REPORT NO. 842

Non-Pathogens in Food
Proficiency Testing Program
Round 15

January 2014

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Ms S Mott, Global Proficiency Ltd (New Zealand). Also our thanks go to Mrs S Giannoulidis, Global Proficiency Pty Ltd (Australia), who arranged for the supply of the samples, and Global Proficiency Ltd (New Zealand) for the production of the samples.

PO Box 7507 Silverwater NSW 2128 AUSTRALIA

CONTENTS

1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the fifteenth of an ongoing series of rounds, involving the microbiological analysis of food samples for a range of non-pathogens.

Proficiency Testing Australia (PTA) conducted the exercise in October / November 2013. The aim of the program was to assess laboratories' ability to competently perform the nominated tests.

The Program Coordinator was Dr M Bunt. The Technical Adviser was Ms 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, one of which did not return results for inclusion in the final report.

(b) Documentation and Testing Methods

Laboratories were provided with two 25 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:

- Standard Plate Count (SPC)
- Coliforms
- *Escherichia coli* (*E. coli*)
- Yeasts
- 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 Sheet*, which was 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, ten 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 five sections (A1–A5). These sections contain the analysis of results reported by laboratories for Standard Plate Count, Coliforms, *E. coli*, 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;
iii) ordered z-score charts; and
iv) a Youden diagram.

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

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

4. STATISTICAL DESIGN OF THE PROGRAM

Samples PTA 1 and PTA 2 differed by up to half a log for Standard Plate Count, Coliforms, *E. coli*, Yeasts and Moulds. These samples have not been paired when analysed.

5. OUTLIER RESULTS

Robust z-scores have been used to assess each laboratory's testing performance. When calculated from single results, z-scores are used to detect excessively large or excessively small results in comparison to the consensus value (the median). Any result with an absolute z-score greater than or equal to three (*i.e.* ≤ -3.0 or ≥ 3.0) is classified as an outlier. For further details on the calculation and interpretation of robust z-scores, please see the *Guide to Proficiency Testing Australia (2012)*.

The following table summarises the results submitted by participants for the program.

Table A: Summary Statistics for All Tests

Test	Method	Summary Statistics	PTA 1	PTA 2
SPC (log_{10})	All Methods Pooled	Number of Results	12	12
		Median	3.981	3.914
		Normalised IQR	0.122	0.185
		Uncertainty (Median)	0.044	0.067
Coliforms (log_{10})	Pour Plate / Petrifilm™	Number of Results	11	11
		Median	2.699	2.663
		Normalised IQR	0.524	0.388
		Uncertainty (Median)	0.198	0.146
Yeasts (log_{10})	All Methods Pooled	Number of Results	11	11
		Median	2.699	2.778
		Normalised IQR	0.351	0.178
		Uncertainty (Median)	0.132	0.067
Moulds (log_{10})	All Methods Pooled	Number of Results	11	11
		Median	3.301	3.477
		Normalised IQR	0.160	0.192
		Uncertainty (Median)	0.061	0.072

Table B: Summary of Statistical Outliers and False / Unsatisfactory Results

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

Test	Method	Outliers		False Negative / Unsatisfactory Results
		PTA 1	PTA 2	
SPC	All Methods Pooled	6	-	-
Coliforms	MPN			-
	Pour Plate / Petrifilm™	-	8A	-
	Other			
E. coli	MPN			6 (×2)
	Pour Plate			-
	Petrifilm™			-
	Other			
Yeasts	All Methods Pooled	8B	-	-
Moulds	All Methods Pooled	-	-	-

Notes:

1. The results reported are for $\log_{10}$(cfu/g) and $\log_{10}$(MPN/g).
2. All the methods were pooled when analysing the SPC, Yeasts and Moulds results. A target CV was used to calculate the z-scores for the Yeasts results for sample PTA 1.
3. The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results. Summary statistics and z-scores were only calculated for the pooled Pour Plate and Petrifilm™ results for Coliforms. Target CVs were used to calculate the z-scores for the pooled Pour Plate and Petrifilm™ results for Coliforms.
4. Summary statistics and z-scores were not calculated for the *E. coli* results.
5. The uncertainty of the median was calculated as: $\sqrt{\frac{\pi}{2}} \times \frac{normIQR}{\sqrt{n}}$.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Round 15 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *E. coli* and a non-faecal coliform as the coliform organisms present in the sample, whereas sample PTA 2 contained *E. coli* only as the test organism for the coliform tests.

Included also were other bacterial species to contribute to the Standard Plate Count, but not interfere with the tests for the indicator organisms. A *Saccharomyces* species was incorporated as the yeast in each sample, and a species of *Penicillium* was used as the mould organism. Both Yeasts and Moulds were assessed separately.

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 / unsatisfactory 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

Of the nine laboratories that participated in the program, eight (89%) submitted results for inclusion in the final report. Of these eight laboratories, three (38%) submitted results where more than one method was used for a specific test, while five laboratories (63%) provided results for all five tests. The return rate for all tests is as follows:

• Standard Plate Count	8 out of 8	100%
• Coliforms	7 out of 8	88%
• *E. coli*	6 out of 8	75%
• Yeasts	7 out of 8	88%
• Moulds	7 out of 8	88%

6.2 Performance summary

One or more statistical outliers or unsatisfactory results were reported by two laboratories (25%) for this round of the Non-Pathogens in Food program. For comparison, 30% of the participants reported outlier results in Round 14 of the Non-Pathogens in Food program (see Report No. 821 for more details).

A total of 112 results were analysed in this round of the program. Of these results, five (4%) were identified as outliers or unsatisfactory results. For comparison, 5% of the results analysed in Round 14 of the Non-Pathogens in Food program were outliers (see Report No. 821 for more details).

6.3 Standard Plate Count

Of the eight laboratories that tested for Standard Plate Count, one laboratory tested using more than one method. Eight laboratories tested using Pour Plate, including two laboratories that submitted two sets of results. One laboratory tested using Petrifilm™ and submitted two sets of results.

All the methods were pooled when analysing the results.

The robust CVs of 3.07% and 4.74% for the results for this round are higher than the values of 2.79% and 2.08%, obtained for the results in Round 14 of this program, for samples containing the same organisms at similar levels (see Report No. 821).

Laboratory 6 (using the Pour Plate method) reported an outlier for sample PTA 1 for the submission of a high bias result. There were no outliers reported for sample PTA 2.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 4 of this report), which was calculated for each test and/or method within a test. For the SPC test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
SPC - All methods pooled	3.981 ± 0.044	3.914 ± 0.067

The Reproducibility MU for each sample ($\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
SPC - All methods pooled	0.27	0.38

Laboratories may use this MU data as a comparison to internal estimations.

Only one laboratory reported MUs associated with their test results in this round, as a percentage of the total result.

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

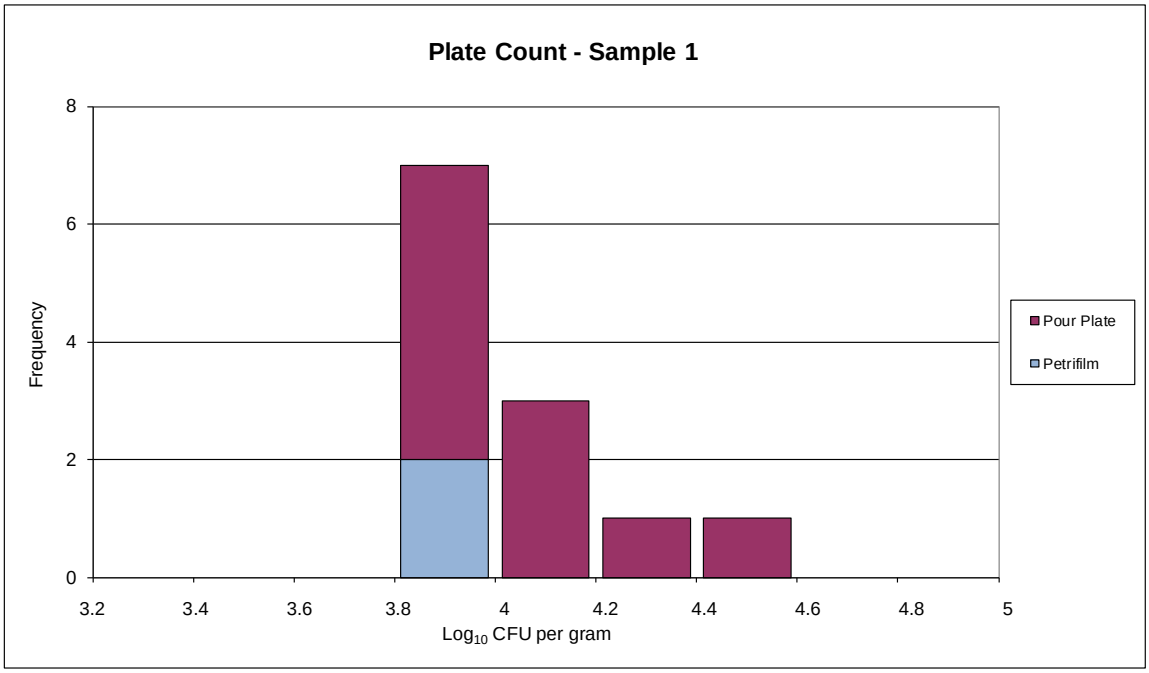


Figure TA-1. SPC $\log_{10}$ cfu/g results for sample PTA 1.

