for sample PTA 2 were further from the median than their stated uncertainty, and are recommended to be examined.

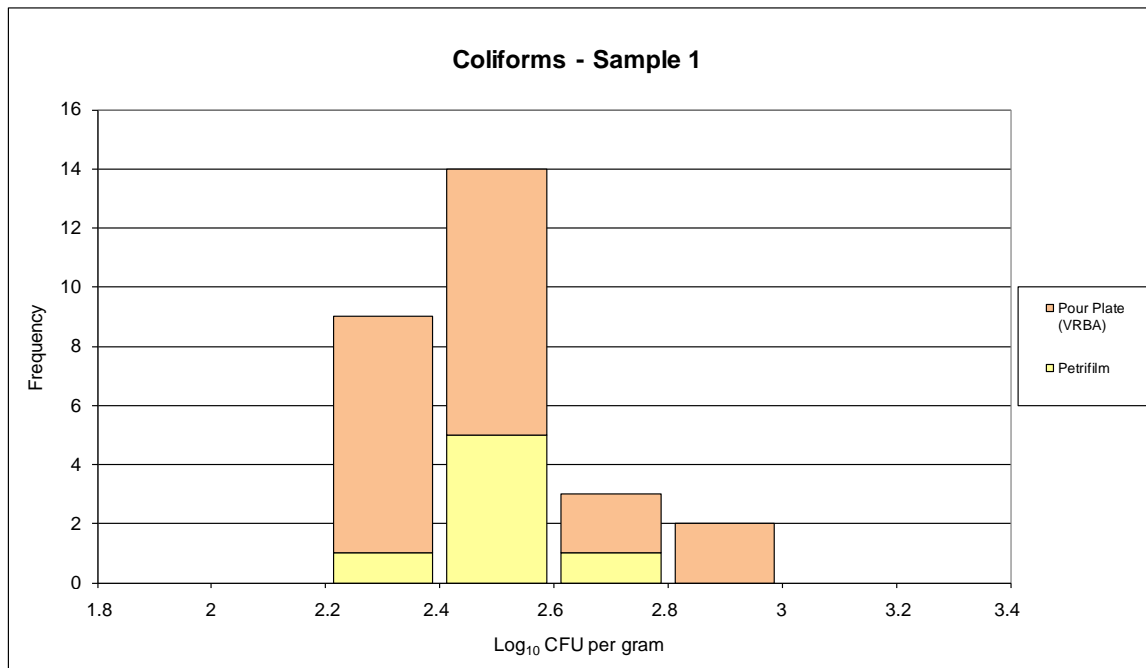


Figure TA-3. Coliforms log₁₀ cfu/g results for sample PTA 1.

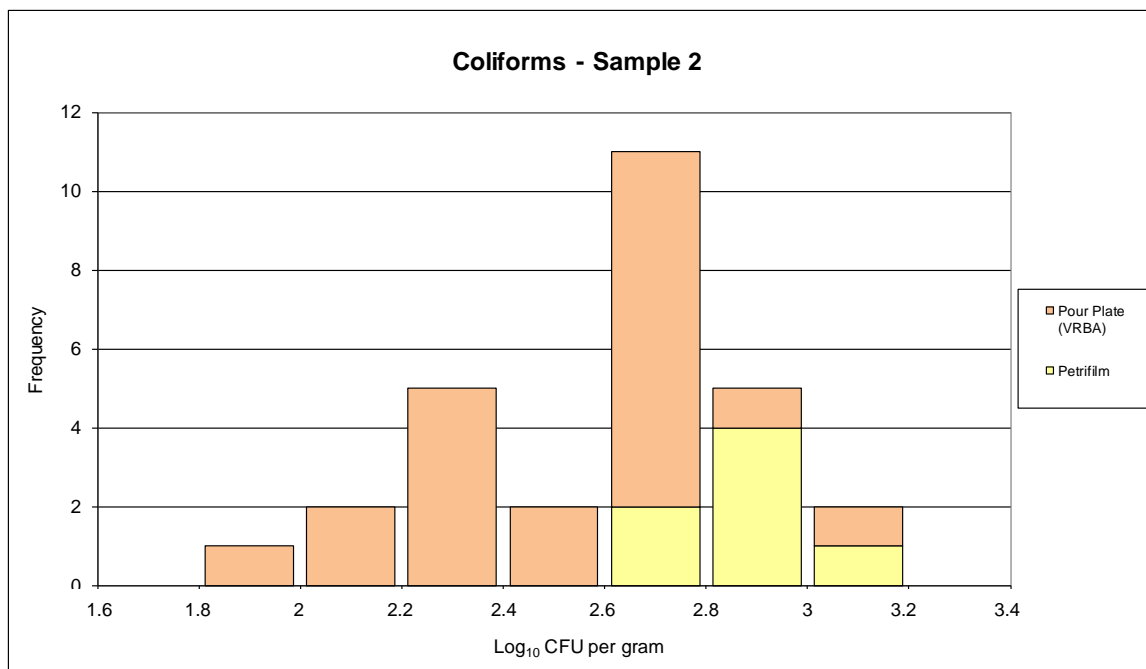


Figure TA-4. Coliforms log₁₀ cfu/g results for sample PTA 2.

6.5 *E. coli*

Of the four laboratories that submitted results for *E. coli*, three laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results. One laboratory tested using the MPN method. The Petrifilm™ results were analysed against the pooled Pour Plate, Petrifilm™ and HGMF results from the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.

The robust CV of 7.6% for the Petrifilm™ results for sample PTA 2 for this round is higher than the values of 5.2% and 3.6%, obtained for the Petrifilm™ results in Round 23 of this program, for samples containing similar organisms at similar levels (see Report No. 1060).

There were no outliers reported for the Petrifilm™ results for sample PTA 2. Sample PTA 1 did not contain *E. coli*.

A graph showing the differentiation of methods used for *E. coli* testing is included in Figure TA-5 below. 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.

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 / Petrifilm™ / HGMF	-	2.480 ± 0.071

The Global Proficiency Ltd DairyChek Microbiology program does not offer MPN testing as an option, so z-scores could not be calculated for the MPN results for *E. coli*. In the Instructions to Participants, it is requested that certain tests, including Coliforms / *E. coli* are not tested using MPN techniques, due to low participant numbers. However, laboratory 4A (using the MPN method) reported a false positive result for sample PTA 1, which should be investigated, as this sample did not contain *E. coli*. This sample in fact contained a thermotolerant strain of *K. pneumoniae*, which should have given negative results in the confirmatory testing procedures.

One laboratory reported MUs associated with their test results in this round for *E. coli*. The ranges of the reported results with their associated MU for the *E. coli* methods related well to the medians and their associated uncertainties in this round.

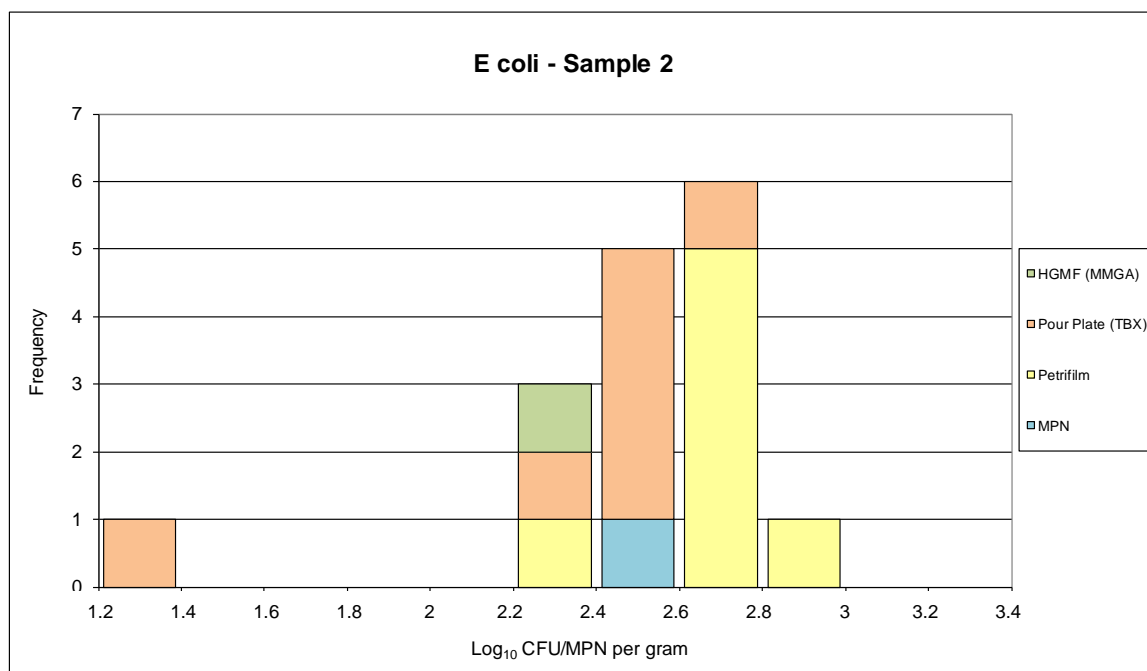


Figure TA-5. *E. coli* log₁₀ cfu or MPN/g results for sample PTA 2.

6.6 Enterobacteriaceae

A total of seven laboratories submitted results for Enterobacteriaceae. Two of these laboratories used more than one method. Five laboratories tested using Pour Plate, including one laboratory that submitted three sets of results and two laboratories that submitted two sets of results. Four laboratories tested using Petrifilm™, including two laboratories that submitted two sets of results. The Pour Plate and Petrifilm™ results 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 4.6% and 11.6% for the results for this round are higher than the values of 5.5% and 3.1%, obtained for the results in Round 23 of this program, for samples containing similar organisms at similar levels (see Report No. 1060).

There were no outliers reported for either sample.

Graphs showing the differentiation of methods used for Enterobacteriaceae testing are included in Figures TA-6 and TA-7 below. 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.

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 / Petrifilm™	2.465 ± 0.045	2.620 ± 0.120

Two laboratories reported MUs associated with their test results in this round for Enterobacteriaceae. Laboratory 7 submitted two sets of results for two different methods, all with very low uncertainties; while results for sample PTA 1 and their associated uncertainties were within the median and its uncertainty, both sets of results for both methods (VRBGA and Petrifilm™) for sample PTA 2 were further from the median than their stated uncertainty, and are recommended to be examined.

