

**REPORT NO. 821**

**Non-Pathogens in Food  
Proficiency Testing Program  
Round 14**

**August 2013**

**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.

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## 1. FOREWORD

This report summarises the results of a proficiency testing program involving the analysis of milk powder. It constitutes the fourteenth 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 June / July 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 Ms W Fajloun, PTA Quality Coordinator.

## 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. Laboratories from the following states and countries participated:

3	VIC
2	QLD
1	NSW
2	INDONESIA
1	PHILIPPINES
1	OMAN

### (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.*  $\leq -3.0$  or  $\geq 3.0$ ) 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 <sub>10</sub> )	All Methods Pooled	Number of Results	17	17
		Median	3.947	4.051
		Normalised IQR	0.110	0.084
		Uncertainty (Median)	0.034	0.026
Coliforms (log <sub>10</sub> )	Pour Plate / PetriFilm™	Number of Results	12	12
		Median	2.599	2.866
		Normalised IQR	0.081	0.110
		Uncertainty (Median)	0.029	0.040
<i>E. coli</i> (log <sub>10</sub> )	MPN	Number of Results	7	7
		Median	2.968	3.176
		Normalised IQR	0.620	0.693
		Uncertainty (Median)	0.294	0.328
Yeasts (log <sub>10</sub> )	All Methods Pooled	Number of Results	16	16
		Median	2.900	2.778
		Normalised IQR	0.262	0.180
		Uncertainty (Median)	0.082	0.056
Moulds (log <sub>10</sub> )	All Methods Pooled	Number of Results	16	16
		Median	3.562	3.515
		Normalised IQR	0.091	0.101
		Uncertainty (Median)	0.029	0.032

**Table B: Summary of Statistical Outliers and False Results**

Test	Method	Outliers		False Negatives
		PTA 1	PTA 2	
SPC	All Methods Pooled	-	-	-
Coliforms	MPN			-
	Pour Plate / Petrifilm™	10A, 10B	10A, 10B	-
	Other			-
<i>E. coli</i>	MPN	-	9	-
	Pour Plate			-
	Petrifilm™			-
	Other			-
Yeasts	All Methods Pooled	-	-	-
Moulds	All Methods Pooled	1, 10A, 10B	-	-

Notes:

1. The results reported are for log<sub>10</sub> (cfu/g) and log<sub>10</sub> (MPN/g).
2. All the methods were pooled when analysing the SPC, Yeasts and Moulds results.
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.
4. Summary statistics and z-scores were only calculated for the MPN results for *E. coli*. Target CVs were used to calculate the z-scores for the *E. coli* (MPN) 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 14 of the Non-Pathogens in Food Proficiency Testing Program consisted of a two-sample set. Sample PTA 1 contained *E. coli* as the only coliform organism present in the sample, whereas sample PTA 2 contained a mix of *E. coli* and a non-faecal coliform as the test organisms 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 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, three (30%) submitted results where more than one method was used for a specific test, while seven laboratories (70%) provided results for all five tests. The return rate for all tests is as follows:

• Standard Plate Count	10 out of 10	100%
• Coliforms	10 out of 10	100%
• <i>E. coli</i>	7 out of 10	70%
• Yeasts	10 out of 10	100%
• Moulds	10 out of 10	100%

### 6.2 Performance summary

One or more statistical outliers were reported by three laboratories (30%) for this round of the Non-Pathogens in Food program. For comparison, 44% of the participants reported outlier results or false negatives in Round 13 of the Non-Pathogens in Food program (see Report No. 793 for more details).

A total of 150 results were analysed in this round of the program. Of these results, eight (5%) were identified as outliers. For comparison, 9% of the results analysed in Round 13 of the Non-Pathogens in Food program were outliers or false negative results (see Report No. 793 for more details).

### 6.3 Standard Plate Count

Of the ten laboratories that tested for Standard Plate Count, two laboratories tested using more than one method. Ten laboratories tested using Pour Plate, including four laboratories that submitted two sets of results. Two laboratories tested using Petrifilm™, including one laboratory that submitted two sets of results.

All the methods were pooled when analysing the results.

The robust CVs of 2.79% and 2.08% for the results for this round compare well with the values of 2.68% and 3.28%, obtained for the results in Round 13 of this program, for samples containing the same organisms at similar levels (see Report No. 793).

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 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	$3.947 \pm 0.034$	$4.051 \pm 0.026$
SPC Pour Plate	$3.978 \pm 0.042$	$4.065 \pm 0.028$

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

	PTA 1	PTA 2
SPC All methods pooled	0.22	0.20
SPC Pour Plate	0.23	0.23

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. These MUs were reported as  $\pm \log_{10}$  values and were very close to the Reproducibility MUs given for both samples as above.



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.

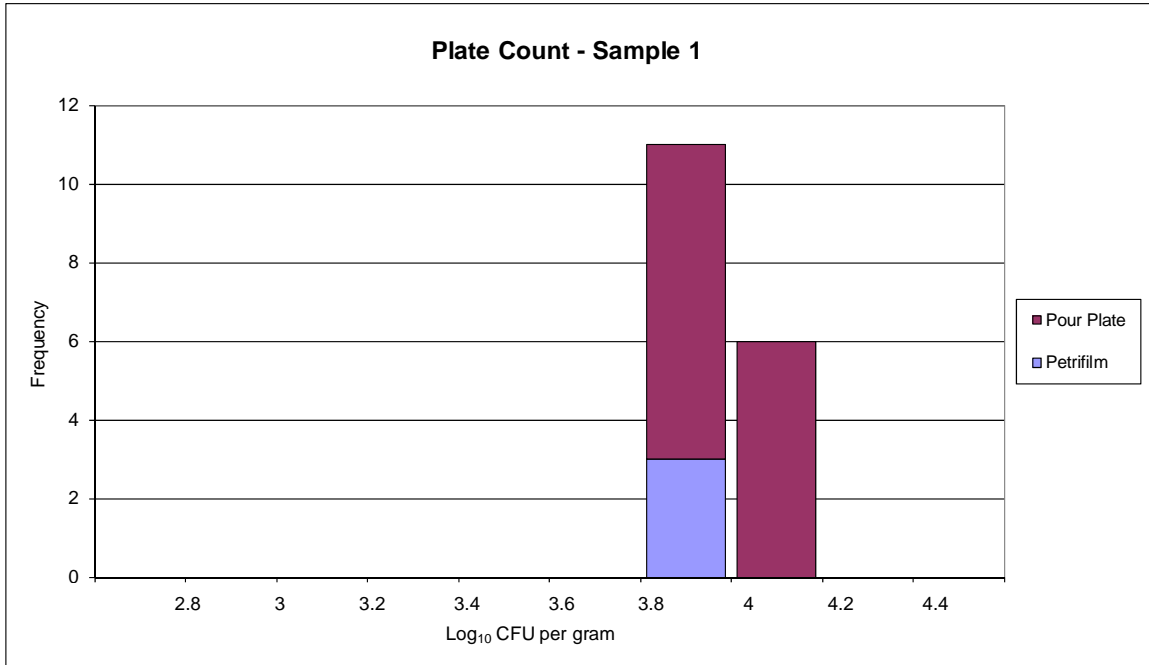


Figure TA-1. SPC  $\log_{10}$  cfu/g results for sample PTA 1.