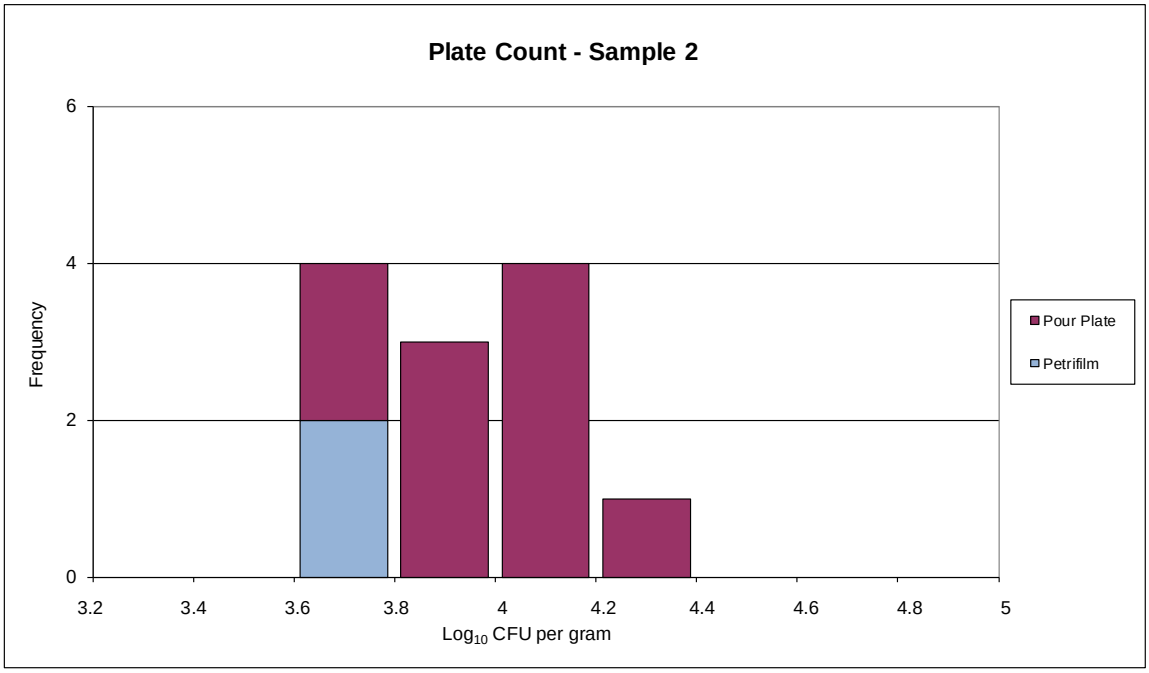


Figure TA-2. SPC $\log_{10}$ cfu/g results for sample PTA 2.

6.4 Coliforms

A total of seven laboratories submitted results for Coliforms. Three of these laboratories used more than one method. Three laboratories tested using MPN. Six laboratories tested using Pour Plate, including two laboratories that submitted two sets of results. Two laboratories tested using the Petrifilm™ method, including one laboratory that submitted two sets of results. The Pour Plate and Petrifilm™ results were pooled for analysis. There were not enough Coliforms results reported using methods other than Pour Plate or Petrifilm™ to analyse.

For the pooled Pour Plate / Petrifilm™ results, the robust CVs for the samples were 19.43% and 14.56%. These were considered too high to evaluate the performance of the participants in this round and a target CV of 10.00% was chosen to calculate the z-scores for both samples.

For the pooled Pour Plate / Petrifilm™ results, laboratory 8A (using the Pour Plate method) reported an outlier for sample PTA 2. There were no outliers reported for sample PTA 1.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 4 of this report), which was calculated for each test and/or method within a test. For the Coliforms via Pour Plate / Petrifilm™ test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Coliforms - Pour Plate / Petrifilm™	2.699 ± 0.198	2.663 ± 0.146

The Reproducibility MU for each sample ($\log_{10}$ cfu/g) was as follows (outliers removed):

	PTA 1	PTA 2
Coliforms - Pour Plate / Petrifilm™	0.89	0.71

Laboratories may use this MU data as a comparison to internal estimations.

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

Graphs showing the differentiation of methods used for Coliforms testing are included in Figures TA-3 and TA-4 below. These graphs show the distribution of results from all methods used in this round and are included for interest purposes only. Of note, are the wide spread of results for the Pour plate methods on both samples, however the smaller data sets also need consideration when interpreting results.

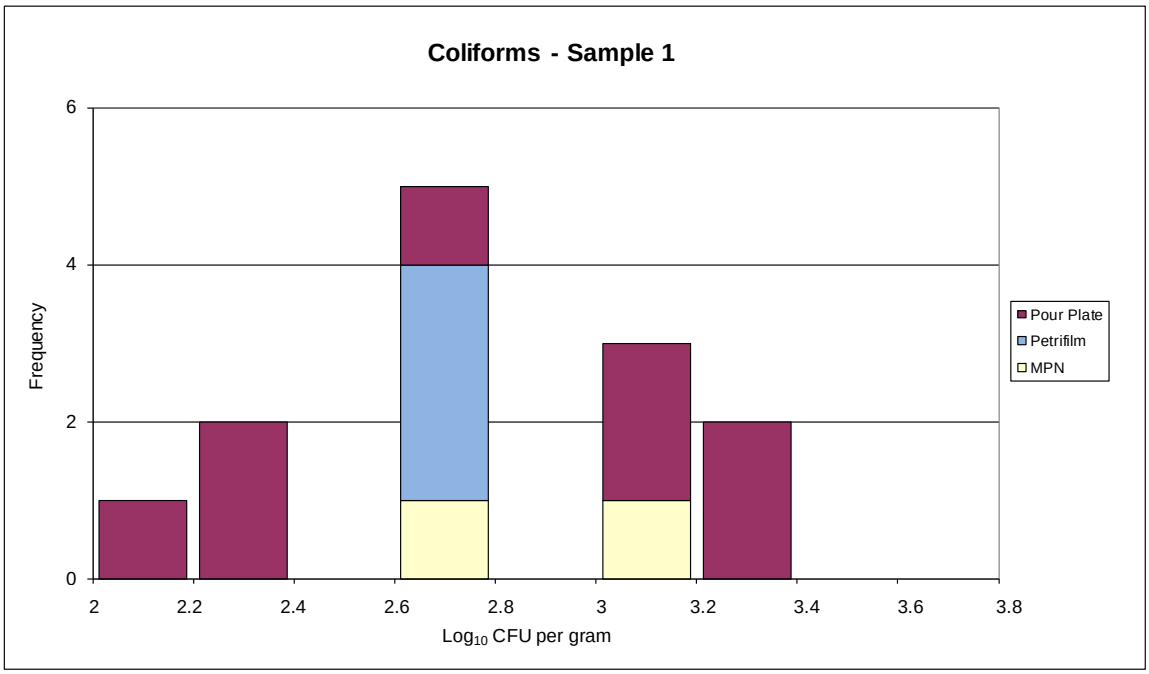


Figure TA-3. Coliforms $\log_{10}$ cfu/MPN/g results for sample PTA 1.

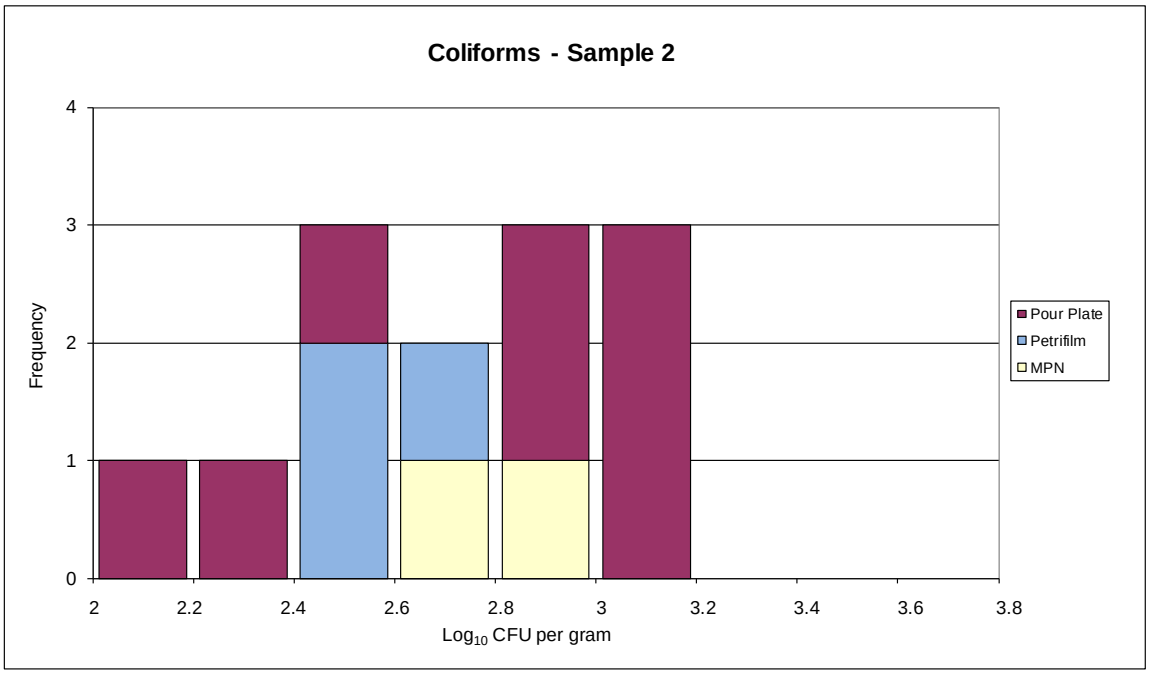


Figure TA-4. Coliforms $\log_{10}$ cfu/MPN/g results for sample PTA 2.

6.5 *E. coli*

Of the six laboratories that submitted results for *E. coli*, one laboratory tested using more than one method. Five laboratories tested using MPN. Two laboratories tested using Pour Plate. One laboratory tested using the Petrifilm™ method. There were not enough *E. coli* results reported using any method to analyse separately.

Laboratory 6 reported MPN results of 0.11 MPN/g and 0.07 MPN/g for samples PTA 1 and PTA 2, respectively. This laboratory was contacted and they confirmed that their results had been recorded correctly. These results cannot possibly be whole counts and they have therefore been analysed as unsatisfactory results.

None of the laboratories reported MUs associated with their test results in this round, although one laboratory did report a confidence limit however this was one value, and not a range as would be expected.

Graphs showing the differentiation of methods used for *E. coli* testing have not been included as the round could not be assessed due to insufficient results.

6.6 Yeasts

A total of seven laboratories submitted results for Yeasts. One of these laboratories tested using more than one method. Seven laboratories tested using Pour Plate, including two laboratories that submitted two sets of results. One laboratory tested using the Petrifilm™ method and submitted two sets of results.

One laboratory reported using AS 5013.29-2009 for their testing. Three laboratories used ISO 6611-2004. Three laboratories used other methods.

All the methods were pooled when analysing the results.