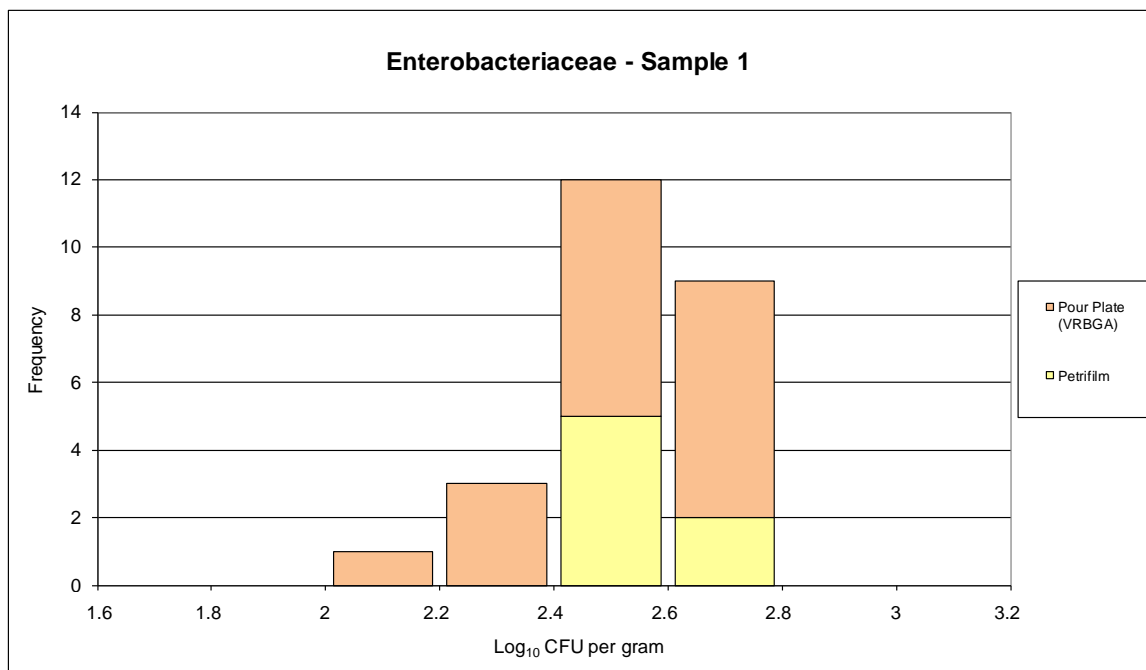


Figure TA-6. Enterobacteriaceae log₁₀ cfu/g results for sample PTA 1.

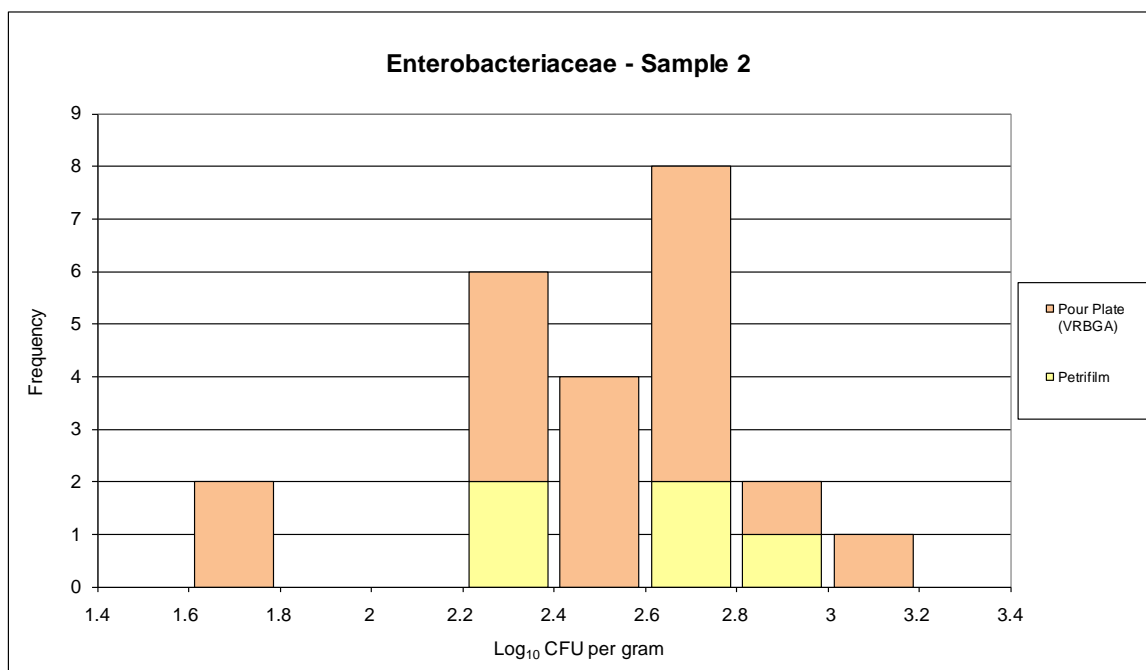


Figure TA-7. Enterobacteriaceae log₁₀ cfu/g results for sample PTA 2.

6.7 Coagulase-positive *Staphylococci*

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

The robust CV of 4.3% for the Spread Plate and Petrifilm™ results for sample PTA 1 for this round is higher than the value of 3.3%, obtained for the Spread Plate and Petrifilm™ results in Round 23 of this program, for samples containing similar organisms at similar levels (see Report No. 1060).

There were no outliers reported for the Spread Plate and Petrifilm™ results for sample PTA 1. Sample PTA 2 did not contain Coagulase-positive *Staphylococci*.

A graph showing the distribution of results for Coagulase-positive *Staphylococci* testing is included in Figure TA-8 below. 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.

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 / Petrifilm™	2.860 ± 0.051	-

The Global Proficiency Ltd DairyChek Microbiology program does not offer MPN testing as an option, so z-scores could not be calculated for the MPN results for Coagulase-positive *Staphylococci*. In the Instructions to Participants, it is requested that certain tests, including Coagulase-positive *Staphylococci* are not tested using MPN techniques, due to low participant numbers. However, laboratory 4A (using the MPN method) reported a false positive result for sample PTA 2, which should be investigated, as this sample did not contain Coagulase-positive *Staphylococci*. The sample did, however, contain a strain of the **Coagulase-negative *Staphylococcus epidermidis***.

One laboratory reported MUs associated with their test results in this round for Coagulase-positive *Staphylococci*. The ranges of the reported results with their associated MU for the Coagulase-positive *Staphylococci* methods related well to the medians and their associated uncertainties in this round.

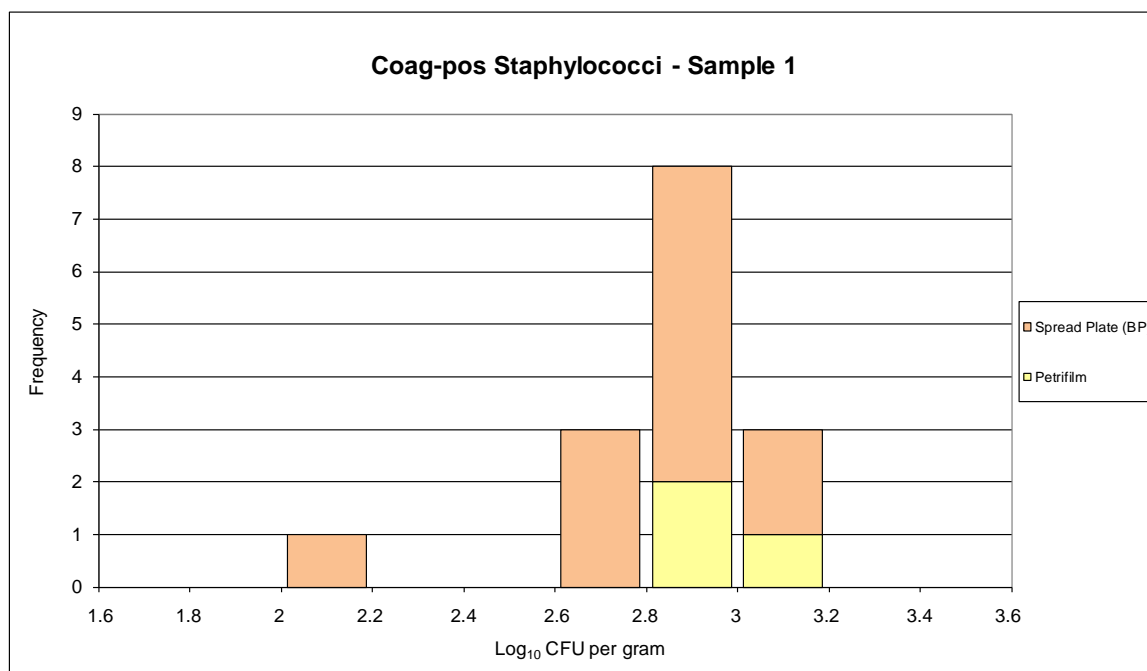


Figure TA-8. Coagulase-positive *Staphylococci* log₁₀ cfu/g results for sample PTA 1.

6.8 *B. cereus*

All of the four laboratories that submitted results for *B. cereus* tested using Spread Plate, including one laboratory that submitted two sets of results.

Z-scores and summary statistics were not calculated for *B. cereus* due to an insufficient number of results reported for either this program or the Global Proficiency Ltd DairyChek Microbiology program.

One laboratory reported MUs associated with their test results in this round for *B. cereus*.

6.9 Yeasts

A total of ten laboratories submitted results for Yeasts. One of these laboratories used more than one method. Five laboratories tested using Pour Plate, including one laboratory that submitted three sets of results and one laboratory that submitted two sets of results. Four laboratories tested using Spread Plate, including one laboratory that submitted three sets of results and two laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™. All the methods were pooled for analysis.

The robust CVs of 12.6% and 5.3% for this round are higher than the value of 3.9%, obtained in Round 23 of this program (see Report No. 1060). The high CV for sample PTA 1 is due to the lower level of Yeasts in the sample.

Laboratories 7A, 7B and 9 (using the Spread Plate method) and laboratory 1 (using the Petrifilm™ method) reported outliers for sample PTA 2. There were no outliers reported for sample PTA 1. Laboratory 1 (using both the Pour Plate and

Petrifilm™ methods) obtained false negative results for sample PTA 1, which should be investigated. The levels of yeasts in these samples are intended for plating 1mL volumes of the homogenate (10^{-1}), so those laboratories using Spread Plate techniques really need to include a 1mL volume spread over 3-5 plates as one of their dilution series.

Graphs showing the distribution of results for Yeast testing for sample PTA 1 and PTA 2 are included in Figures TA-9 and TA-10 below. 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.

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 / Petrifilm™	1.968 ± 0.080	2.712 ± 0.043

Two laboratories reported MUs associated with their test results in this round for Yeasts. It is recommended that laboratory 3 re-examines their test results or their MU calculations for sample PTA 2, as their results were further from the median than their stated uncertainty (taking into consideration the uncertainty associated with the median). Laboratory 7 submitted two sets of results, all with very low uncertainties; while results for sample PTA 1 and their associated uncertainties were within the median and its uncertainty, both sets of results for sample PTA 2 were further from the median than their stated uncertainty, and should be examined.