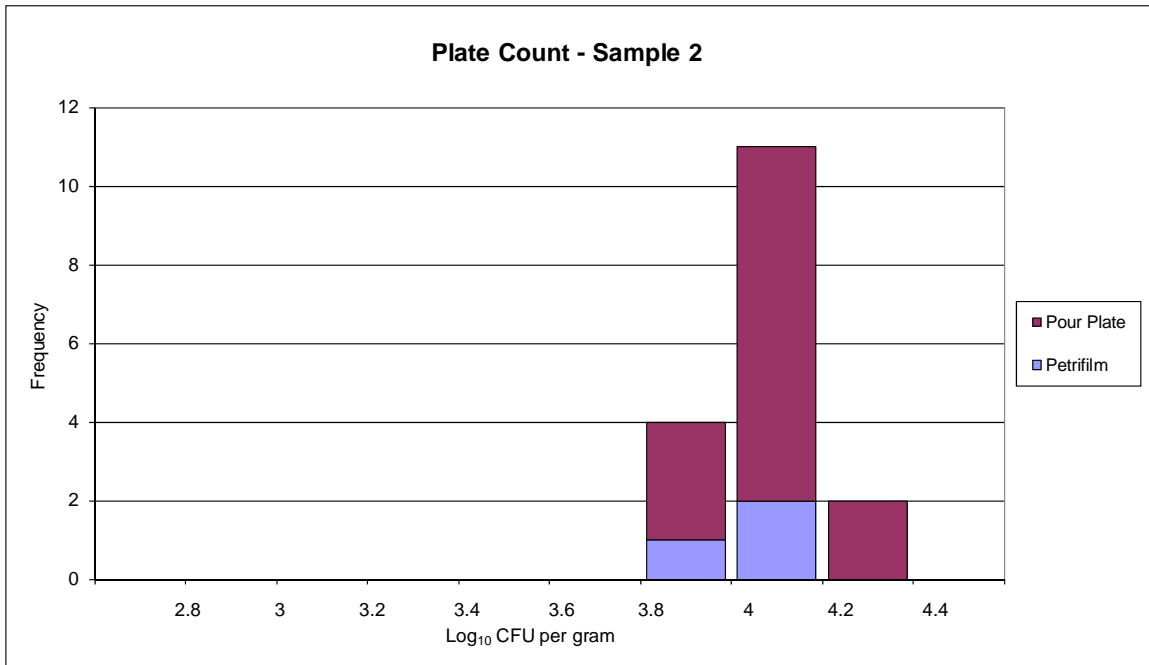


Figure TA-2. SPC  $\log_{10}$  cfu/g results for sample PTA 2.

## 6.4 Coliforms

A total of ten laboratories submitted results for Coliforms. Two of these laboratories used more than one method. Four laboratories tested using MPN, including one laboratory that submitted two sets of results. Seven laboratories tested using Pour Plate, including three laboratories that submitted two sets of results. One laboratory tested using the Petrifilm™ method and 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 this round, the robust CVs of 3.12% and 3.84% are lower than the values of 10.83% and 10.46%, obtained for the results in Round 13 of this program, for samples containing the same organisms at similar levels (see Report No. 793).

For the pooled Pour Plate / Petrifilm™ results, laboratories 10A and 10B (using the Pour Plate method) reported outliers for both samples.

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™)	2.599 ± 0.029	2.866 ± 0.040

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

	PTA 1	PTA 2
Coliforms - Pour Plate / Petrifilm™ pooled	0.34	0.47
Coliforms - Pour Plate	0.38	0.52

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, for the Pour Plate method. These MUs were reported as  $\pm \log_{10}$  values and were about half the Reproducibility MUs given for both samples as above.

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.

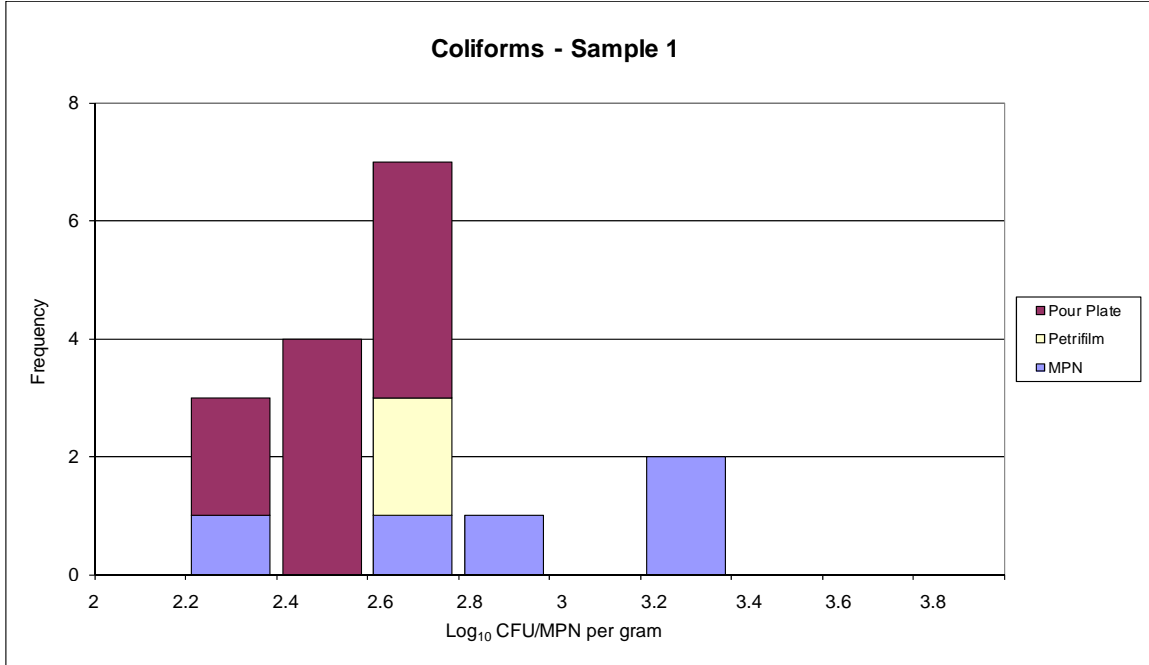


Figure TA-3. Coliforms log<sub>10</sub> cfu/MPN/g results for sample PTA 1.