The robust CVs for this round are 12.99% and 6.40% for sample PTA 1 and sample PTA 2, respectively. As the robust CV for sample PTA 1 is considerably higher than the robust CV for sample PTA 2, and is higher than the CVs of previous rounds for Yeasts, a target CV was used to calculate the robust z-scores for sample PTA 1. The target value of the CV used was 6.40%, the same as the robust CV obtained for sample PTA 2.

Laboratory 8B (using the Petrifilm™ method) reported an outlier for sample PTA 1. There were no outliers reported for sample PTA 2.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 4 of this report), which was calculated for each 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 - All methods pooled	2.699 ± 0.132	2.778 ± 0.067

The Reproducibility MU for each sample for the pooled results ($\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Yeasts - All methods pooled	0.66	0.45

Laboratories may use this MU data as a comparison to internal estimations.

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

Graphs showing the differentiation of methods used for Yeasts testing are included in Figures TA-5 and TA-6 below. These graphs show the distribution of results from the methods used in this round and are included for interest purposes only.

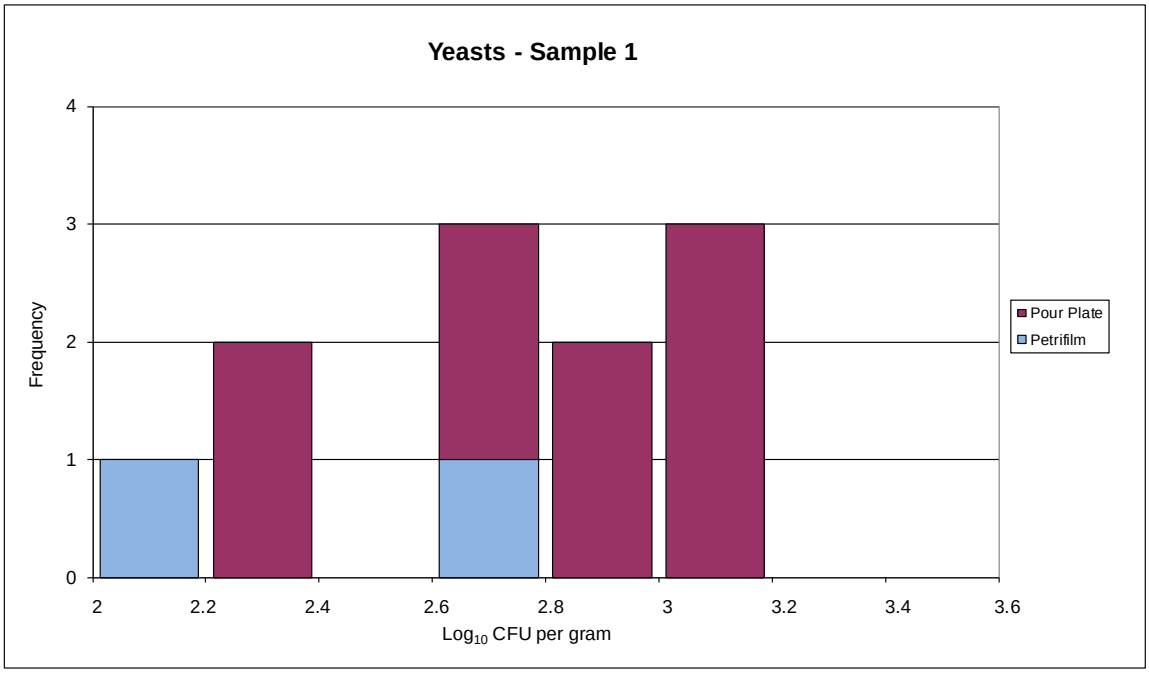


Figure TA-5. Yeasts $\log_{10}$ cfu/g results for sample PTA 1.

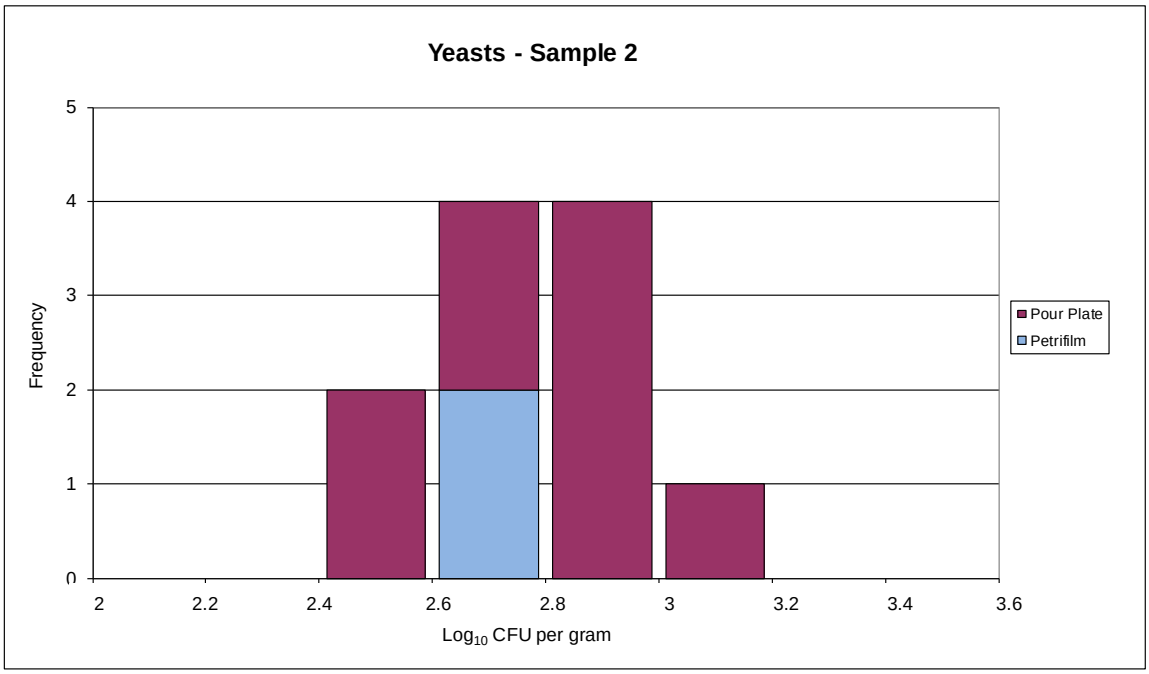


Figure TA-6. Yeasts $\log_{10}$ cfu/g results for sample PTA 2.

6.7 Moulds

Of the seven laboratories that submitted results for Moulds, one laboratory tested using more than one method. Seven laboratories tested using Pour Plate, including two laboratories that submitted two sets of results. One laboratory tested using the Petrifilm™ method and submitted two sets of results.

One laboratory reported using AS 5013.29-2009 for their testing. Three laboratories used ISO 6611-2004. Three laboratories used other methods.

All the methods were pooled when analysing the results.

The robust CVs of 4.85% and 5.51% for this round are higher than the values of 2.57% and 2.87%, obtained in Round 14 of this program, for samples containing the same organisms at similar levels (see Report No. 821).

There were no outliers reported for either sample.

Confidence in the medians can be expressed as the uncertainty of the median (as defined in page 4 of this report), which was calculated for each test. For the Moulds test, the median and associated standard error (se) for each sample (expressed in $\log_{10}$ cfu/g) was as follows:

	PTA 1	PTA 2
Moulds - All methods pooled	3.301 ± 0.061	3.477 ± 0.072

The Reproducibility MU for each sample for the pooled results ($\log_{10}$ cfu/g) was as follows (outliers removed):

	PTA 1	PTA 2
Moulds - All methods pooled	0.31	0.32

Laboratories may use this MU data as a comparison to internal estimations.

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

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

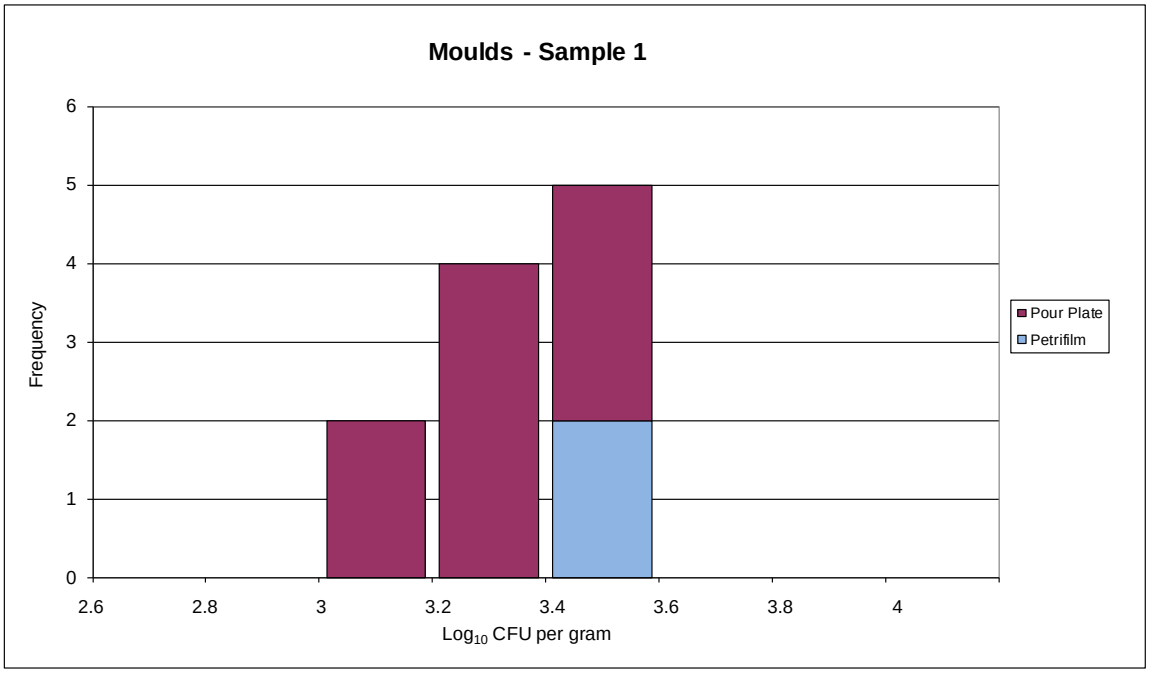


Figure TA-7. Moulds $\log_{10}$ cfu/g results for sample PTA 1.