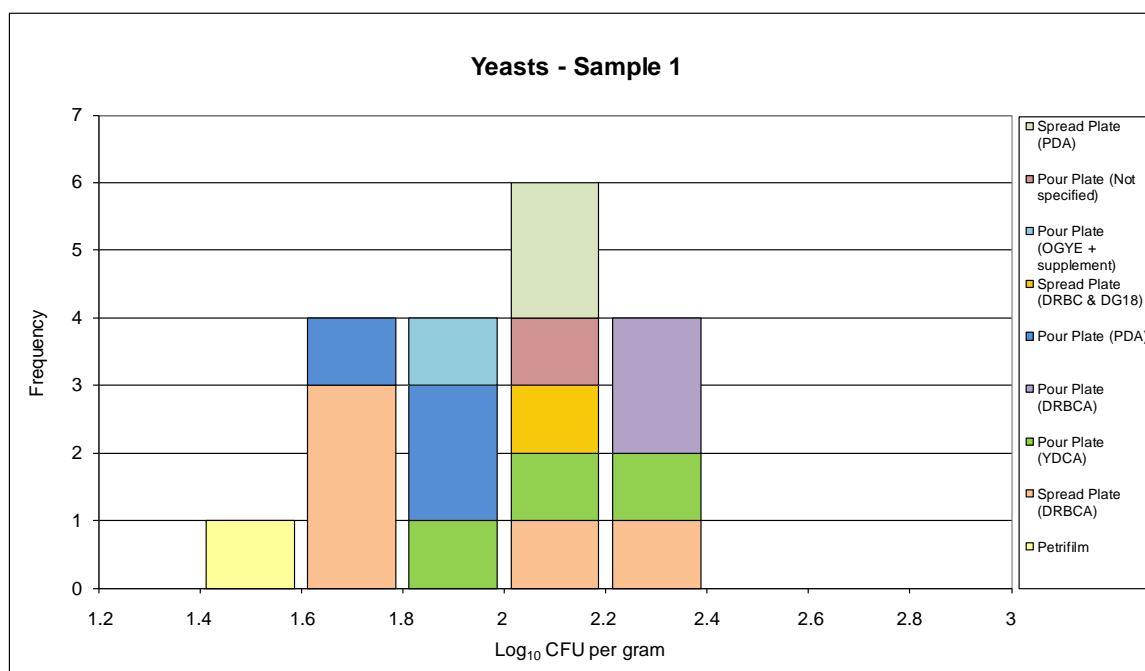


Figure TA-9. Yeasts \log_{10} cfu/g results for sample PTA 1.

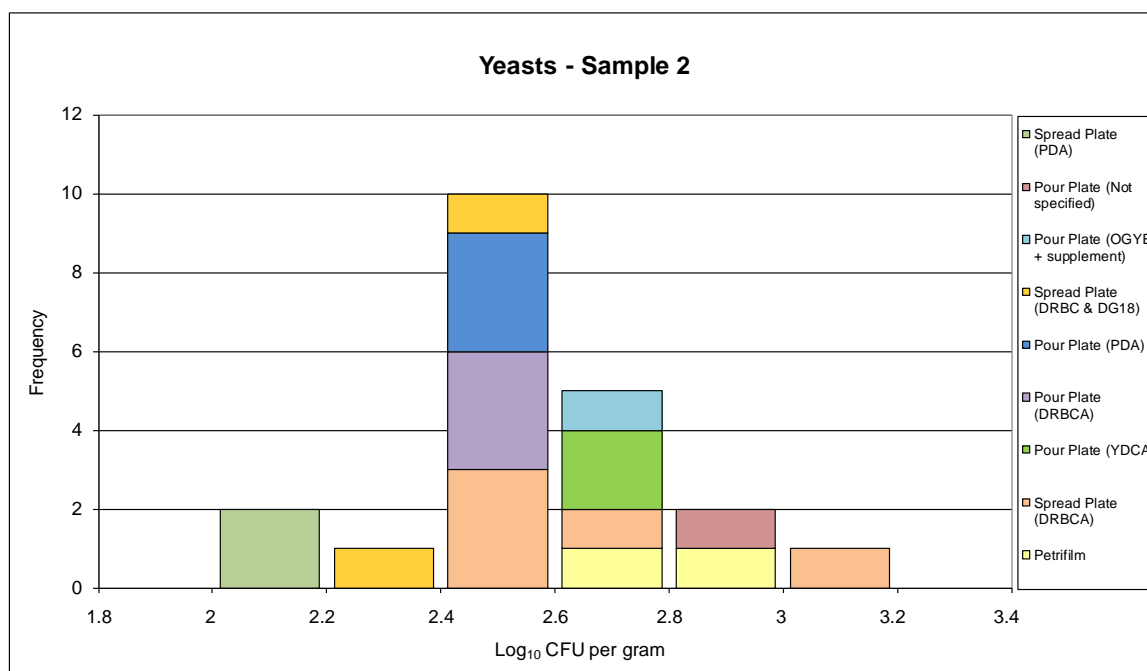


Figure TA-10. Yeasts log₁₀ cfu/g results for sample PTA 2.

6.10 Moulds

A total of ten laboratories submitted results for Moulds. One of these laboratories used more than one method. Five laboratories tested using Pour Plate, including one laboratory that submitted three sets of results and one laboratory that submitted two sets of results. Four laboratories tested using Spread Plate, including one laboratory that submitted three sets of results and two laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™. All the methods were pooled for analysis.

The robust CVs of 4.3% and 7.1% for this round compare well with the values of 5.8% and 6.0%, obtained in Round 23 of this program, for samples containing the same organisms at similar levels (see Report No. 1060).

Laboratory 3 (using the Petrifilm™ method) reported an outlier for sample PTA 1. There were no outliers reported for sample PTA 2.

Graphs showing the distribution of results for Mould testing for sample PTA 1 and PTA 2 are included in Figures TA-11 and TA-12 below. 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.

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 / Petrifilm™	2.828 ± 0.036	2.908 ± 0.061

Two laboratories reported MUs associated with their test results in this round for Moulds. It is recommended that laboratory 3 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). Laboratory 7 submitted two sets of results with very low uncertainties; while most results and their associated uncertainties were within the median and its uncertainty, the results for sample PTA 1 for result sets A and B were further from the median than their stated uncertainty, and should be examined.

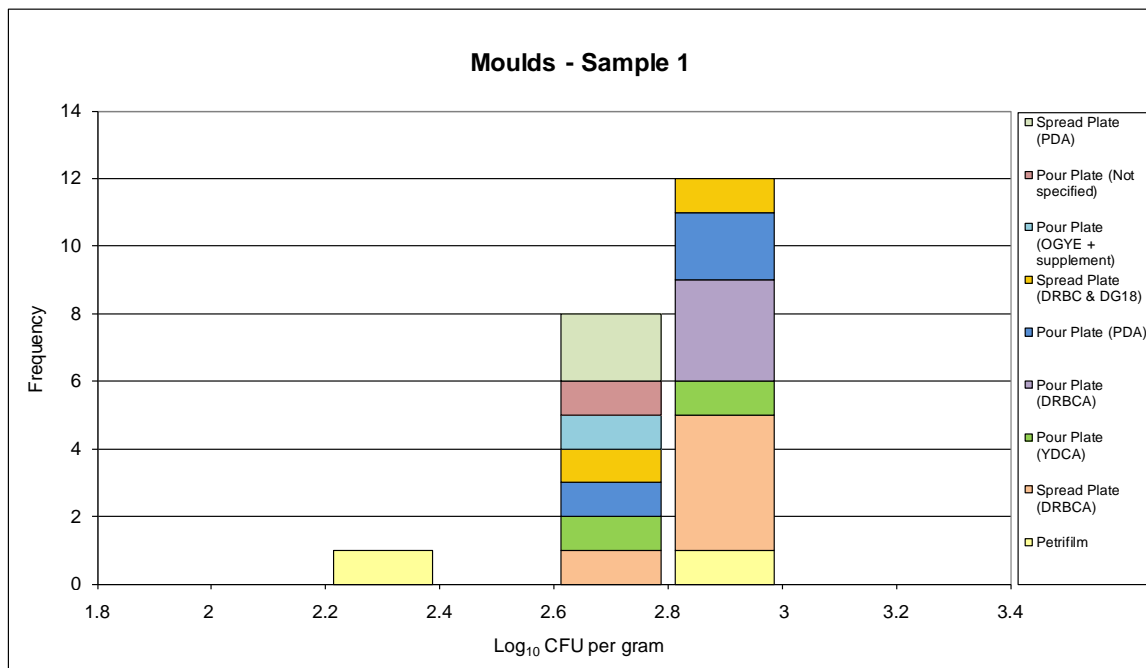


Figure TA-11. Moulds log₁₀ cfu/g results for sample PTA 1.

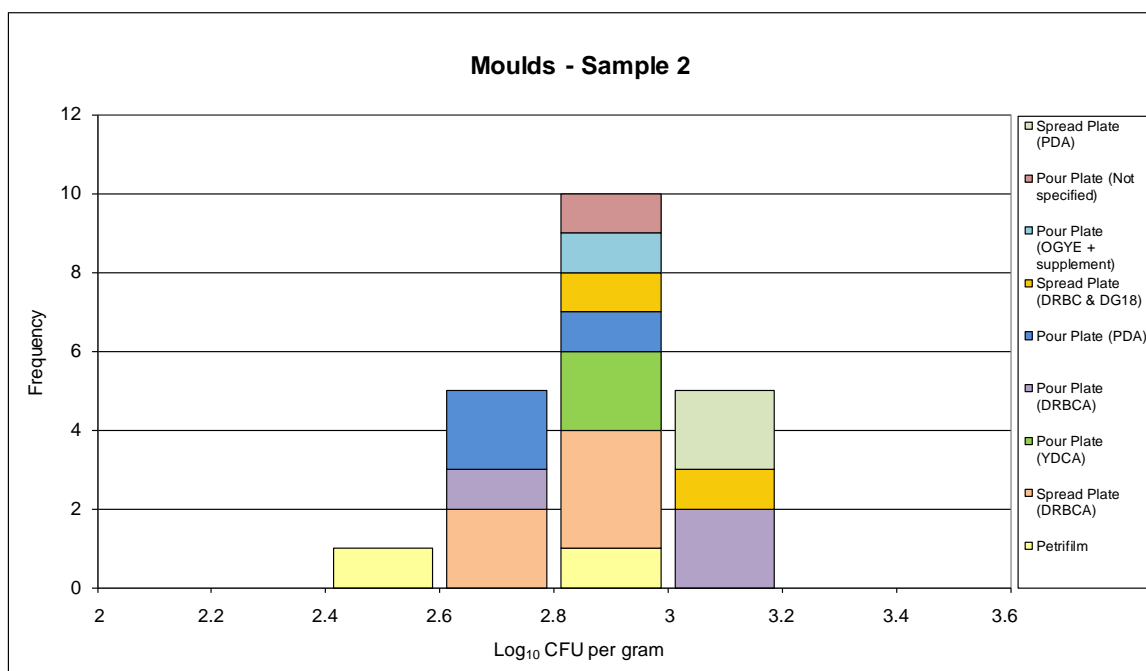


Figure TA-12. Moulds log₁₀ cfu/g results for sample PTA 2.