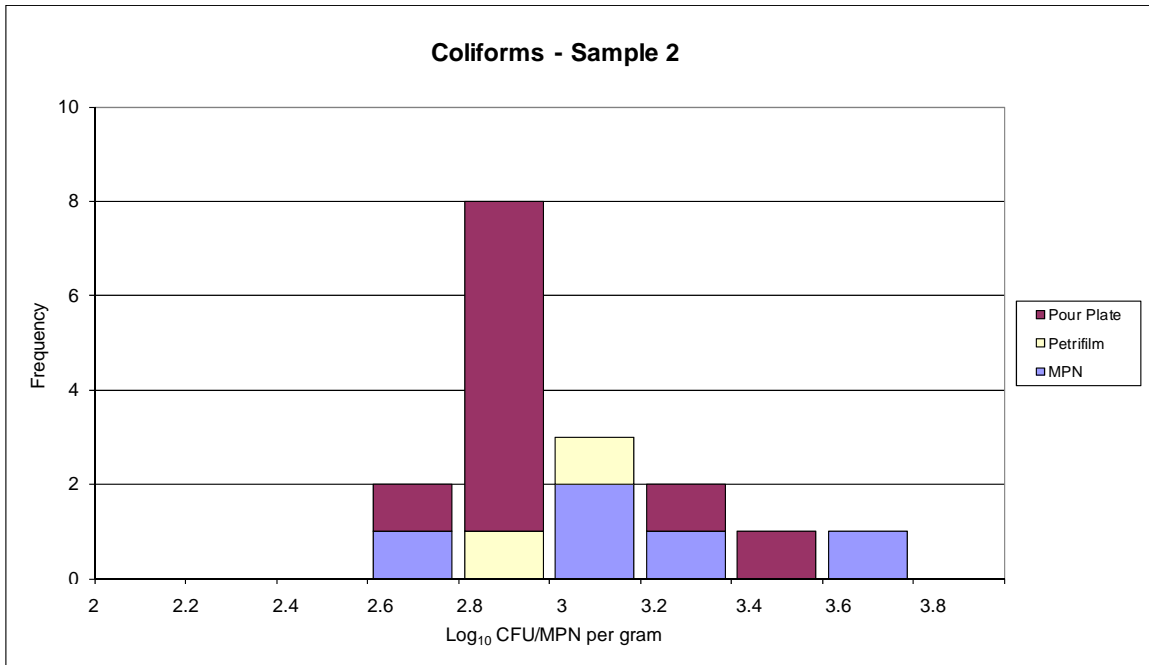


Figure TA-4. Coliforms log<sub>10</sub> cfu/MPN/g results for sample PTA 2.

## 6.5 *E. coli*

Of the seven laboratories that submitted results for *E. coli*, one laboratory tested using more than one method. Six laboratories tested using MPN, including one laboratory that submitted two sets of results. Two laboratories tested using Pour Plate. There were not enough *E. coli* results reported using methods other than MPN to analyse separately.

For the MPN method, the robust CVs for the samples were 20.89% and 21.82%. 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.

There were no outliers reported for sample PTA 1 for the MPN results, while laboratory 9 reported an outlier 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 *E. coli* via MPN test, the median and associated standard error (se) for each sample (expressed in  $\log_{10}$  MPN/g) was as follows:

	PTA 1	PTA 2
<i>E. coli</i> (MPN)	2.968 ± 0.294	3.176 ± 0.328

The Reproducibility MU for each sample for the MPN test has not been included due to the smaller number of participants and wide spread of data. For this reason also, method differentiation graphs have not been presented.

Only one laboratory reported MUs associated with their test results in this round. These MUs were reported as  $\pm \log_{10}$  values.

## 6.6 Yeasts

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

Four laboratories reported using AS 5013.29-2009 for their testing. Two laboratories used ISO 6611-2004. Three laboratories used other methods. One laboratory did not specify a method for testing.

All the methods were pooled when analysing the results.

The robust CVs of 9.04% and 6.49% for this round compare well with the values of 4.87% and 8.81%, obtained in Round 13 of this program, for samples containing the same organisms at similar levels (see Report No. 793).

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 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	2.900 ± 0.082	2.778 ± 0.056

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	0.47	0.33
Yeasts Pour Plate	0.51	0.39

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, for the Spread Plate method. These MUs were reported as  $\pm \log_{10}$  values and were about half the Reproducibility MUs given for both samples as above.

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.