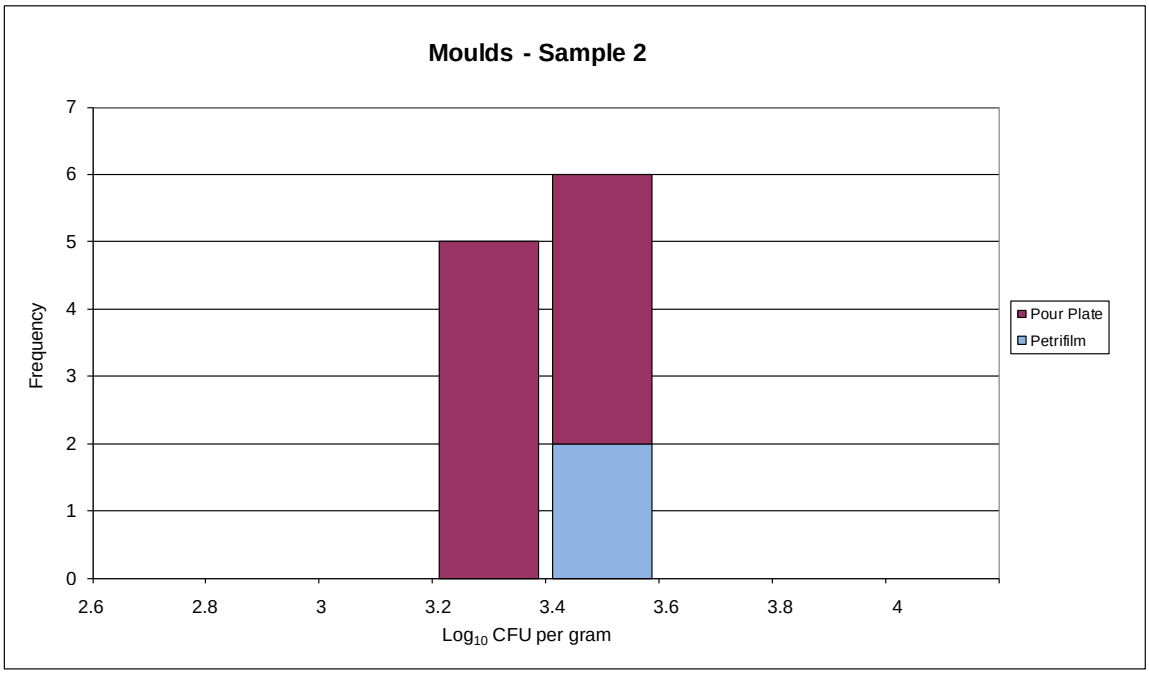


Figure TA-8. Moulds $\log_{10}$ cfu/g results for sample PTA 2.

7. REFERENCES

1. *Guide to Proficiency Testing Australia (2012)*. (This document is located on the PTA website at www.pta.asn.au under Programs / Documents).
2. *AS 5013.3 (2009) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - Most probable number technique.*
3. *AS 5013.4 (2009) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms – Colony-count technique.*
4. *AS 5013.5 (2004) Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony count technique at 30C.*
5. *AS 5013.9 (2009) Food microbiology - Examination for specific organisms - Coliforms and Escherichia coli by the triplicate tube detection method.*
6. *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.*
7. *AS 5013.29 (2009) Food microbiology - Examination for specific organisms - Colony count of yeasts and moulds.*
8. *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*.
9. *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*.

APPENDIX A

Summary of Results

Section A1

Standard Plate Count

A1.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2	
2	18000	4.26	-	8400	3.92	-	2.24	0.06	PP
3	13000	4.11	-	11000	4.04	-	1.08	0.69	PP
4	8600	3.93	-	11000	4.04	-	-0.38	0.69	PP
5A	12900	4.11	-	11100	4.05	-	1.06	0.71	PP
5B	13700	4.14	-	11400	4.06	-	1.27	0.77	PP
6	30000	4.48	-	8000	3.90	-	4.05 §	-0.06	PP
7	7600	3.88	3.32%	7250	3.86	3.32%	-0.82	-0.29	PP
8A	9765	3.99	-	4625	3.67	-	0.07	-1.34	PP
8A	7470	3.87	-	4500	3.65	-	-0.88	-1.41	Pfm
8B	9150	3.96	-	6250	3.80	-	-0.16	-0.64	PP
8B	9400	3.97	-	6050	3.78	-	-0.07	-0.71	Pfm
9	9200	3.96	-	17000	4.23	-	-0.14	1.71	PP

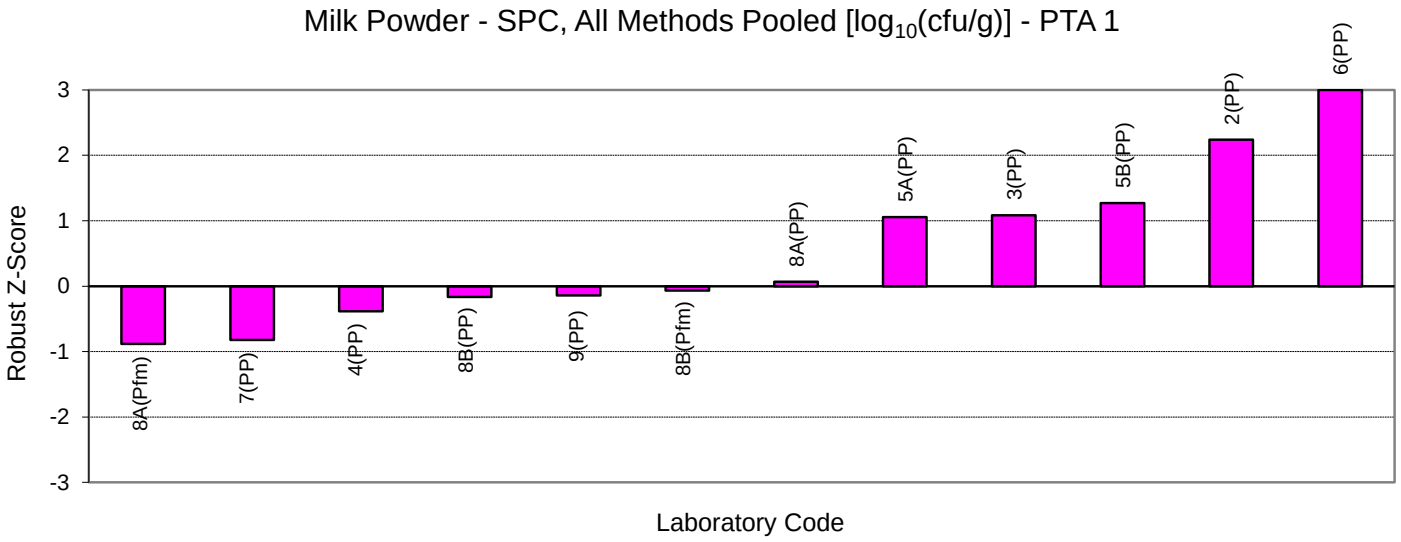
Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	12	12
Median	3.981	3.914
Normalised IQR	0.122	0.185
Uncertainty (Median)	0.044	0.067
Robust CV	3.07%	4.74%
Minimum	3.87	3.65
Maximum	4.48	4.23
Range	0.60	0.58

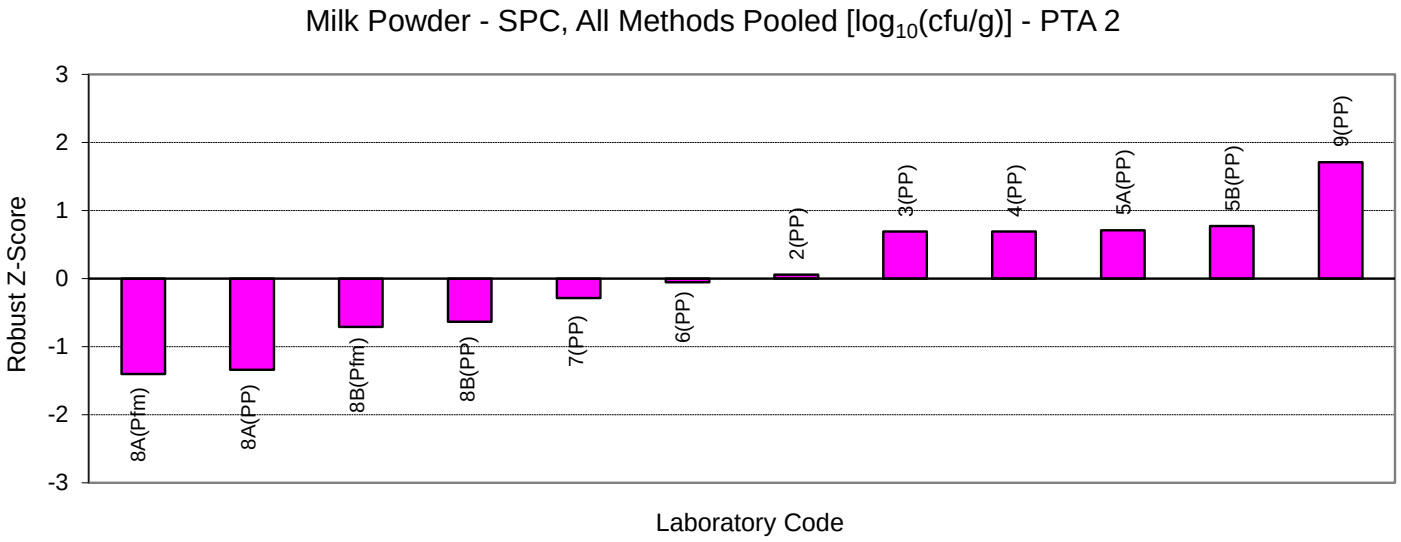
Method	Number of Results
PP = Pour Plate	10
Pfm = Petrifilm™	2
Oth = Other	0