6.11 Total Yeasts and Moulds

A total of six laboratories submitted results for Total Yeasts and Moulds. Two of these laboratories used more than one method. Five laboratories tested using Pour Plate, including one laboratory that submitted three sets of results and one laboratory that submitted two sets of results. One laboratory tested using Spread Plate. One laboratory tested using Petrifilm™. One laboratory tested using the Tempo automated system manufactured by Bioré, which provides a combined result for Yeasts and Moulds. All the methods were pooled for analysis.

The robust CVs of 4.7% and 5.0% for this round compare well with the values of 5.5% and 5.2%, obtained in Round 23 of this program, for samples containing the same organisms at similar levels (see Report No. 1060).

There were no outliers reported for either sample.

Graphs showing the distribution of results for Total Yeasts and Moulds testing for sample PTA 1 and PTA 2 are included in Figures TA-13 and TA-14 on the pages following. 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.

For the plating techniques, five different types of media were used in this round for Yeasts and Moulds testing including OGYE (Oxytetracycline-Glucose-Yeast Extract agar), DRBCA (Dichloran-Rose Bengal-Chloramphenicol agar) – this was used via both the Pour Plate and Spread Plate techniques; YDCA (Yeast Extract-Dextrose-Chloramphenicol agar), PDA (Potato Dextrose agar) and 3M™ Petrifilm™ Yeast and Mould Count plates. The media differ in the antibiotics incorporated in the medium and the final pH to inhibit bacterial growth, as well as the inclusion of other compounds to inhibit spreading moulds.

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

	PTA 1	PTA 2
Total Yeasts and Moulds - Pour Plate / Spread Plate / Petrifilm™ / Tempo	2.989 ± 0.053	3.104 ± 0.058

One laboratory reported MUs associated with their test results in this round for Total Yeasts and Moulds. It is recommended that laboratory 9 re-examines their test results or their MU calculations for sample PTA 2, as their results were further from the median than their stated uncertainty (taking into consideration the uncertainty associated with the median).

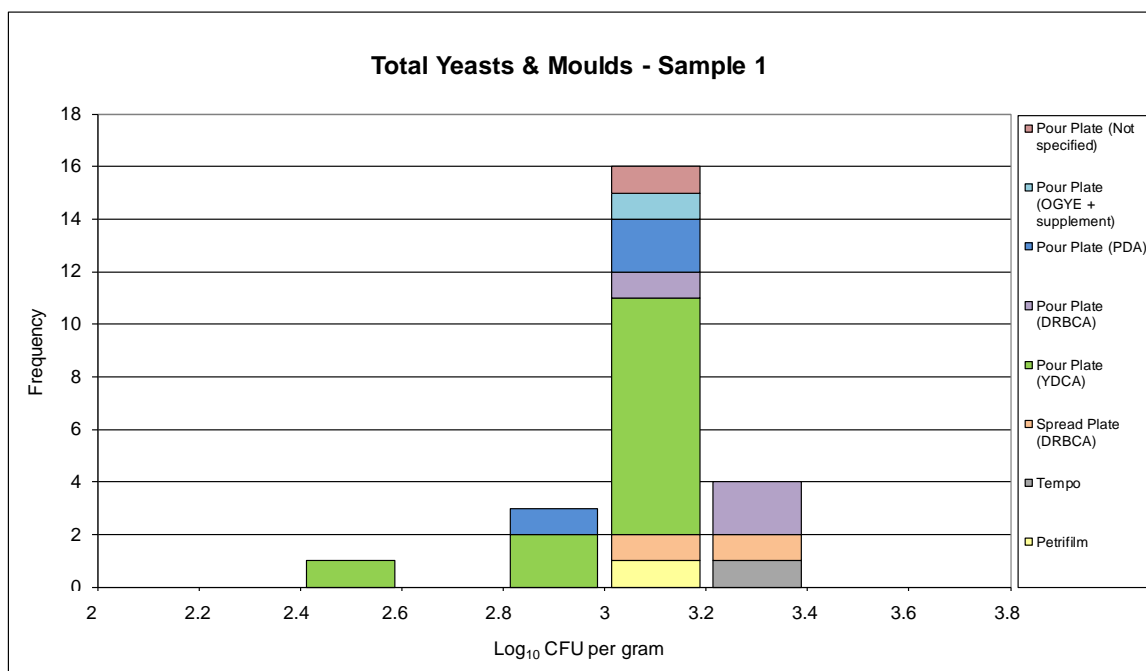


Figure TA-13. Total Yeasts & Moulds log₁₀ cfu/g results for sample PTA 1.

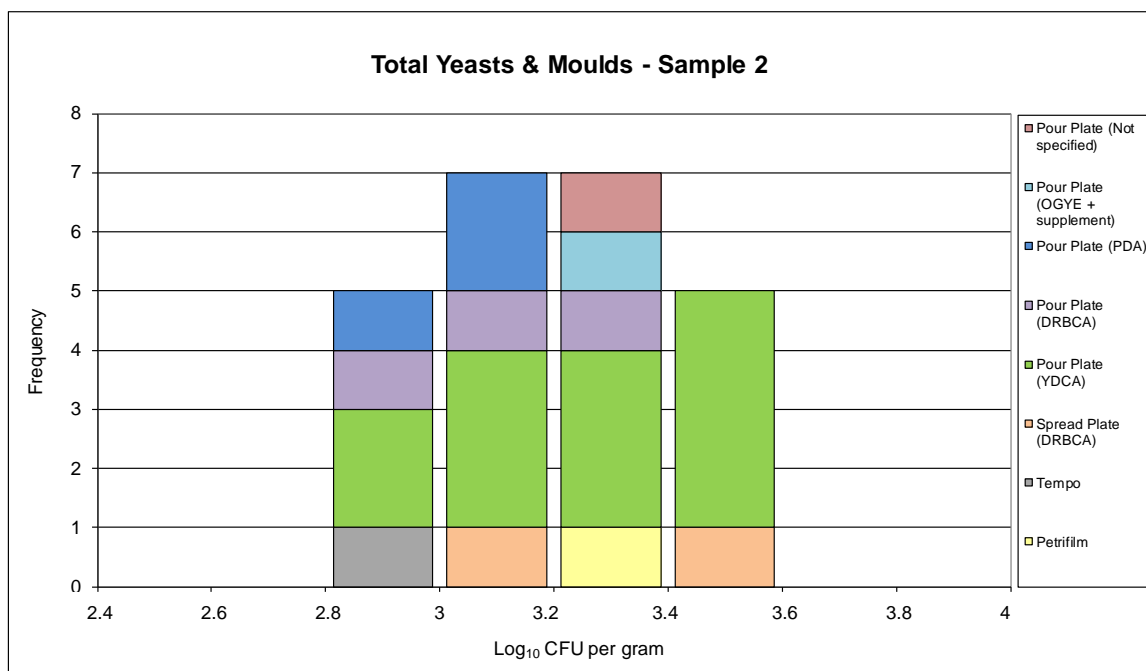


Figure TA-14. Total Yeasts & Moulds log₁₀ cfu/g results for sample PTA 2.

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 30°C (ISO 7932: 2004, MOD)*.
4. AS 5013.4 (2009) *Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms – Colony-count technique*.
5. AS 5013.5 (2016) *Food microbiology - Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Colony count at 30°C by the pour plate technique*.
6. AS 5013.9 (2009) *Food microbiology - Examination for specific organisms - Coliforms and Escherichia coli by the triplicate tube detection method*.
7. AS 5013.12.1 (2004) *Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Technique using Baird-Parker agar medium*.
8. AS 5013.15 (2006) *Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive Escherichia coli - Most probable number technique*.
9. AS 5013.29 (2009) *Food microbiology - Examination for specific organisms - Colony count of yeasts and moulds*.
10. ISO 6611 (2004) / IDF 94 (2004) *Milk and milk products - Enumeration of colony-forming units of yeasts and/or moulds - Colony-count technique at 25 degrees C*.
11. ISO 6888-1:1999/Amd.2:2018 *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) – Part 1: Technique using Baird-Parker agar medium*.
12. ISO 7932 (2004) *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive Bacillus cereus - Colony-count technique at 30 degrees C*.
13. ISO 16649-2 (2001) *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*.
14. ISO 21528-2 (2017) *Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count technique*.

APPENDIX A

Summary of Results

Section A1

Aerobic Plate Count

A1.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	11000	4.04	-	13250	4.12	-	0.56	1.08	PP	SPCA
1	12250	4.09	-	8650	3.94	-	0.80	-0.33	Pfm	-
2A	5900	3.77	-	8900	3.95	-	-0.80	-0.23	PP	PC
2B	3800	3.58	-	7500	3.88	-	-1.77	-0.80	PP	PC
3	5000.00	3.70	700.00	5200.00	3.72	600.00	-1.17	-2.01	Pfm	-
4A	11000	4.04	-	12000	4.08	-	0.56	0.75	PP	PCA
4B	10000	4.00	-	13000	4.11	-	0.35	1.02	PP	PCA
5	6500	3.81	-	8900	3.95	-	-0.59	-0.23	PP	PCA
5	7200	3.86	-	8100	3.91	-	-0.37	-0.54	Pfm	-
6	5300	3.72	-	14500	4.16	-	-1.04	1.38	PP	-
7A	8000	3.90	0.0039	7000	3.85	0.0088	-0.14	-1.03	PP	PCA
7A	8200	3.91	0.0019	7200	3.86	0.0045	-0.08	-0.93	Pfm	-
7B	8200	3.91	0.0039	7400	3.87	0.0088	-0.08	-0.84	PP	PCA
7B	8300	3.92	0.0019	7000	3.85	0.0045	-0.06	-1.03	Pfm	-
8A	8700	3.94	-	11350	4.05	-	0.05	0.57	PP	PCA
8B	7300	3.86	-	8200	3.91	-	-0.34	-0.50	PP	PCA
8C	6000	3.78	-	8500	3.93	-	-0.77	-0.38	PP	PCA
9	6400	3.81	0.15	11000	4.04	0.15	-0.62	0.47	PP	PCA
10A	190000	5.28	-	150000	5.18	-	6.80 §	9.09 §	PP	TPC
10B	100750	5.00	-	165000	5.22	-	5.41 §	9.40 §	PP	TPC
10C	131000	5.12	-	140000	5.15	-	5.99 §	8.86 §	PP	TPC