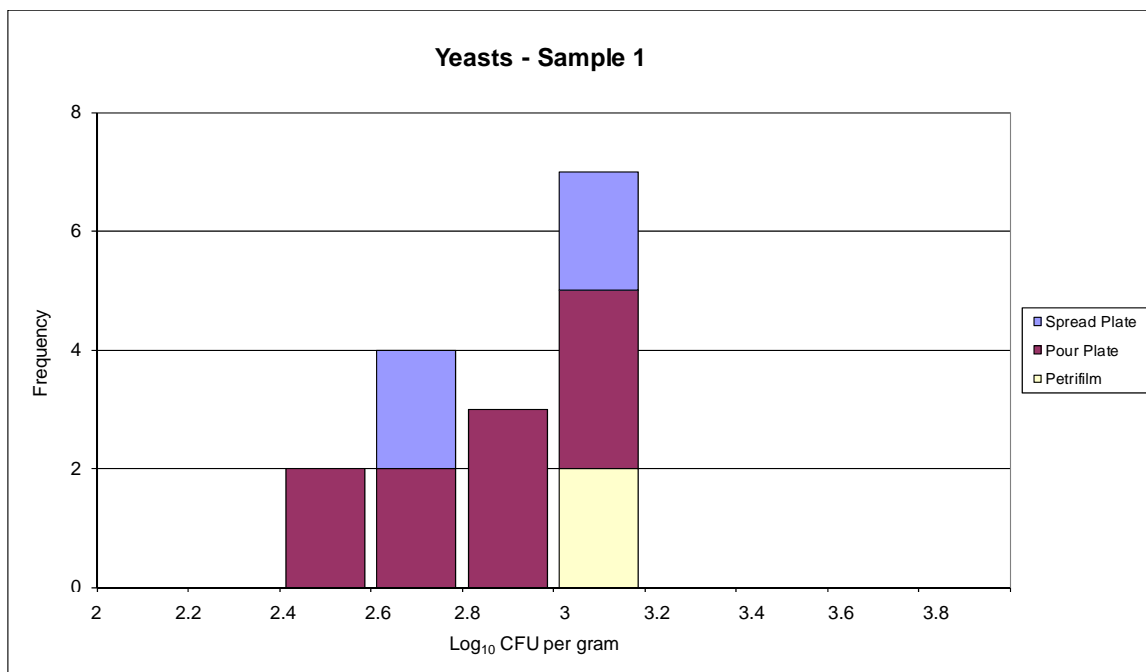


Figure TA-5. Yeasts log<sub>10</sub> cfu/g results for sample PTA 1.

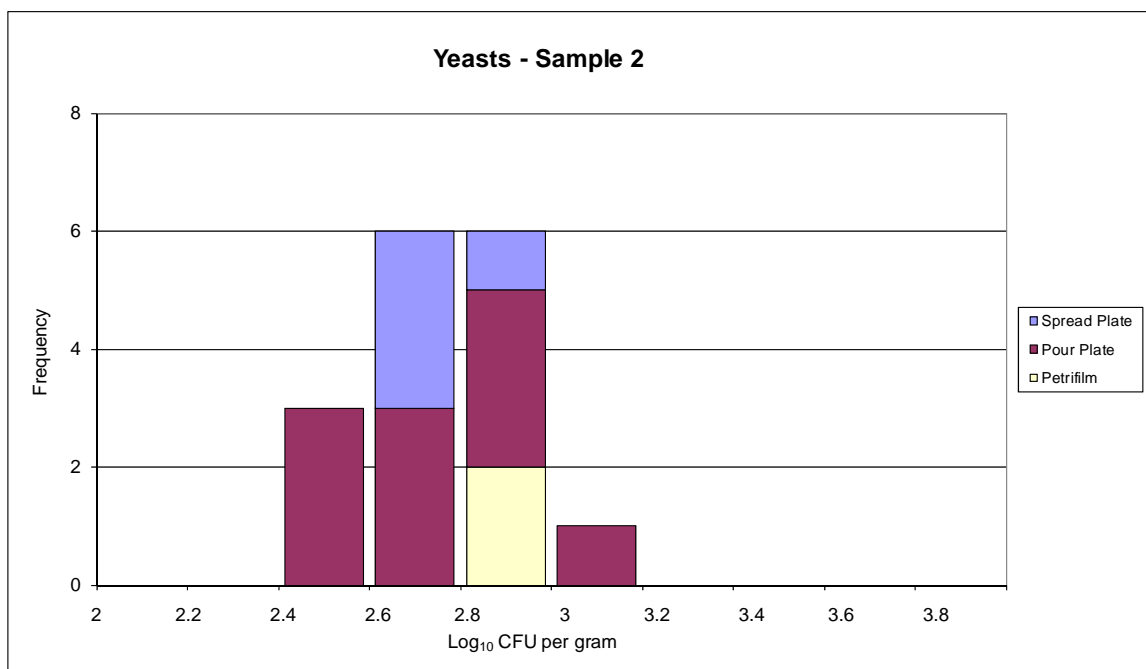


Figure TA-6. Yeasts log<sub>10</sub> cfu/g results for sample PTA 2.

## 6.7 Moulds

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

Four laboratories reported using AS 5013.29-2009 for their testing. Two laboratories used ISO 6611-2004. Three laboratories used other methods. One laboratory did not specify a method for testing.

All the methods were pooled when analysing the results.

The robust CVs of 2.57% and 2.87% for this round compare well with the values of 4.10% and 2.32%, obtained in Round 13 of this program, for samples containing the same organisms at similar levels (see Report No. 793).

Laboratory 1 (using the Spread Plate method) and laboratories 10A and 10B (using the Pour Plate method) obtained outliers 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 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	3.562 ± 0.029	3.515 ± 0.032

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	0.46	0.28
Moulds Pour Plate	0.42	0.25

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, for the Spread Plate method. These MUs were reported as  $\pm \log_{10}$  values and were similar to the Reproducibility MUs given for sample PTA 2 and about half the Reproducibility MUs given for sample PTA 1, as above.

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.

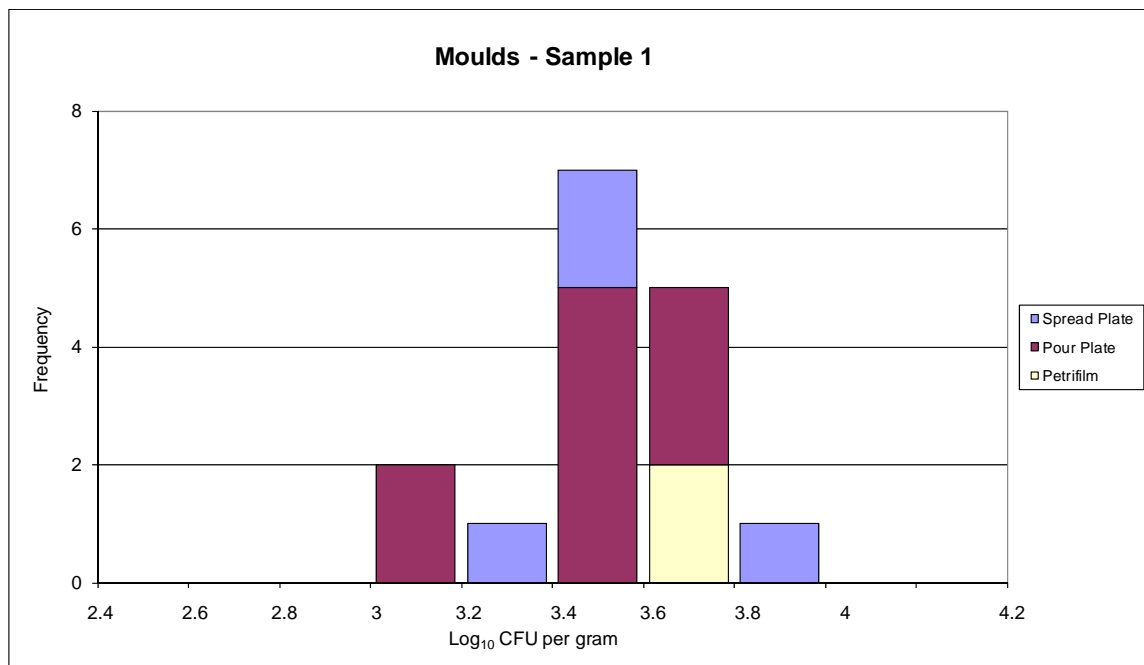


Figure TA-7. Moulds log<sub>10</sub> cfu/g results for sample PTA 1.