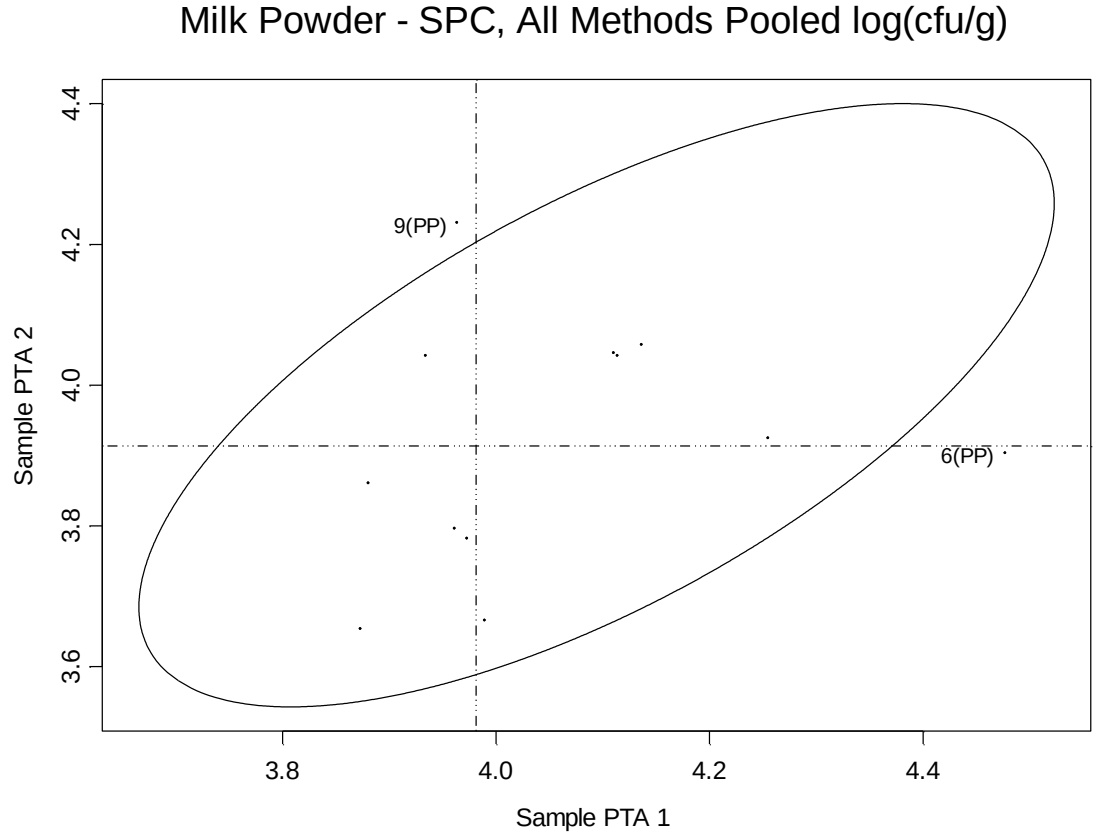
Notes:

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. All the methods were pooled when analysing the SPC results.
3. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
4. The Youden diagram on the following page is provided for information only.

A1.2


Milk Powder - SPC, All Methods Pooled [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
8A(Pfm)
7(PP)
4(PP)
8B(PP)
9(PP)
8B(Pfm)
8A(PP)
5A(PP)
3(PP)
5B(PP)
2(PP)
6(PP)
Laboratory Code


Milk Powder - SPC, All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
8A(Pfm)
8A(PP)
8B(Pfm)
8B(PP)
7(PP)
6(PP)
2(PP)
3(PP)
4(PP)
5A(PP)
5B(PP)
9(PP)
Laboratory Code


Milk Powder - SPC, All Methods Pooled log(cfu/g)
Sample PTA 2
4.4
4.2
4.0
3.8
3.6
9(PP)
6(PP)
3.8
4.0
4.2
4.4
Sample PTA 1

Section A2

Coliforms

A2.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2	
3	1400	3.15	-	690	2.84	-	1.66	0.66	PP
4	510	2.71	-	270	2.43	-	0.03	-0.87	PP
4	410	2.61	-	460	2.66	-	-0.32	0.00	Pfm
5A	1810	3.26	-	1230	3.09	-	2.07	1.60	PP
5B	1720	3.24	-	1410	3.15	-	1.99	1.83	PP
7	1560	3.19	-	750	2.88	-	1.83	0.80	PP
8A	150	2.18	-	50	1.70	-	-1.94	-3.62 **§**	PP
8A	450	2.65	-	250	2.40	-	-0.17	-0.99	Pfm
8B	205	2.31	-	200	2.30	-	-1.43	-1.36	PP
8B	500	2.70	-	275	2.44	-	0.00	-0.84	Pfm
9	200	2.30	-	1000	3.00	-	-1.47	1.27	PP

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	11	11
Median	2.699	2.663
Normalised IQR	0.524	0.388
Uncertainty (Median)	0.198	0.146
Robust CV	19.43%	14.56%
Target CV	10.00%	10.00%
Minimum	2.18	1.70
Maximum	3.26	3.15
Range	1.08	1.45

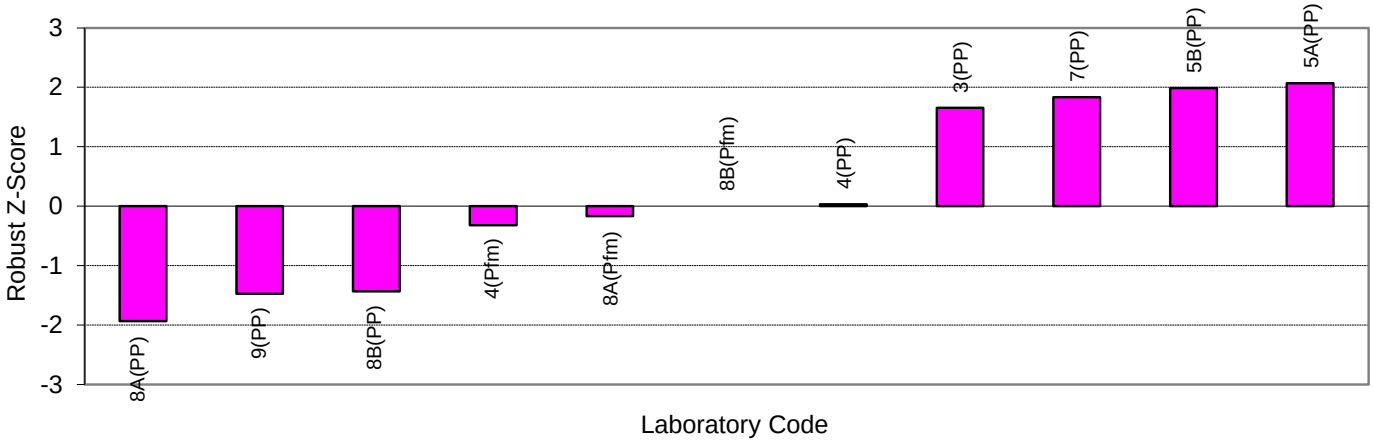
Method	Number of Results
PP = Pour Plate	8
Pfm = Petrifilm™	3

Notes:

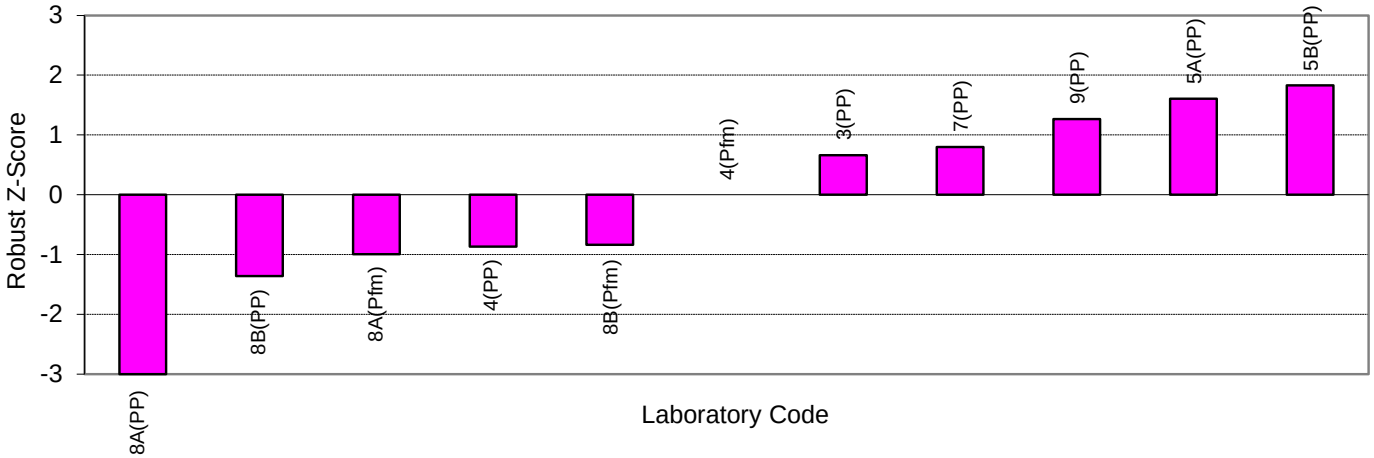
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
3. A target CV of 10% was used to calculate the robust z-scores for both samples.
4. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
5. The Youden diagram on the following page is provided for information only.