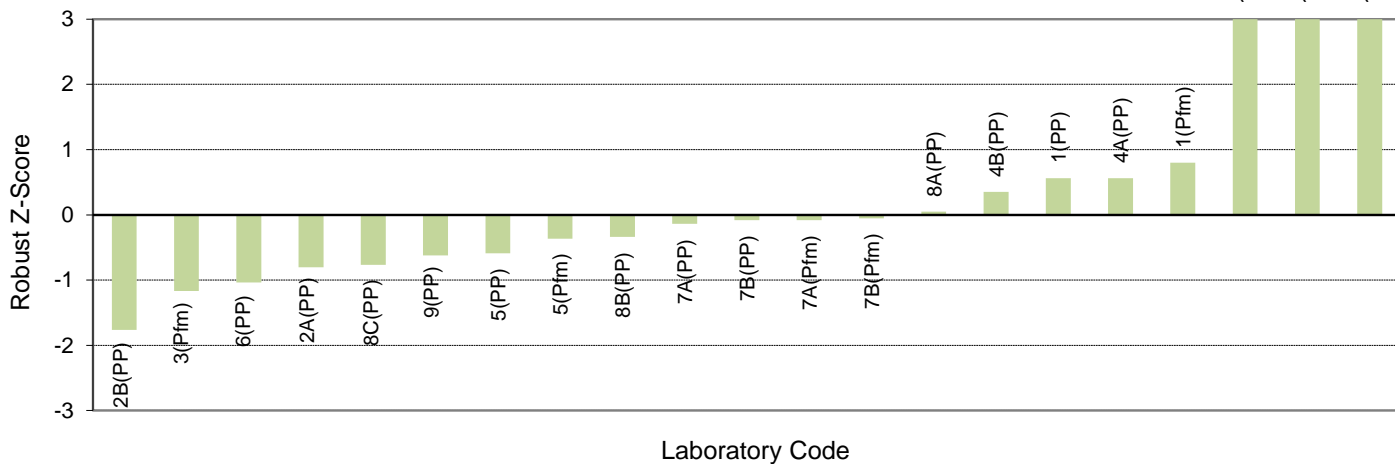
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	12	12
Median	3.930	3.980
Normalised IQR	0.198	0.132
Uncertainty (Median)	0.072	0.048
Robust CV	5.0%	3.3%
Minimum	3.70	3.83
Maximum	4.93	4.20
Range	1.23	0.37

Notes:

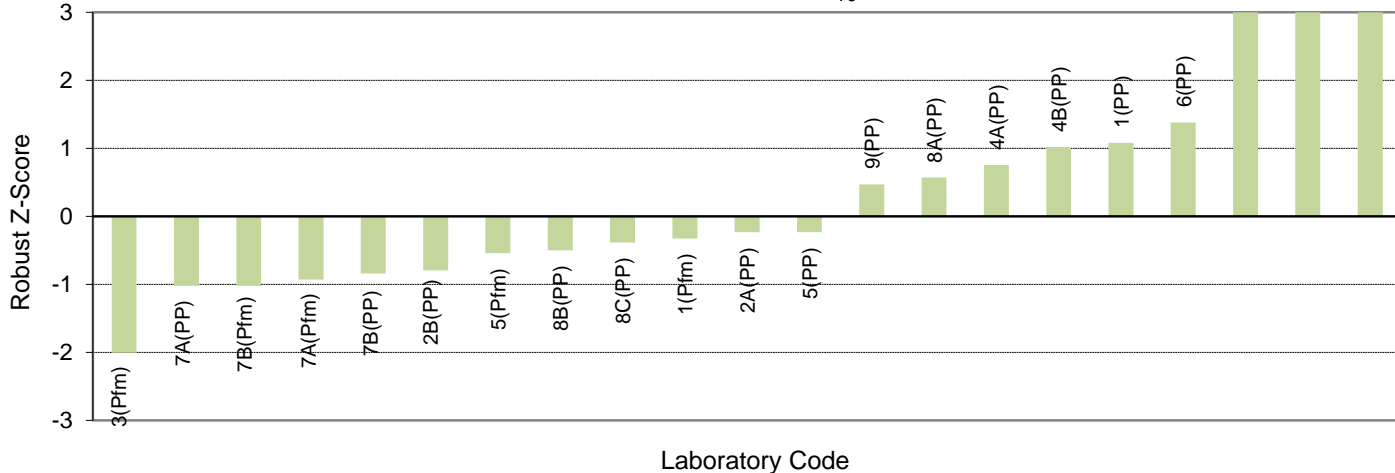
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Aerobic Plate Count results.
4. Z-scores and summary statistics (including the number of results) 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™ [$\log_{10}(\text{cfu/g})$] - PTA 1



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



Section A2

Coliforms

A2.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	353	2.55	-	677	2.83	-	0.81	0.74	Pfm	-
2A	280	2.45	-	640	2.81	-	0.12	0.64	Pfm	-
2B	260	2.41	-	610	2.79	-	-0.10	0.55	Pfm	-
3	200.00	2.30	30.00	405.00	2.61	15.00	-0.89	-0.23	Pfm	-
4A	300	2.48	-	250	2.40	-	0.33	-1.14	PP	VRBA
4B	300	2.48	-	300	2.48	-	0.33	-0.80	PP	VRBA
5	300	2.48	-	710	2.85	-	0.33	0.83	PP	VRBA
5	340	2.53	-	830	2.92	-	0.70	1.13	Pfm	-
6	420	2.62	-	1090	3.04	-	1.34	1.64	PP	-
7A	240	2.38	0.0110	170	2.23	0.0173	-0.34	-1.87	PP	VRBA
7B	230	2.36	0.0110	150	2.18	0.0173	-0.47	-2.11	PP	VRBA
8A	240	2.38	-	230	2.36	-	-0.34	-1.30	PP	VRBA
8B	190	2.28	-	490	2.69	-	-1.05	0.13	PP	VRBA
8C	280	2.45	-	170	2.23	-	0.12	-1.87	PP	VRBA
10A	490	2.69	-	630	2.80	-	1.80	0.61	PP	VRBA
10B	700	2.85	-	630	2.80	-	2.87	0.61	PP	VRBA
10C	780	2.89	-	490	2.69	-	3.20 §	0.13	PP	VRBA

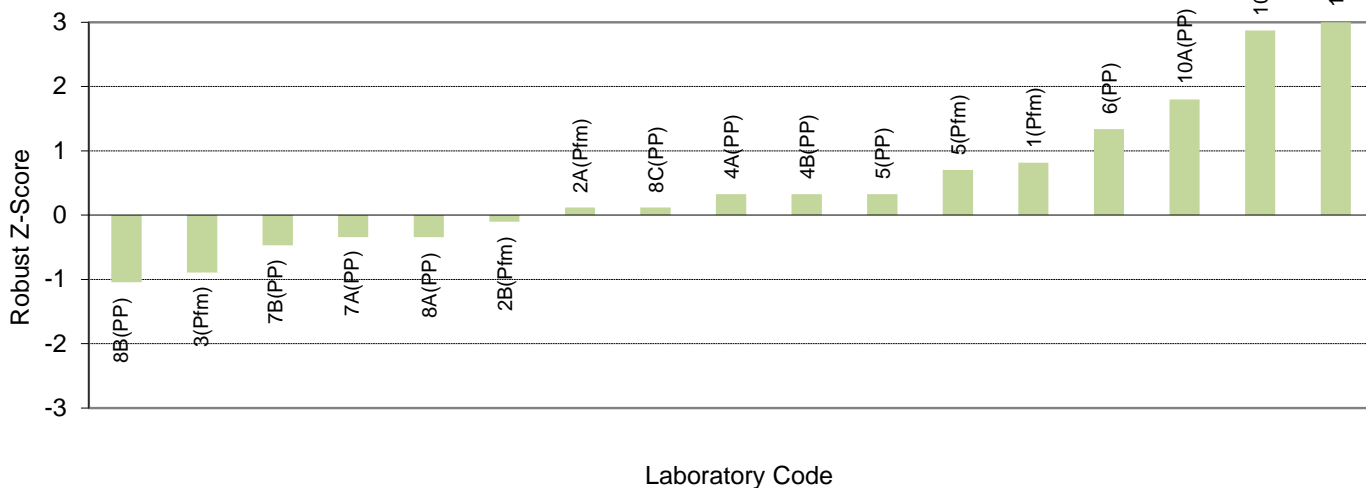
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	11	11
Median	2.430	2.660
Normalised IQR	0.145	0.230
Uncertainty (Median)	0.055	0.087
Robust CV	5.9%	8.6%
Minimum	2.28	1.85
Maximum	2.63	3.00
Range	0.35	1.15

Notes:

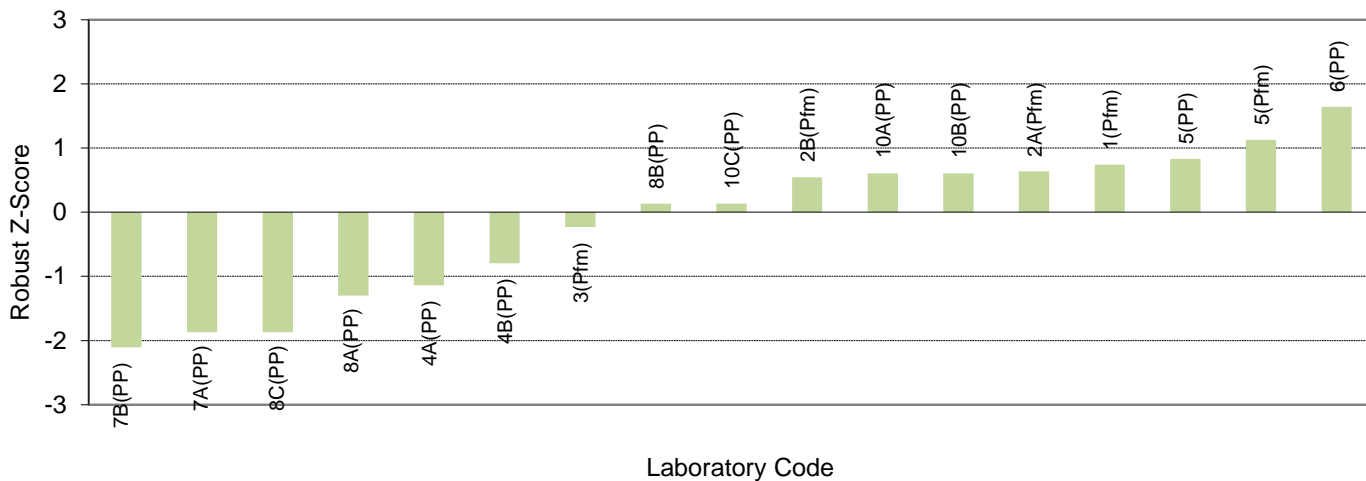
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
- Z-scores and summary statistics (including the number of results) for Coliforms 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™ / Other (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	< 3	-	-	542	2.73	-	-	1.34	Pfm	-
2A	< 10	-	-	540	2.73	-	-	1.34	Pfm	-
2B	< 10	-	-	500	2.70	-	-	1.16	Pfm	-
3	0.00	-	0.00	245.00	2.39	5.00	-	-0.48	Pfm	-

Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	11	11
Median	n/a	2.480
Normalised IQR	n/a	0.189
Uncertainty (Median)	n/a	0.071
Robust CV	n/a	7.6%
Minimum	n/a	1.30
Maximum	n/a	2.90
Range	n/a	1.60

Notes:

1. For the method abbreviation in the table above, Pfm = Petrifilm™.
2. 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.
3. The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
4. Sample PTA 1 did not contain *E. coli*.