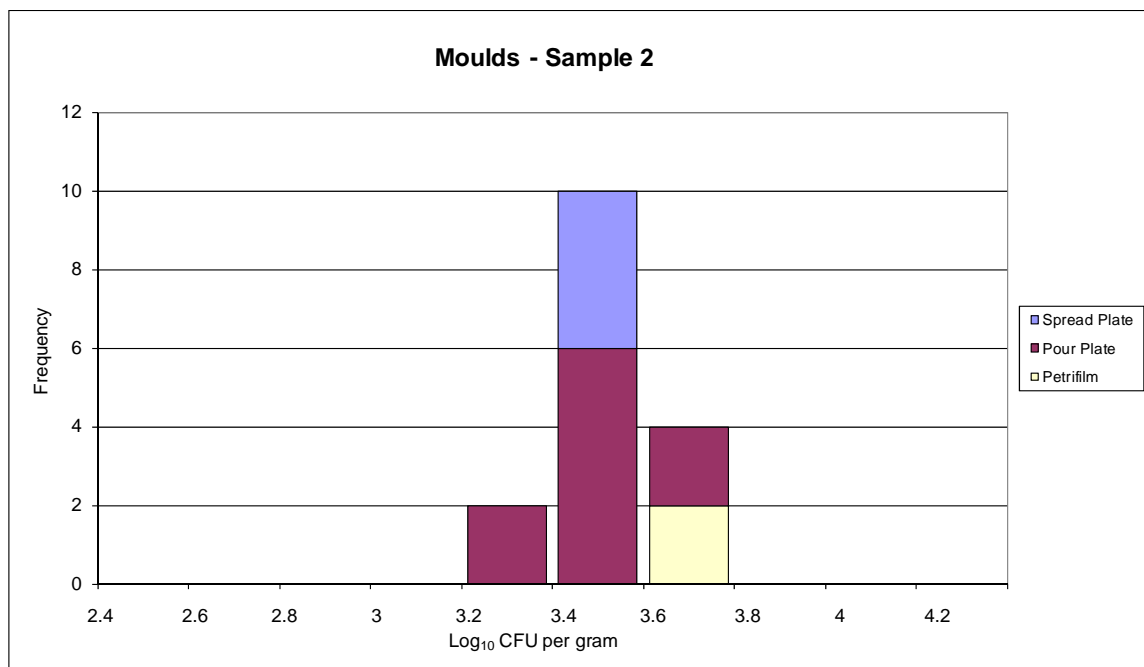


Figure TA-8. Moulds log<sub>10</sub> cfu/g results for sample PTA 2.



## 7. REFERENCES

1. *Guide to Proficiency Testing Australia (2012)*. (This document is located on the PTA website at [www.pta.asn.au](http://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 <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	PTA 1	PTA 2	
1	10000	4.00	-	8800	3.94	-	0.48	-1.26	PP
2	14000	4.15	-	13000	4.11	-	1.81	0.74	PP
3A	7950	3.90	-	12700	4.10	-	-0.42	0.62	PP
3A	7600	3.88	-	12900	4.11	-	-0.60	0.70	Pfm
3B	8850	3.95	-	11250	4.05	-	0.00	0.00	PP
3B	7000	3.85	-	11250	4.05	-	-0.92	0.00	Pfm
4	9400	3.97	± 0.22 (log)	10000	4.00	± 0.22 (log)	0.24	-0.61	PP
4	7600	3.88	± 0.19 (log)	9900	4.00	± 0.19 (log)	-0.60	-0.66	Pfm
5	10000	4.00	-	10000	4.00	-	0.48	-0.61	PP
6A	13100	4.12	-	15700	4.20	-	1.54	1.71	PP
6B	11200	4.05	-	13000	4.11	-	0.93	0.74	PP
7A	6800	3.83	-	7500	3.88	-	-1.04	-2.08	PP
7B	11000	4.04	-	11000	4.04	-	0.86	-0.12	PP
8	9600	3.98	-	12000	4.08	-	0.32	0.33	PP
9	6750	3.83	-	9100	3.96	-	-1.07	-1.09	PP
10A	7100	3.85	-	16000	4.20	-	-0.87	1.81	PP
10B	6400	3.81	-	17000	4.23	-	-1.28	2.12	PP

Statistic	Log <sub>10</sub> PTA 1	Log <sub>10</sub> PTA 2
Number of Results	17	17
Median	3.947	4.051
Normalised IQR	0.110	0.084
Uncertainty (Median)	0.034	0.026
Robust CV	2.79%	2.08%
Minimum	3.81	3.88
Maximum	4.15	4.23
Range	0.34	0.36