A2.2

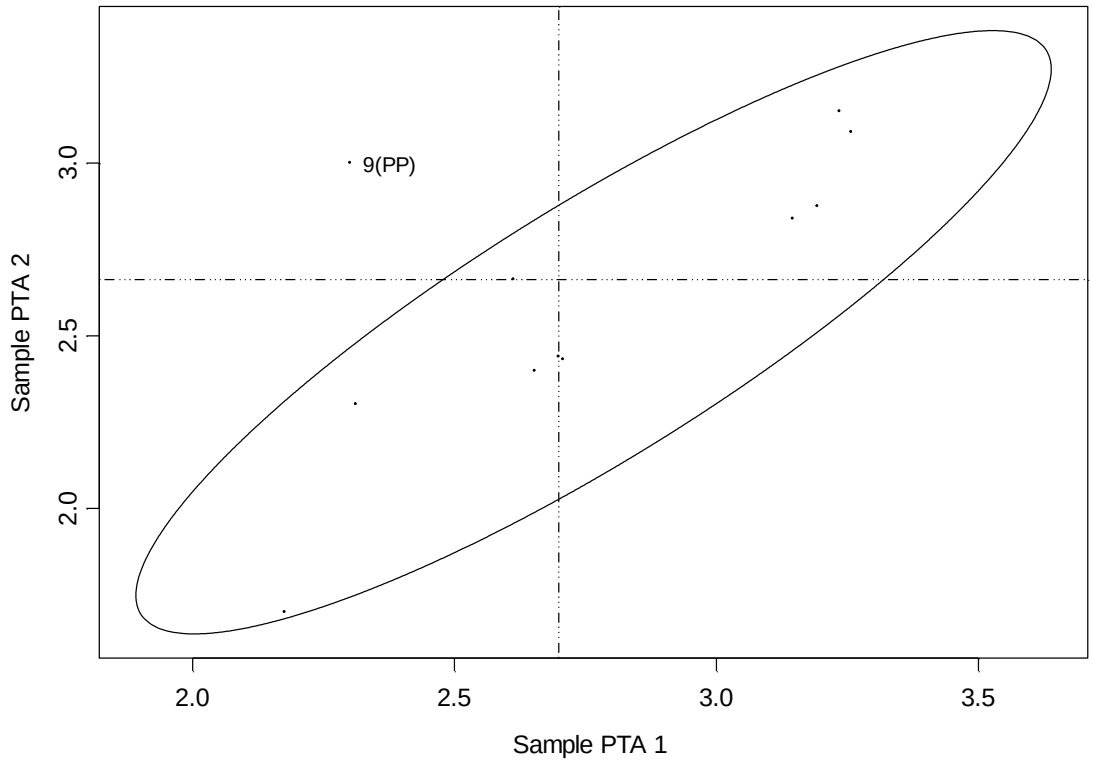
Milk Powder - Coliforms, Pour Plate / Petrifilm [$\log_{10}$(cfu/g)] - PTA 1



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



Milk Powder - Coliforms, Pour Plate / Petrifilm log(cfu/g)



A2.3

Milk Powder – Coliforms, MPN and Other Methods (MPN/g, cfu/g)

Lab Code	PTA 1			PTA 2			Method
	Result	Log_{10}	MU	Result	Log_{10}	MU	
4	430	2.63	-	930	2.97	-	MPN
6	> 11.0	-	-	> 11.0	-	-	MPN
7	1100	3.04	-	460	2.66	-	MPN

Note:

1. There were not enough Coliforms results reported using methods other than Pour Plate or Petrifilm™ to calculate summary statistics or z-scores.

Section A3

E. coli

A3.1

Milk Powder – *E. coli*, All Methods (MPN/g, cfu/g)

Lab Code	PTA 1			PTA 2			Method
	Result	Log_{10}	MU	Result	Log_{10}	MU	
2	910	2.96	-	6600	3.82	-	Pour Plate
3	360	2.56	-	740	2.87	-	MPN
4	430	2.63	-	930	2.97	-	MPN
4	210	2.32	-	220	2.34	-	Pour Plate
4	280	2.45	-	460	2.66	-	Petrifilm™
6	0.11 #	-0.96	-	0.07 #	-1.15	-	MPN
7	1100	3.04	-	460	2.66	-	MPN
9	290	2.46	CL: 90	160	2.20	CL: 40	MPN

Notes:

1. There were not enough *E. coli* results reported using any method to calculate summary statistics or z-scores.
2. # denotes an unsatisfactory result.
3. CL denotes “confidence limit”.

Section A4

Yeasts

A4.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	AS / ISO / Other
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
3	1400	3.15	-	600	2.78	-	2.59	0.00	1	PP
4	170	2.23	-	290	2.46	-	-2.71	-1.78	2	PP
5A	650	2.81	-	830	2.92	-	0.66	0.79	2	PP
5B	830	2.92	-	980	2.99	-	1.27	1.20	2	PP
6	1000	3.00	-	800	2.90	-	1.74	0.70	3	PP
7	1000	3.00	-	1390	3.14	-	1.74	2.05	3	PP
8A	400	2.60	-	600	2.78	-	-0.56	0.00	2	PP
8A	500	2.70	-	550	2.74	-	0.00	-0.21	2	Pfm
8B	235	2.37	-	355	2.55	-	-1.90	-1.28	2	PP
8B	150	2.18	-	400	2.60	-	-3.03 **§**	-0.99	2	Pfm
9	500	2.70	-	800	2.90	-	0.00	0.70	3	PP

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	11	11
Median	2.699	2.778
Normalised IQR	0.351	0.178
Uncertainty (Median)	0.132	0.067
Robust CV	12.99%	6.40%
Target CV	6.40%	6.40%
Minimum	2.18	2.46
Maximum	3.15	3.14
Range	0.97	0.68

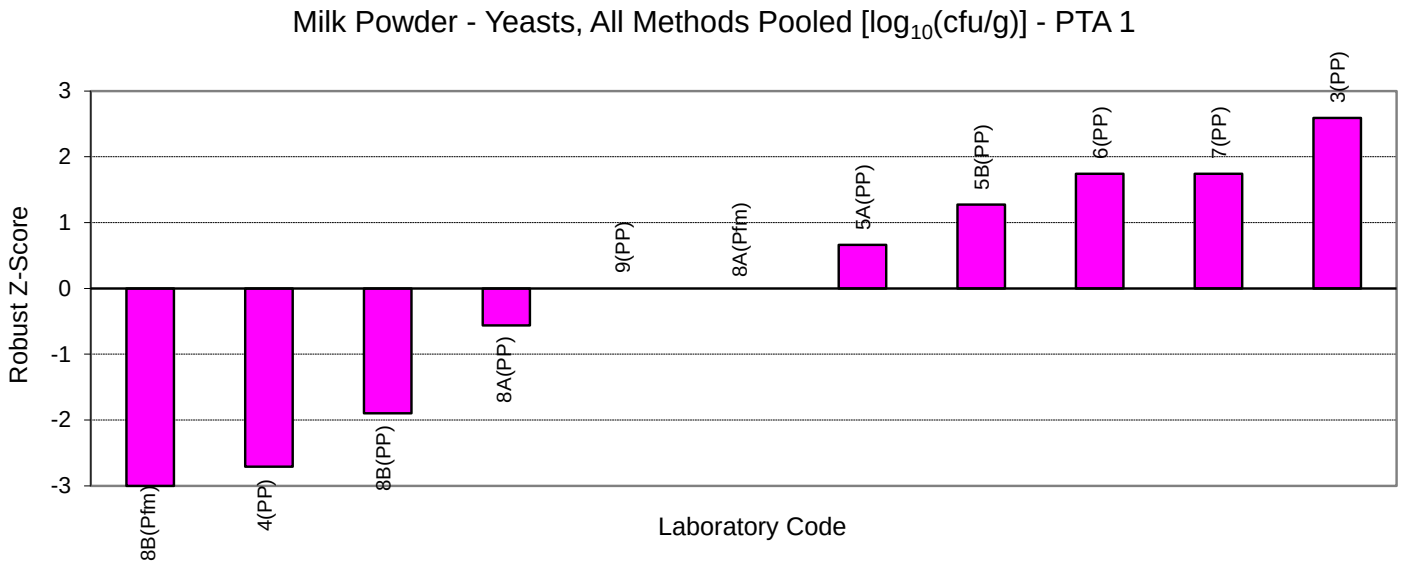
Method	Number of Results
PP = Pour Plate	9
SP = Spread Plate	0
Pfm = Petrifilm™	2

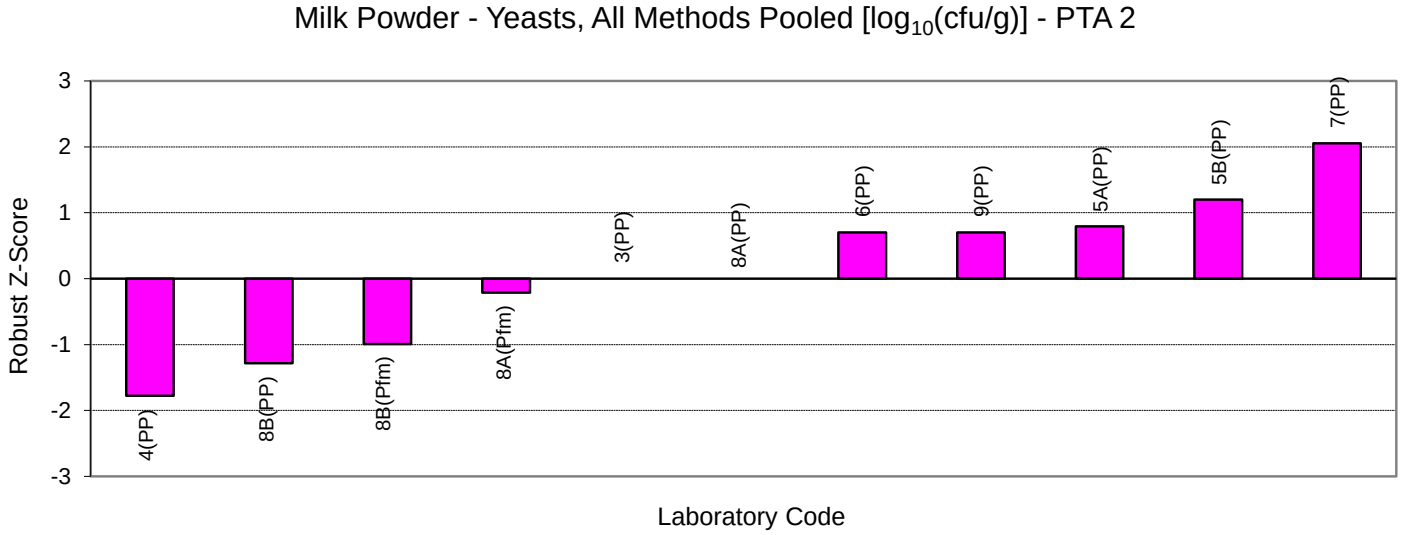
AS / ISO / Other Code	No. of Laboratories
1 = AS 5013.29-2009	1
2 = ISO 6611-2004	3
3 = Other	3