Milk Powder – *E. coli*, MPN (MPN/g)

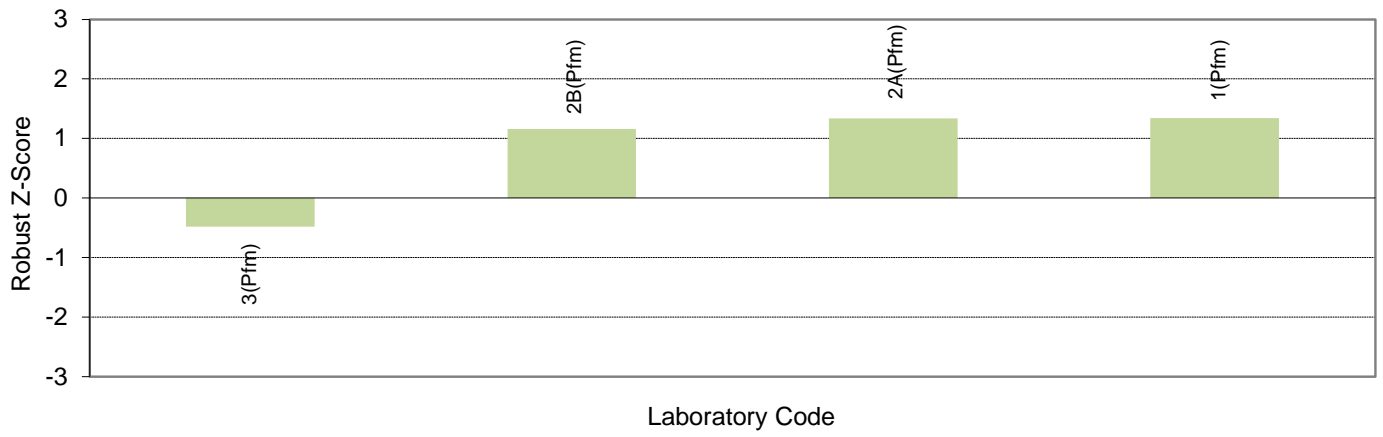
Lab Code	PTA 1			PTA 2			Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	
4A	300 ‡	2.48	-	300	2.48	-	LST

Notes:

1. The Global Proficiency Ltd DairyChek Microbiology program does not offer MPN testing as an option, so z-scores could not be calculated for the MPN results reported for *E. coli*.
2. ‡ denotes a false positive result.

A3.2

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



Section A4

Enterobacteriaceae

A4.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	360	2.56	-	623	2.79	-	0.81	0.58	Pfm	-
2A	340	2.53	-	-	-	-	0.59	-	Pfm	-
2B	410	2.61	-	-	-	-	1.31	-	Pfm	-
4A	350	2.54	-	300	2.48	-	0.70	-0.47	PP	VRBGA
4B	400	2.60	-	380	2.58	-	1.21	-0.13	PP	VRBGA
6	445	2.65	-	1125	3.05	-	1.62	1.42	PP	-
6	415	2.62	-	905	2.96	-	1.35	1.11	Pfm	-
7A	320	2.51	0.0038	160	2.20	0.0294	0.36	-1.37	PP	*
7A	310	2.49	0.0038	160	2.20	0.0294	0.23	-1.37	Pfm	-
7B	310	2.49	0.0038	180	2.26	0.0294	0.23	-1.20	PP	*
7B	320	2.51	0.0038	180	2.26	0.0294	0.36	-1.20	Pfm	-
9	200	2.30	0.22	240	2.38	0.22	-1.45	-0.79	PP	VRBA
10A	530	2.72	-	550	2.74	-	2.29	0.40	PP	VRBD
10B	510	2.71	-	610	2.79	-	2.15	0.55	PP	VRBD
10C	500	2.70	-	660	2.82	-	2.07	0.66	PP	VRBD

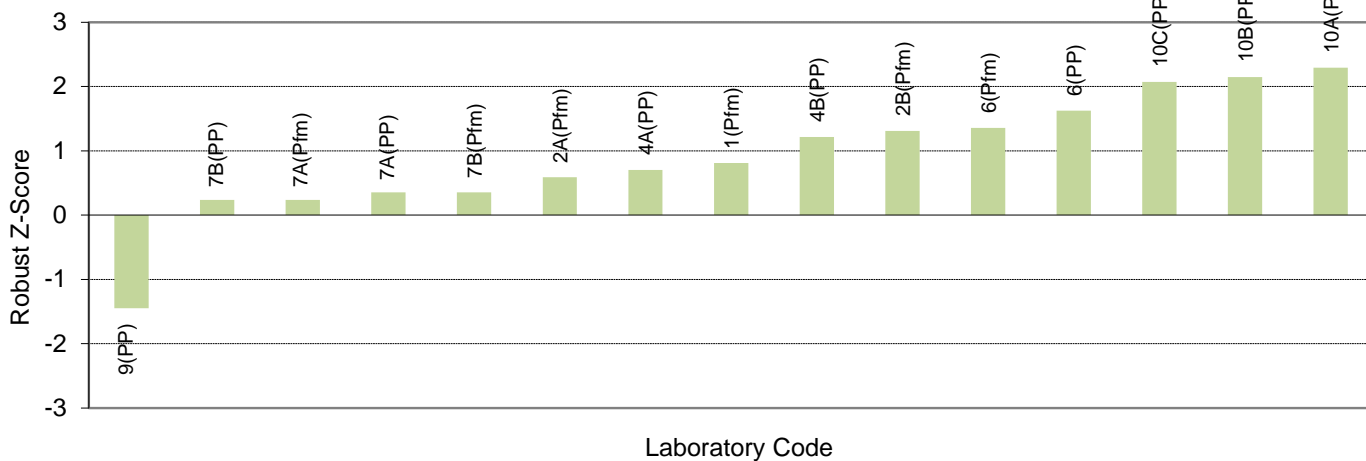
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	10	10
Median	2.465	2.620
Normalised IQR	0.113	0.303
Uncertainty (Median)	0.045	0.120
Robust CV	4.6%	11.6%
Minimum	2.11	1.60
Maximum	2.66	2.76
Range	0.55	1.16

Notes:

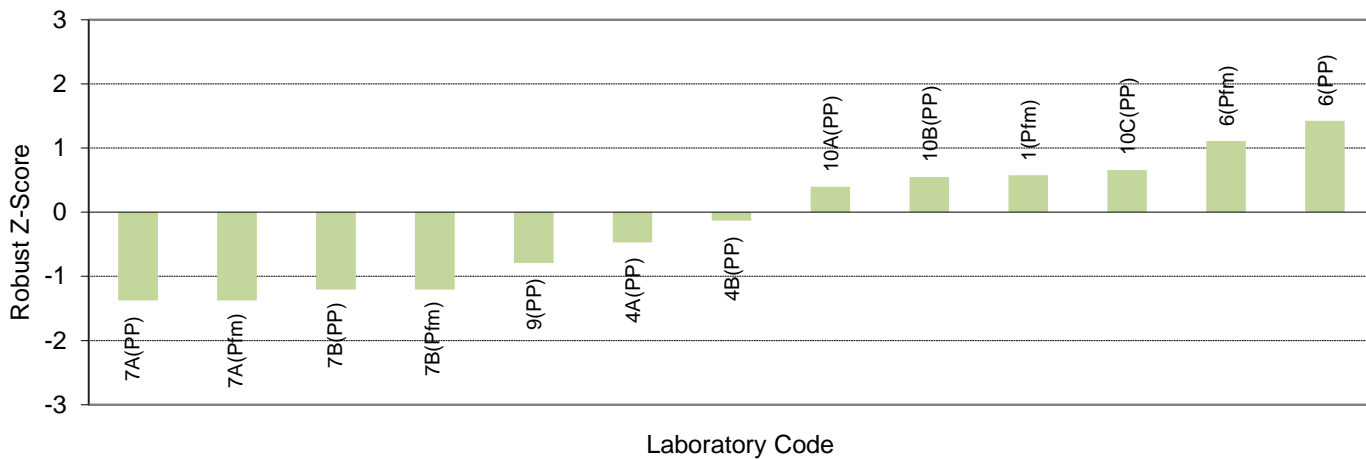
1. * denotes VRBA w/ glucose.
2. For the method abbreviations in the table above, PP= Pour Plate and Pfm = Petrifilm™.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Enterobacteriaceae results.
4. Z-scores and summary statistics (including the number of results) for Enterobacteriaceae were calculated from the results for the Global Proficiency Ltd DairyChek Microbiology program, using the same samples.
5. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A4.2

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



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



Section A5

Coagulase-positive *Staphylococci*

A5.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	PTA 1	PTA 2		
1	1147	3.06	-	< 10	-	-	1.63	-	Pfm	-
2A	1300	3.11	-	< 100	-	-	2.08	-	SP	BP
2B	1400	3.15	-	< 100	-	-	2.34	-	SP	BP
5	430	2.63	-	0	-	-	-1.85	-	SP	BPA
6	970	2.99	-	< 10	-	-	1.04	-	SP	-
9	900	2.95	0.17	< 100	-	0.17	0.77	-	SP	BP

Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	9	8
Median	2.860	n/a
Normalised IQR	0.122	n/a
Uncertainty (Median)	0.051	n/a
Robust CV	4.3%	n/a
Minimum	2.15	n/a
Maximum	2.99	n/a
Range	0.84	n/a

Notes:

- For the method abbreviations in the table above, SP= Spread Plate and Pfm = Petrifilm™.
- The Spread Plate and Petrifilm™ methods were pooled when analysing the Coagulase-positive *Staphylococci* results.
- 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.
- The method used has been appended to the laboratory code on the ordered z-score chart on the following page.
- Sample PTA 2 did not contain Coagulase-positive *Staphylococci*.

Milk Powder – Coagulase-positive *Staphylococci*, MPN (MPN/g)

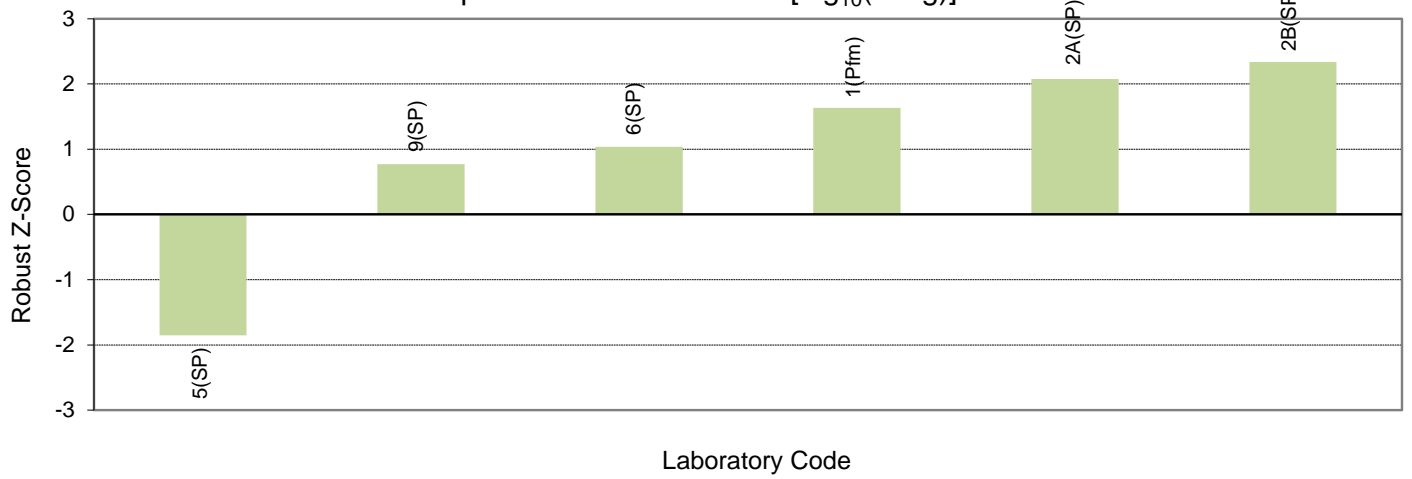
Lab Code	PTA 1			PTA 2			Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	
4A	> 1100	-	-	> 1100 ‡	-	-	MSA

Notes:

- The Global Proficiency Ltd DairyChek Microbiology program does not offer MPN testing as an option, so z-scores could not be calculated for the MPN results reported for Coagulase-positive *Staphylococci*.
- ‡ denotes a false positive result.