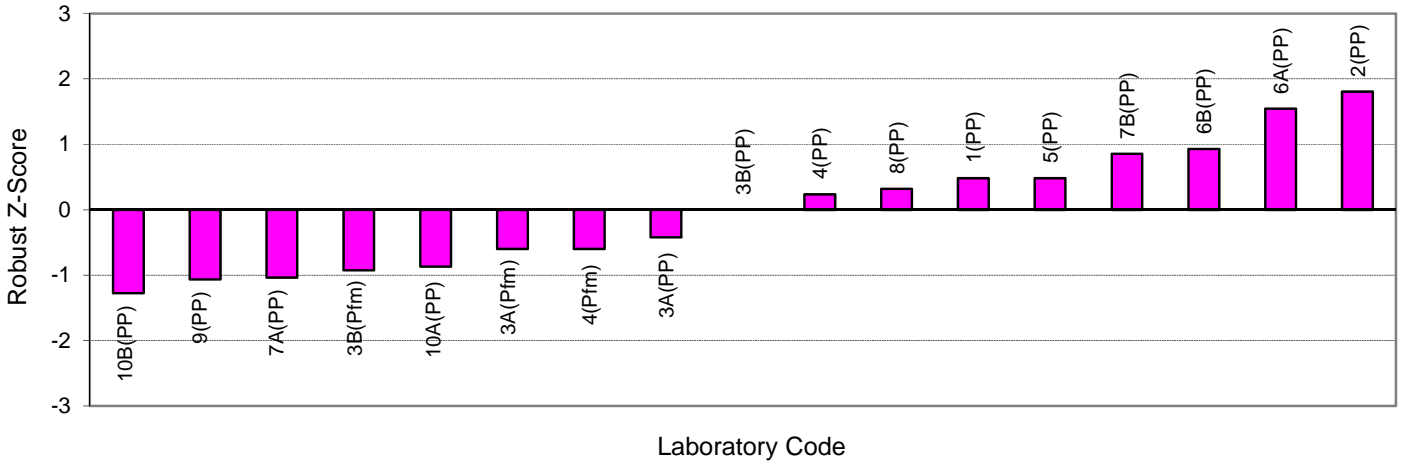
Method	Number of Results
PP = Pour Plate	14
Pfm = Petrifilm™	3
Oth = Other	0

#### Notes:

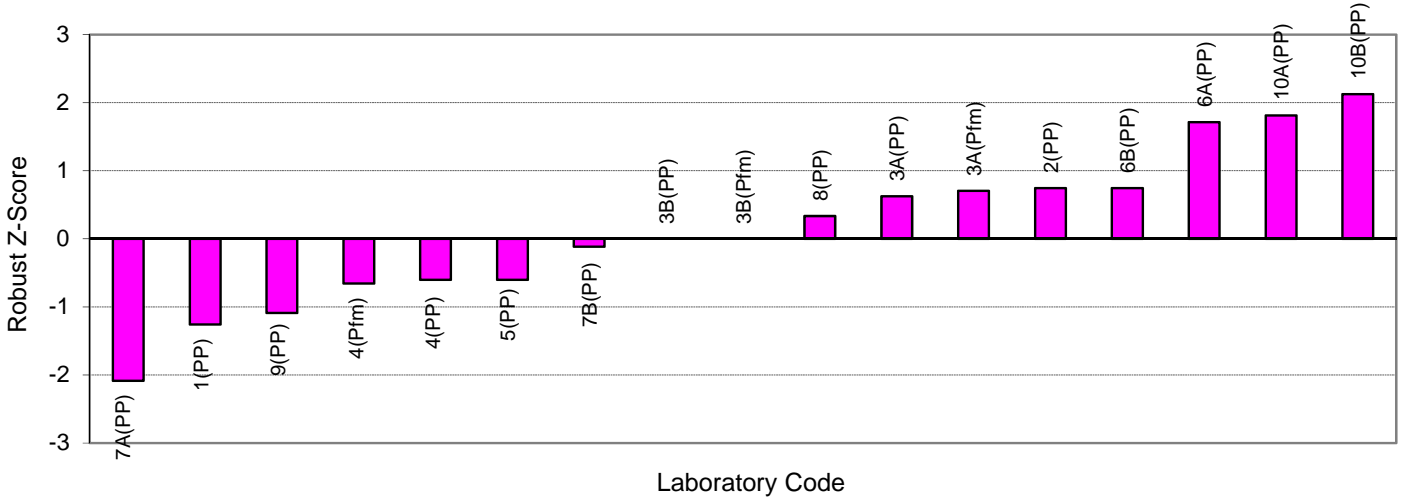
1. All the methods were pooled when analysing the SPC results.
2. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
3. The Youden diagram on the following page is provided for information only.

## A1.2

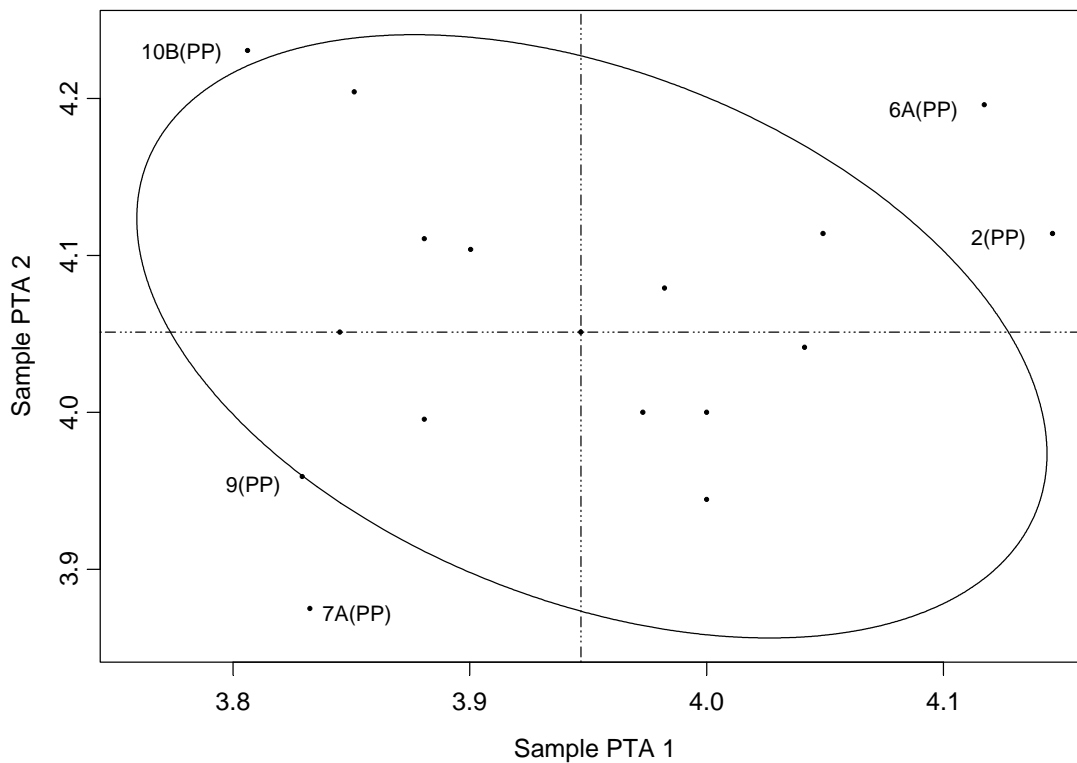
Milk Powder - SPC, All Methods Pooled [ $\log_{10}(\text{cfu/g})$ ] - PTA 1