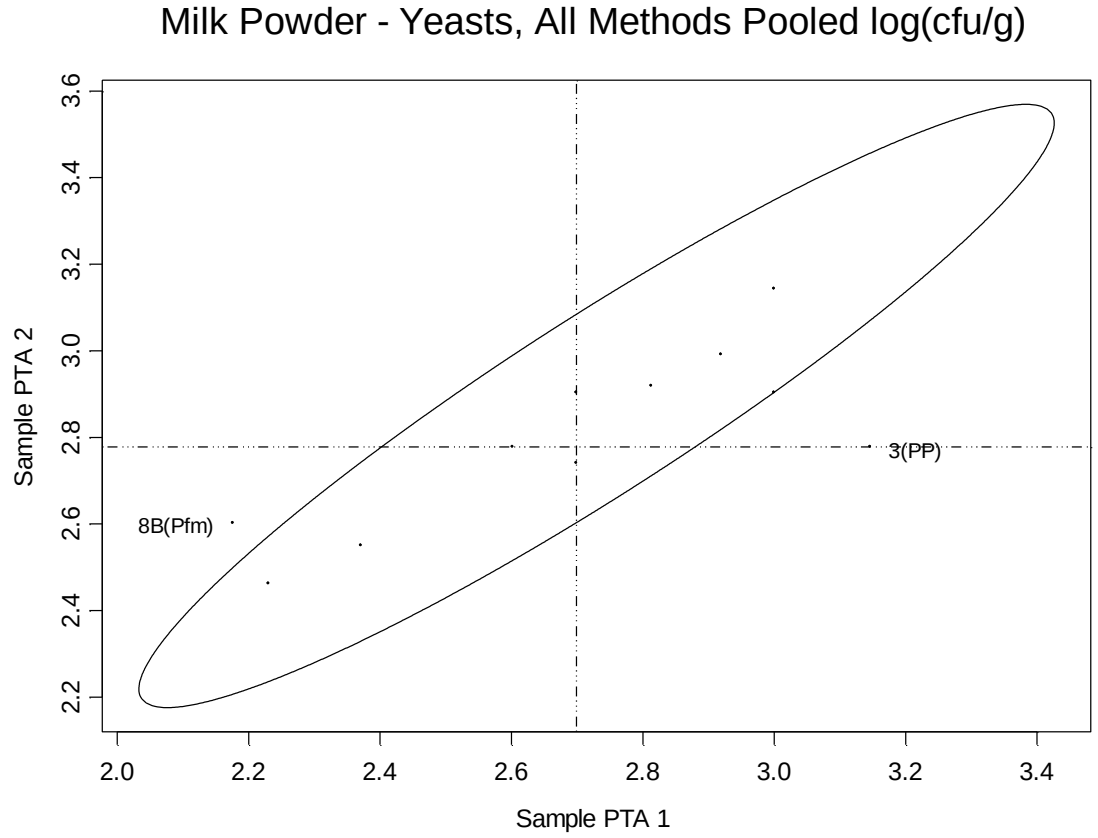
Notes:

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. All the methods were pooled when analysing the Yeasts results.
3. A target CV was used to calculate the robust z-scores for sample PTA 1. The target value of the CV used was the same as the robust CV obtained for sample PTA 2.
4. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
5. The Youden diagram on the following page is provided for information only.

A4.2


Milk Powder - Yeasts, All Methods Pooled [log10(cfu/g)] - PTA 1
Robust Z-Score
3
2
1
0
-1
-2
-3
8B(Pfm)
4(PP)
8B(PP)
8A(PP)
9(PP)
8A(Pfm)
5A(PP)
5B(PP)
6(PP)
7(PP)
3(PP)
Laboratory Code


Milk Powder - Yeasts, All Methods Pooled [log10(cfu/g)] - PTA 2
Robust Z-Score
3
2
1
0
-1
-2
-3
4(PP)
8B(PP)
8B(Pfm)
8A(Pfm)
3(PP)
8A(PP)
6(PP)
9(PP)
5A(PP)
5B(PP)
7(PP)
Laboratory Code


Milk Powder - Yeasts, All Methods Pooled log(cfu/g)
Sample PTA 2
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
8B(Pfm)
3(PP)
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
Sample PTA 1

Section A5

Moulds

A5.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method	AS / ISO / Other
	Result	Log_{10}	MU	Result	Log_{10}	MU	PTA 1	PTA 2		
3	2800	3.45	-	3800	3.58	-	0.91	0.54	1	PP
4	1600	3.20	-	1700	3.23	-	-0.61	-1.29	2	PP
5A	1520	3.18	-	1680	3.23	-	-0.74	-1.31	2	PP
5B	1460	3.16	-	1640	3.21	-	-0.85	-1.37	2	PP
6	2000	3.30	-	3000	3.48	-	0.00	0.00	3	PP
7	1780	3.25	-	2190	3.34	-	-0.32	-0.71	3	PP
8A	2650	3.42	-	3500	3.54	-	0.76	0.35	2	PP
8A	3850	3.59	-	3000	3.48	-	1.78	0.00	2	Pfm
8B	2000	3.30	-	3500	3.54	-	0.00	0.35	2	PP
8B	2750	3.44	-	3500	3.54	-	0.86	0.35	2	Pfm
9	2800	3.45	-	2200	3.34	-	0.91	-0.70	3	PP

Statistic	**Log_{10} PTA 1**	**Log_{10} PTA 2**
Number of Results	11	11
Median	3.301	3.477
Normalised IQR	0.160	0.192
Uncertainty (Median)	0.061	0.072
Robust CV	4.85%	5.51%
Minimum	3.16	3.21
Maximum	3.59	3.58
Range	0.42	0.36

Method	Number of Results
PP = Pour Plate	9
SP = Spread Plate	0
Pfm = Petrifilm™	2

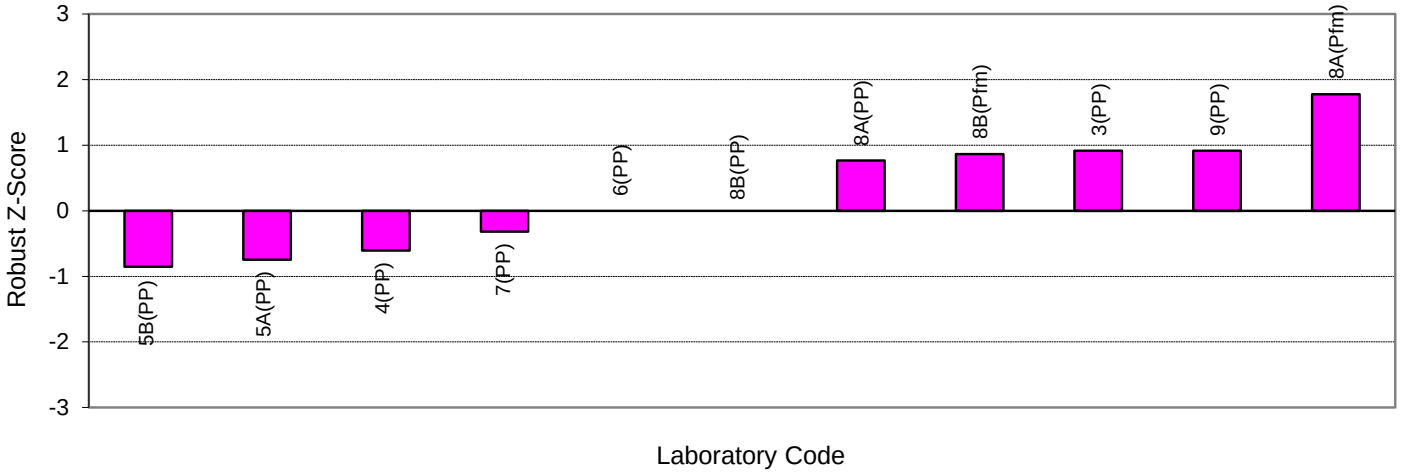
AS / ISO / Other Code	No. of Laboratories
1 = AS 5013.29-2009	1
2 = ISO 6611-2004	3
3 = Other	3

Notes:

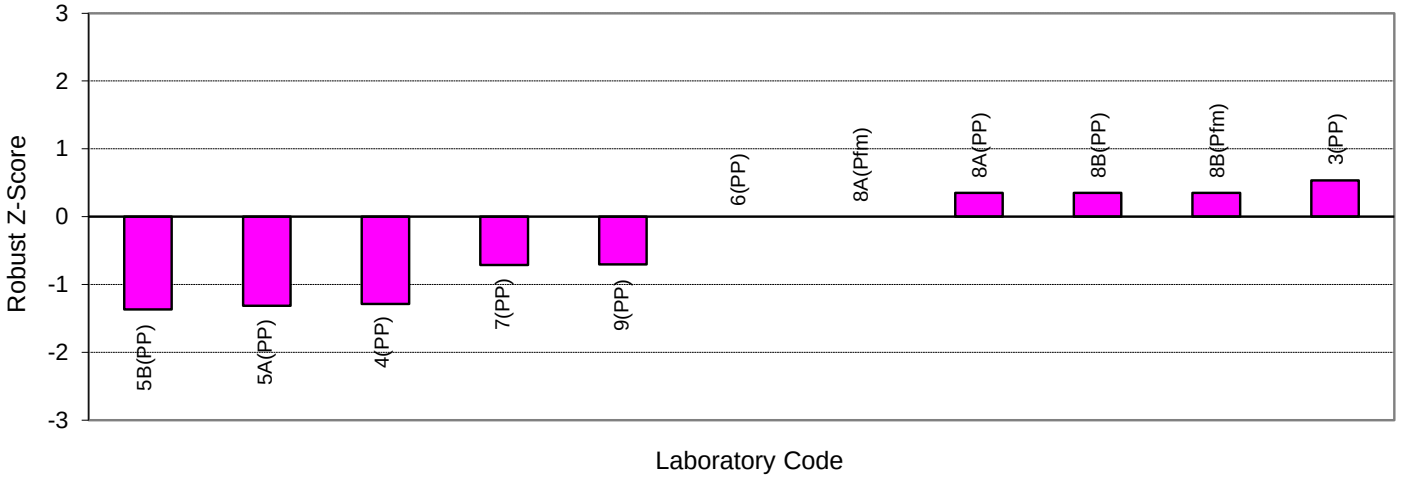
1. All the methods were pooled when analysing the Moulds results.
2. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
3. The Youden diagram on the following page is provided for information only.