A5.2

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



Section A6

Bacillus cereus

A6.1

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

Lab Code	PTA 1			PTA 2			Medium
	Result	Log ₁₀	MU	Result	Log ₁₀	MU	
2A	1900	3.28	-	< 100	-	-	MYP
2B	1600	3.20	-	< 100	-	-	MYP
4A	2000	3.30	-	< 10	-	-	MYP Agar
6	1500	3.18	-	< 100	-	-	-
9	3000	3.48	0.15	< 100	-	0.15	MYP

Notes:

1. Z-scores and summary statistics were not calculated for *Bacillus cereus* due to an insufficient number of results reported for either this program or the Global Proficiency Ltd DairyChek Microbiology program.
2. Sample PTA 2 did not contain *Bacillus cereus*.

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	< 10 †	-	-	545	2.74	-	-	0.17	PP	DRBC
1	< 10 †	-	-	123	2.09	-	-	-4.31 §	Pfm	-
2A	100	2.00	-	300	2.48	-	0.13	-1.62	SP	*
2B	< 100	-	-	600	2.78	-	-	0.46	SP	*
3	85.00	1.93	5.00	745.00	2.87	15.00	-0.16	1.11	Pfm	-
4A	200	2.30	-	470	2.67	-	1.34	-0.27	PP	DRBCA
4B	200	2.30	-	450	2.65	-	1.34	-0.40	PP	DRBCA
5	93	1.97	-	800	2.90	-	0.00	1.33	PP	^
6	110	2.04	-	1010	3.00	-	0.29	2.03	PP	-
7A	100	2.00	0.0290	170	2.23	0.0178	0.13	-3.33 §	SP	PDA
7B	110	2.04	0.0290	160	2.20	0.0178	0.29	-3.52 §	SP	PDA
8A	85	1.93	-	630	2.80	-	-0.16	0.61	PP	PDA
8B	65	1.81	-	400	2.60	-	-0.63	-0.76	PP	PDA
8C	40	1.60	-	530	2.72	-	-1.48	0.09	PP	PDA
9	200	2.30	-	1700	3.23	-	1.34	3.60 §	SP	DRBCA
10A	40	1.60	-	500	2.70	-	-1.48	-0.09	SP	DRBC
10B	40	1.60	-	650	2.81	-	-1.48	0.70	SP	DRBC
10C	40	1.60	-	500	2.70	-	-1.48	-0.09	SP	DRBC

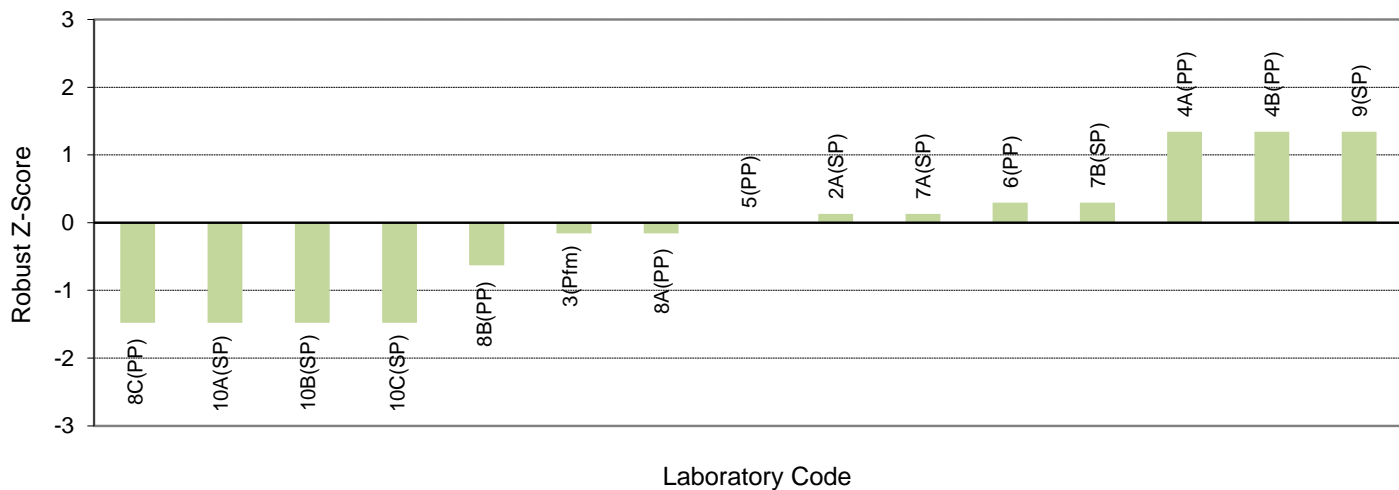
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	15	18
Median	1.968	2.712
Normalised IQR	0.248	0.144
Uncertainty (Median)	0.080	0.043
Robust CV	12.6%	5.3%
Minimum	1.60	2.09
Maximum	2.30	3.23
Range	0.70	1.14

Notes:

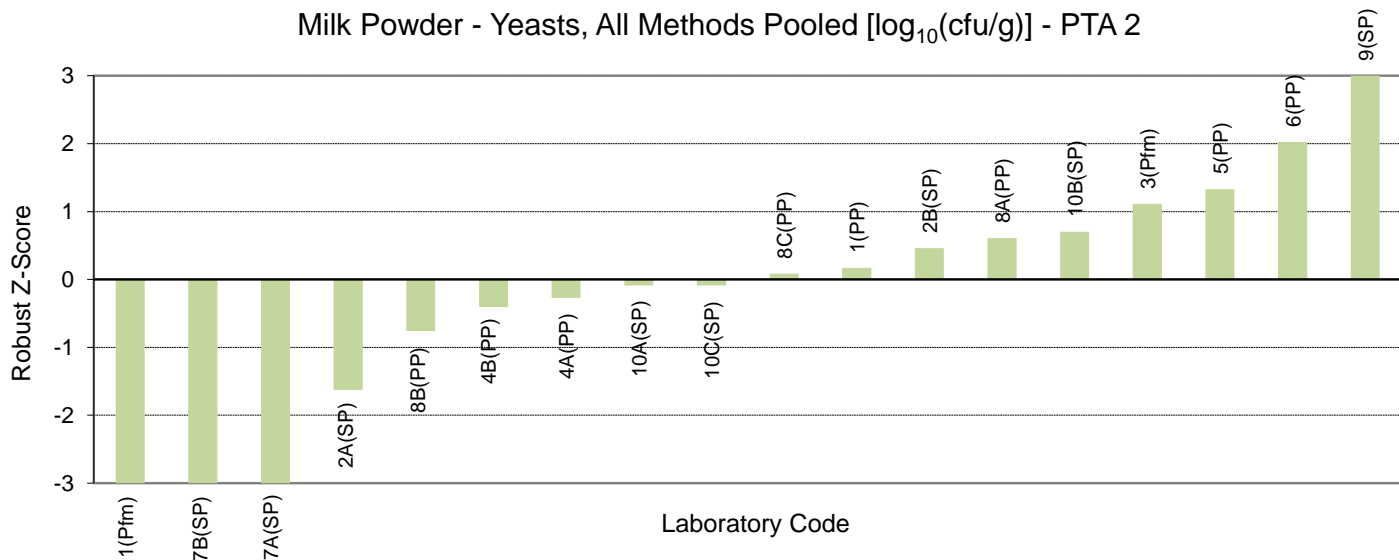
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. † denotes a false negative result.
3. * denotes DRBC and DG18.
4. ^ denotes OGYE + supplement.
5. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
6. All the methods were pooled when analysing the Yeasts results.
7. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A7.2

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



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	975	2.99	-	427	2.63	-	1.33	-1.35	PP	DRBC
1	1025	3.01	-	1075	3.03	-	1.51	0.59	Pfm	-
2A	600	2.78	-	1300	3.11	-	-0.41	0.99	SP	*
2B	700	2.85	-	800	2.90	-	0.14	-0.03	SP	*
3	250.00	2.40	0.00	325.00	2.51	25.00	-3.55 §	-1.92	Pfm	-
4A	800	2.90	-	1200	3.08	-	0.62	0.83	PP	DRBCA
4B	900	2.95	-	1100	3.04	-	1.05	0.64	PP	DRBCA
5	550	2.74	-	950	2.98	-	-0.72	0.34	PP	^
6	525	2.72	-	820	2.91	-	-0.89	0.03	PP	-
7A	490	2.69	0.0047	1100	3.04	0.0175	-1.13	0.64	SP	PDA
7B	480	2.68	0.0047	1200	3.08	0.0175	-1.21	0.83	SP	PDA
8A	655	2.82	-	640	2.81	-	-0.09	-0.49	PP	PDA
8B	515	2.71	-	535	2.73	-	-0.96	-0.87	PP	PDA
8C	690	2.84	-	625	2.80	-	0.09	-0.54	PP	PDA
9	900	2.95	-	900	2.95	-	1.05	0.22	SP	DRBCA
10A	700	2.85	-	500	2.70	-	0.14	-1.01	SP	DRBC
10B	600	2.78	-	560	2.75	-	-0.41	-0.78	SP	DRBC
10C	700	2.85	-	640	2.81	-	0.14	-0.49	SP	DRBC