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



Milk Powder - SPC, All Methods Pooled  $\log(\text{cfu/g})$



## **Section A2**

### **Coliforms**

## A2.1

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

Lab Code	PTA 1			PTA 2			Z-Scores		Method
	Result	Log <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	PTA 1	PTA 2	
2	390	2.59	-	960	2.98	-	-0.10	1.05	PP
3A	375	2.57	-	660	2.82	-	-0.31	-0.42	PP
3A	405	2.61	-	675	2.83	-	0.10	-0.34	Pfm
3B	430	2.63	-	705	2.85	-	0.42	-0.16	PP
3B	450	2.65	-	1050	3.02	-	0.67	1.41	Pfm
4	430	2.63	± 0.21 (log)	730	2.86	± 0.21 (log)	0.42	-0.03	PP
5	280	2.45	-	740	2.87	-	-1.88	0.03	PP
6A	470	2.67	-	710	2.85	-	0.90	-0.14	PP
6B	500	2.70	-	740	2.87	-	1.23	0.03	PP
8	360	2.56	-	530	2.72	-	-0.53	-1.29	PP
10A	170	2.23	-	2700	3.43	-	-4.55 §	5.13 §	PP
10B	180	2.26	-	2100	3.32	-	-4.24 §	4.14 §	PP

Statistic	Log <sub>10</sub> PTA 1	Log <sub>10</sub> PTA 2
Number of Results	12	12
Median	2.599	2.866
Normalised IQR	0.081	0.110
Uncertainty (Median)	0.029	0.040
Robust CV	3.12%	3.84%
Minimum	2.23	2.72
Maximum	2.70	3.43
Range	0.47	0.71

#### Method

PP = Pour Plate

Pfm = Petrifilm™

#### Number of Results

10

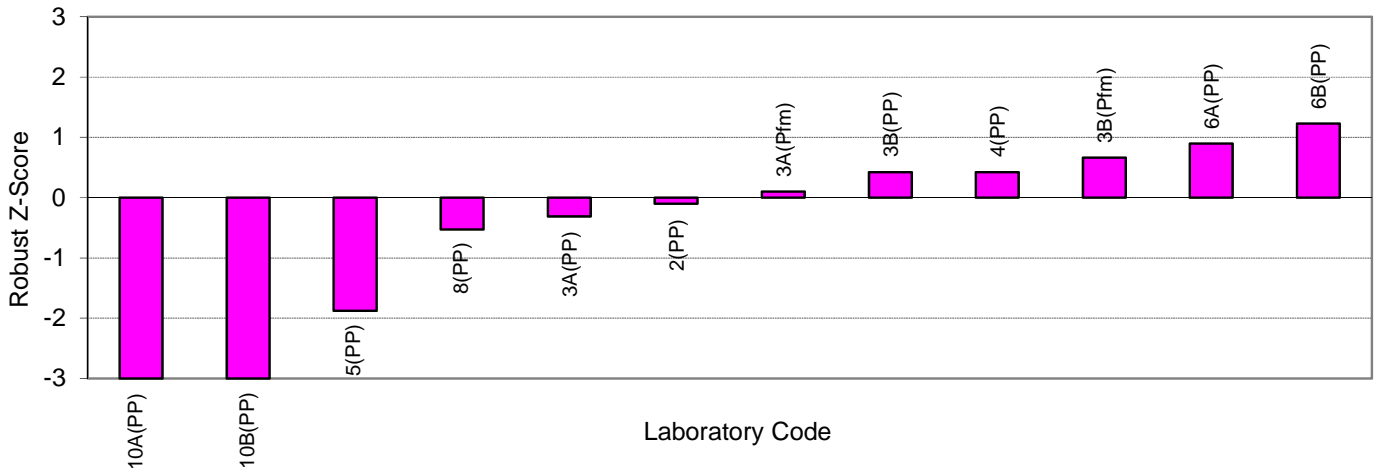
2

#### Notes:

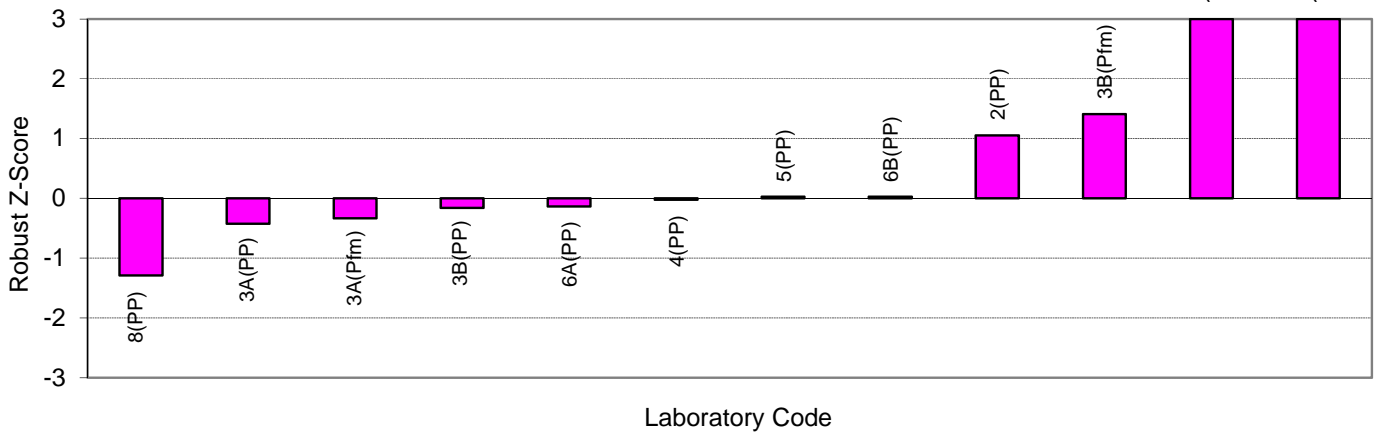
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- The Pour Plate and Petrifilm™ methods were pooled when analysing the Coliforms results.
- The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
- The Youden diagram on the following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