A5.2

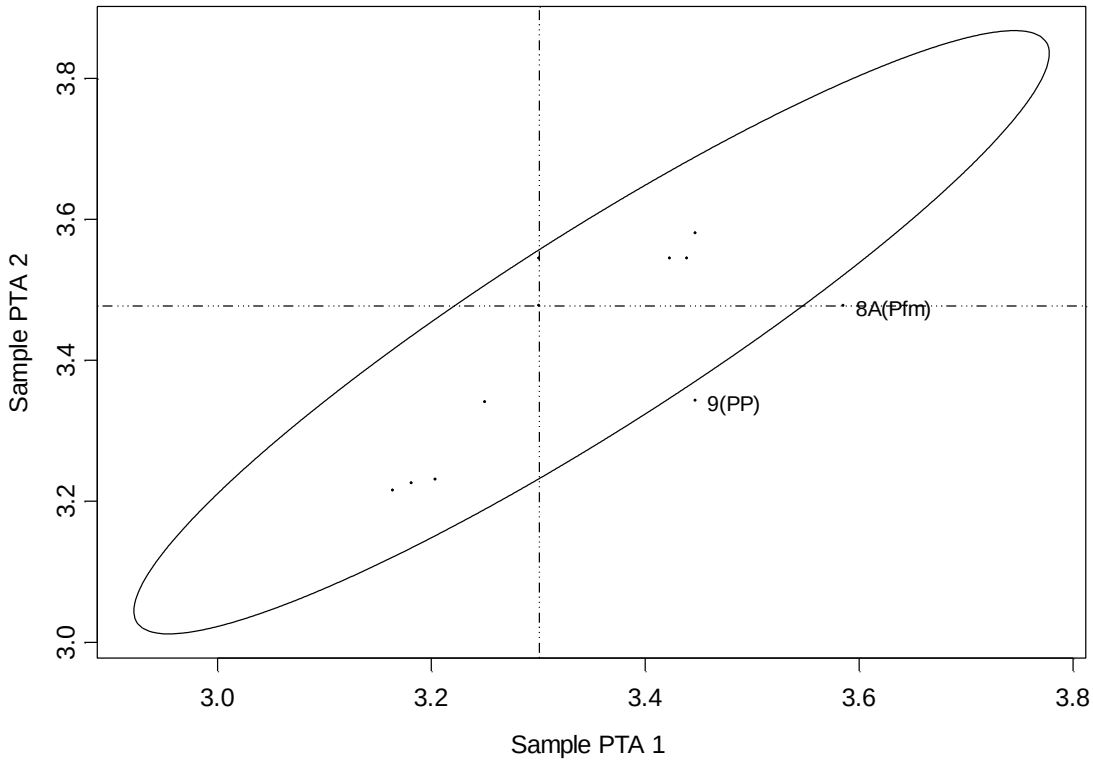
Milk Powder - Moulds, All Methods Pooled [log_{10}(cfu/g)] - PTA 1



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



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



APPENDIX B

Homogeneity and Stability Testing

HOMOGENEITY TESTING

Samples from PTA 1, chosen at random, were retained for homogeneity testing by Global Proficiency Ltd (New Zealand). These samples were tested for Standard Plate Count, Yeasts and Moulds.

Standard Plate Count

The samples from PTA 1 were tested in duplicate for Standard Plate Count using 0.1 mL volumes spread plated onto Plate Count agar with incubation at 30 °C for 24-48 hours. The results of this homogeneity testing appear in the following table.

Standard Plate Count (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
14	8700	3.94	11000	4.04
25	9500	3.98	9600	3.98
33	8400	3.92	10000	4.00
45	9400	3.97	11000	4.04
64	7100	3.85	8000	3.90
107	11000	4.04	10000	4.00
113	7900	3.90	9500	3.98
116	8900	3.95	9500	3.98
118	9800	3.99	10000	4.00
119	9500	3.98	10000	4.00

Yeasts

The samples from PTA 1 were tested in duplicate for Yeasts using 0.1 mL volumes spread plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBCA) with incubation at 20-25 °C for 5 days. The results of this homogeneity testing appear in the following table.

Yeasts (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
14	1200	3.08	1600	3.20
25	1200	3.08	1400	3.15
33	1200	3.08	1700	3.23
45	1000	3.00	800	2.90
64	1200	3.08	1700	3.23
107	1000	3.00	1000	3.00
113	1700	3.23	1200	3.08
116	1200	3.08	1200	3.08
118	800	2.90	1200	3.08
119	900	2.95	1100	3.04

Moulds

The samples from PTA 1 were tested in duplicate for Moulds using 0.1 mL volumes spread plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBCA) with incubation at 20-25 °C for 5 days. The results of this homogeneity testing appear in the following table.

Moulds (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
14	3400	3.53	3400	3.53
25	3800	3.58	3300	3.52
33	3800	3.58	3800	3.58
45	3800	3.58	4000	3.60
64	4200	3.62	3600	3.56
107	4200	3.62	3400	3.53
113	3300	3.52	3400	3.53
116	3800	3.58	3500	3.54
118	3800	3.58	3900	3.59
119	4200	3.62	4600	3.66

Comments on the Homogeneity Testing

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 negatives cannot be attributed to sample variability.

STABILITY TESTING

Three sets of 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 Standard Plate Count, Yeasts and Moulds and were tested 3 days after dispatch.

Standard Plate Count

The samples from PTA 1 were tested in duplicate for Standard Plate Count using 0.1 mL volumes spread plated onto Plate Count agar with incubation at 30 °C for 24-48 hours. The results of this stability testing appear in the following table.

Standard Plate Count (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
35	8500	3.93	8500	3.93
48	8300	3.92	8800	3.94
81	7700	3.89	8900	3.95

Yeasts

The samples from PTA 1 were tested in duplicate for Yeasts using 0.1 mL volumes spread plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBCA) with incubation at 20-25 °C for 5 days. The results of this stability testing appear in the following table.

Yeasts (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
35	550	2.74	400	2.60
48	500	2.70	450	2.65
81	650	2.81	500	2.70

Moulds

The samples from PTA 1 were tested in duplicate for Moulds using 0.1 mL volumes spread plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBCA) with incubation at 20-25 °C for 5 days. The results of this stability testing appear in the following table.

Moulds (cfu/g)				
PTA 1				
Sample	Result A	Log_{10} A	Result B	Log_{10} B
35	3300	3.52	3100	3.49
48	3200	3.51	3200	3.51
81	3700	3.57	3500	3.54

Comments on the Stability Testing

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 Sheet

PROFICIENCY TESTING AUSTRALIA

Non-Pathogens in Food
Proficiency Testing Program
Round 15, October 2013

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. 25 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:

- Standard Plate Count
- Coliforms
- *E. coli*
- Yeasts
- Moulds

❖ 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. Samples may contain up to 5,000 cfu/g coliforms, 2,000 cfu/g *E. coli*, 5,000 cfu/g yeasts, 5,000 cfu/g moulds and 30,000 cfu/g aerobic mesophilic organisms per gram. **Results should not be reported as "greater than …." or "less than ….", as such data cannot be statistically analysed**.

❖ Laboratories are encouraged to use the methods listed in the ***Results Sheet***. For each of the tests being performed, the laboratory may report results for as many of the methods listed as desired.

❖ Laboratories may use methods other than those listed for each test if they wish. Results using other methods are to be reported in the blank row included for each test. The method used should be clearly written in the ***Method*** column of the ***Results Sheet***.

- 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 ± value in log format (preferred), a range if reported in standard form, e.g. 7.5×10^3 cfu/g or confidence limits if MPN tables are used.

Instructions

You have been supplied with freeze dried vials and accompanying whole milk powder matrices in foil laminate sachets. Please find below instructions for the re-hydration and preparation of the freeze-dried vials and steps for the preparation of the matrix.

1. Re-hydrate the freeze-dried vials by adding 3.0 mL of sterile diluent (*e.g.* 0.1% (w/v) peptone and 0.85% (w/v) NaCl (ISO 6887-1)) at room temperature.

2. Allow standing at room temperature for 10 minutes.

3. Mix the vial contents using a vortex mixer for 15 seconds.

4. Aseptically open the sachets. Weigh out 10 g for each sample. Add 90 mL diluent. Mix to dissolve the milk powder. Add 1 mL of the rehydrated vial contents and homogenize/mix. This is now your prepared homogenate, i.e. simulated sample, and should be referred to as 10^{-1}. Continue as per your Standard methods.

5. Report results on the attached ***Results Sheet*** to the specified number of significant figures. Laboratories should report their results in the row corresponding to the method used for each particular test.

6. Return Results Sheets, either by mail, facsimile or email to:

Mark Bunt
Proficiency Testing Australia
PO Box 7507
Silverwater NSW 2128
AUSTRALIA

Telephone: + 61 2 9736 8397 (1300 782 867)
Fax: + 61 2 9743 6664
Email: mbunt@pta.asn.au

All results should arrive at the above address by no later than **Friday 8 November 2013**. Results reported later than this date may not be analysed in the final report.

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

C2.1

PROFICIENCY TESTING AUSTRALIA

Non-Pathogens in Food Proficiency Testing Program
Round 15, October 2013
RESULTS SHEET

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		
SPC	*2 sig. figures* **(cfu/g)**						Pour Plate
							Petrifilm™
Coliforms	*2 sig. figures* **(cfu or MPN/g)**						MPN
							Pour Plate
							Petrifilm™
E. coli	*2 sig. figures* **(cfu or MPN/g)**						MPN
							Pour Plate
							Petrifilm™
Yeasts	*2 sig. figures* **(cfu/g)**						Pour Plate
							Spread Plate
							Petrifilm™
Moulds	*2 sig. figures* **(cfu/g)**						Pour Plate
							Spread Plate
							Petrifilm™

For Yeasts and Moulds, please tick which method was used for testing:

AS 5013.29-2009 ❒

ISO 6611-2004 ❒

Other:

Note: If a method other than those specified above was used for a particular test, please record results and provide details of the method used in the blank row for that test.

Print Name: _____ Signature & Date: _____

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