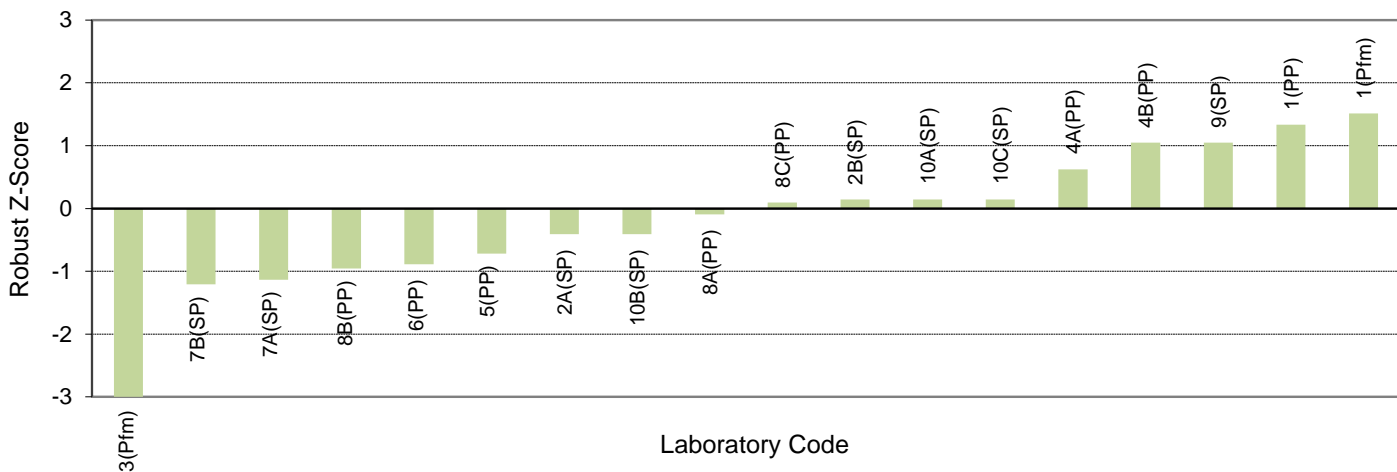
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	18	18
Median	2.828	2.908
Normalised IQR	0.121	0.207
Uncertainty (Median)	0.036	0.061
Robust CV	4.3%	7.1%
Minimum	2.40	2.51
Maximum	3.01	3.11
Range	0.61	0.60

Notes:

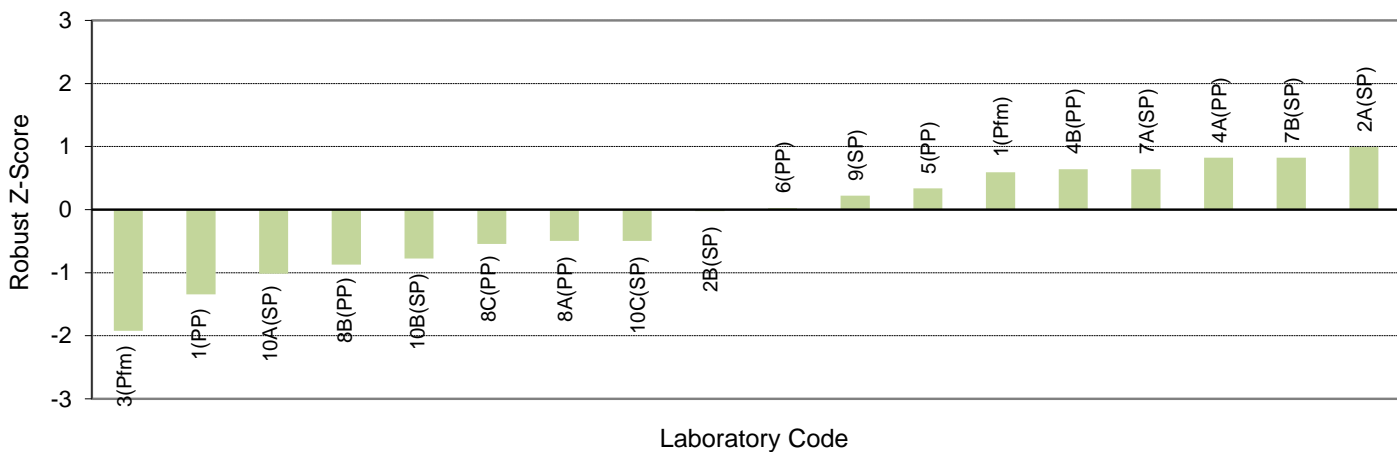
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- * denotes DRBC and DG18.
- ^ denotes OGYE + supplement.
- For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate and Pfm = Petrifilm™.
- All the methods were pooled when analysing the Moulds results.
- The method used has been 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		
1	975	2.99	-	972	2.99	-	0.00	-0.75	PP	DRBC
1	1025	3.01	-	1198	3.08	-	0.16	-0.16	Pfm	-
4A	1000	3.00	-	1670	3.22	-	0.08	0.77	PP	DRBCA
4B	1100	3.04	-	1550	3.19	-	0.38	0.56	PP	DRBCA
4C	1100	3.04	-	710	2.85	-	0.38	-1.64	Oth	-
5	650	2.81	-	1750	3.24	-	-1.26	0.90	PP	^
6	635	2.80	-	1830	3.26	-	-1.34	1.03	PP	-
8A	740	2.87	-	1270	3.10	-	-0.86	0.00	PP	PDA
8B	580	2.76	-	935	2.97	-	-1.62	-0.86	PP	PDA
8C	730	2.86	-	1155	3.06	-	-0.90	-0.27	PP	PDA
9	1100	3.04	0.17	2600	3.41	0.17	0.38	2.02	SP	DRBCA

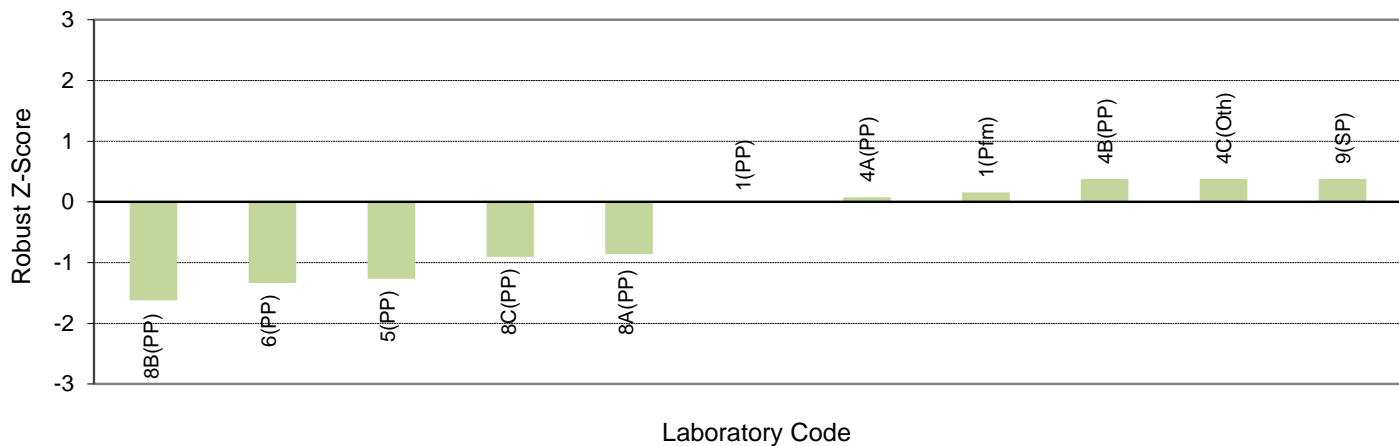
Statistic	Log ₁₀ PTA 1	Log ₁₀ PTA 2
Number of Results	11	11
Median	2.989	3.104
Normalised IQR	0.139	0.154
Uncertainty (Median)	0.053	0.058
Robust CV	4.7%	5.0%
Minimum	2.76	2.85
Maximum	3.04	3.41
Range	0.28	0.56

Notes:

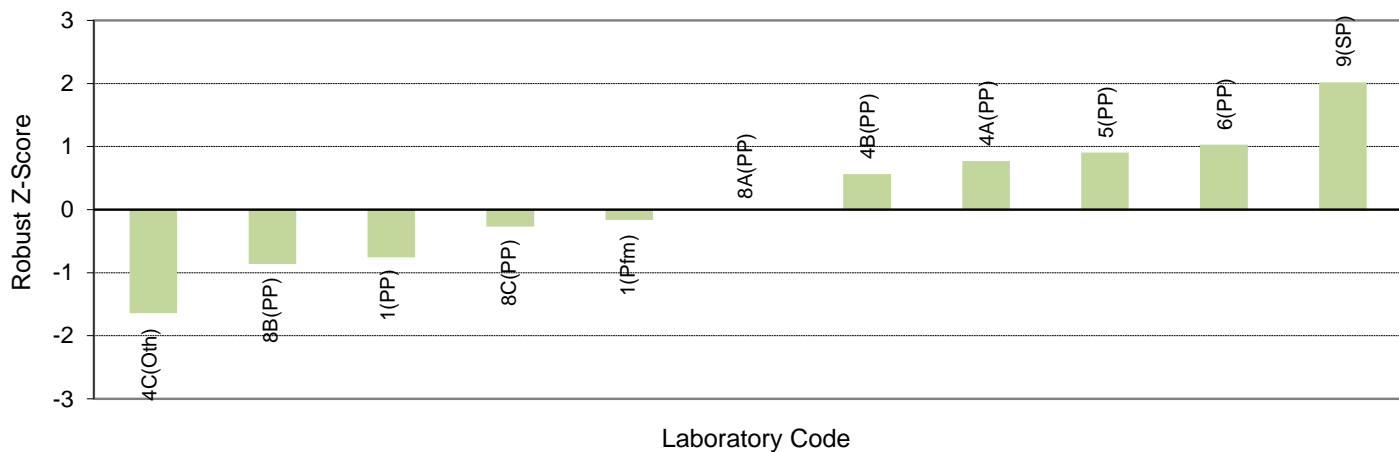
1. ^ denotes OGYE + supplement.
2. For the method abbreviations in the table above, PP= Pour Plate, SP = Spread Plate, Pfm = Petrifilm™ and Oth = Other.
3. The other method used by laboratory 4C for their Total Yeasts and Moulds results was the Tempo automated system.
4. All the methods were pooled when analysing the Total Yeasts and Moulds results.
5. The method used has been appended to the laboratory code on the ordered z-score charts on the following page.

A9.2

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



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



APPENDIX B

Homogeneity and Stability Testing

B1.1

Homogeneity Testing

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

Aerobic Plate Count (cfu/g)				
PTA 2				
Sample	Result A	Log ₁₀ A	Result B	Log ₁₀ B
86	9100	3.96	7800	3.89
449	6800	3.83	8000	3.90
525	9700	3.99	9400	3.97
554	7900	3.90	8000	3.90
619	9700	3.99	10000	4.00

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

Stability Testing

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

Aerobic Plate Count (cfu/g)				
PTA 2				
Sample	Result A	Log ₁₀ A	Result B	Log ₁₀ B
103	8000	3.90	8900	3.95
173	7500	3.88	8000	3.90
214	7800	3.89	8700	3.94

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 24, May 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 | • <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, 2,000 cfu/g Coagulase-positive *Staphylococci*, 8,000 *Bacillus cereus*, 3,000 cfu/g yeasts and moulds, and 15,000 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
--

All results should arrive at the above address by no later than **Tuesday 29 May 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.

PROFICIENCY TESTING AUSTRALIA
Non-Pathogens in Food Proficiency Testing Program
Round 24, May 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)						<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 24, May 2018
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-----