## A2.2

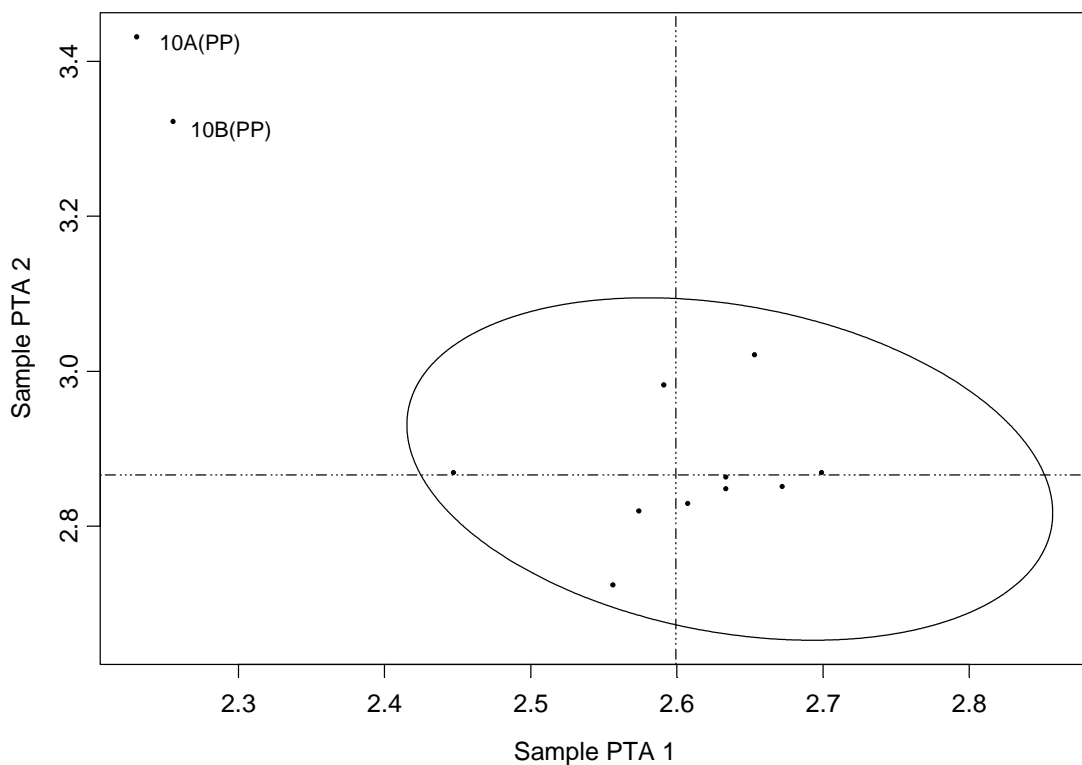
Milk Powder - Coliforms, Pour Plate / Petrifilm [ $\log_{10}(\text{cfu/g})$ ] - PTA 1



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



Milk Powder - Coliforms, Pour Plate / Petrifilm  $\log(\text{cfu/g})$





### A2.3

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

Lab Code	PTA 1			PTA 2			Method
	Result	Log <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	
1	460	2.66	-	1100	3.04	-	MPN
2	930	2.97	-	2400	3.38	-	MPN
7A	2400	3.38	-	4600	3.66	-	MPN
7B	2100	3.32	-	1500	3.18	-	MPN
9	240	2.38	-	430	2.63	-	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*, MPN (MPN/g)

Lab Code	PTA 1			PTA 2			Z-Scores	
	Result	Log <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	PTA 1	PTA 2
1	460	2.66	-	460	2.66	-	-1.03	-1.62
2	930	2.97	-	1700	3.23	-	0.00	0.17
4	460	2.66	± 0.18 (log)	460	2.66	± 0.18 (log)	-1.03	-1.62
5	4300	3.63	-	4300	3.63	-	2.24	1.44
7A	2400	3.38	-	4600	3.66	-	1.39	1.53
7B	2100	3.32	-	1500	3.18	-	1.19	0.00
9	240	2.38	-	150	2.18	-	-1.98	-3.15 §

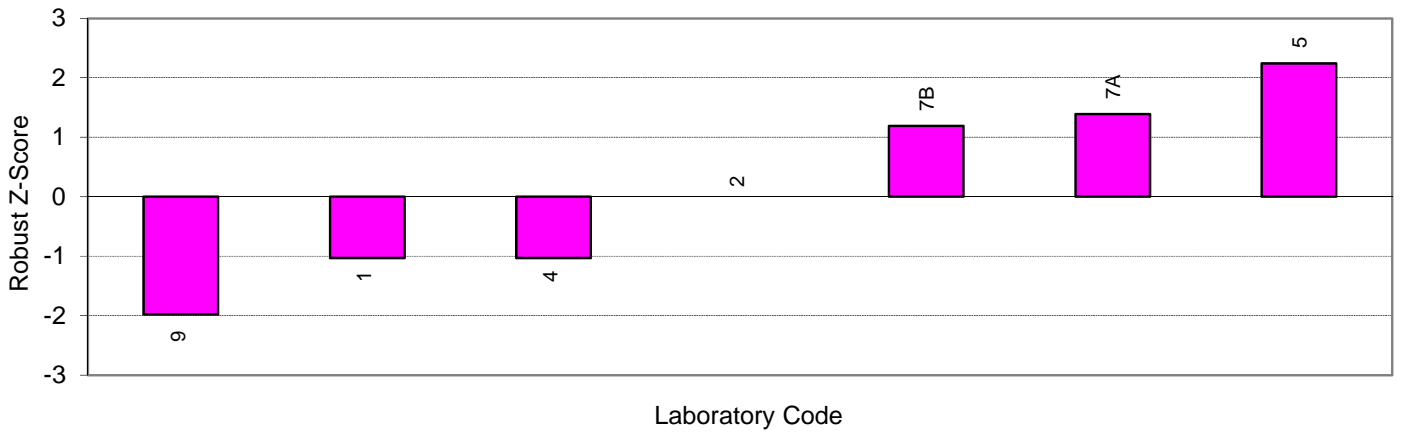
Statistic	Log <sub>10</sub> PTA 1	Log <sub>10</sub> PTA 2
Number of Results	7	7
Median	2.968	3.176
Normalised IQR	0.620	0.693
Uncertainty (Median)	0.294	0.328
Robust CV	20.89%	21.82%
Target CV	10.00%	10.00%
Minimum	2.38	2.18
Maximum	3.63	3.66
Range	1.25	1.49

**Notes:**

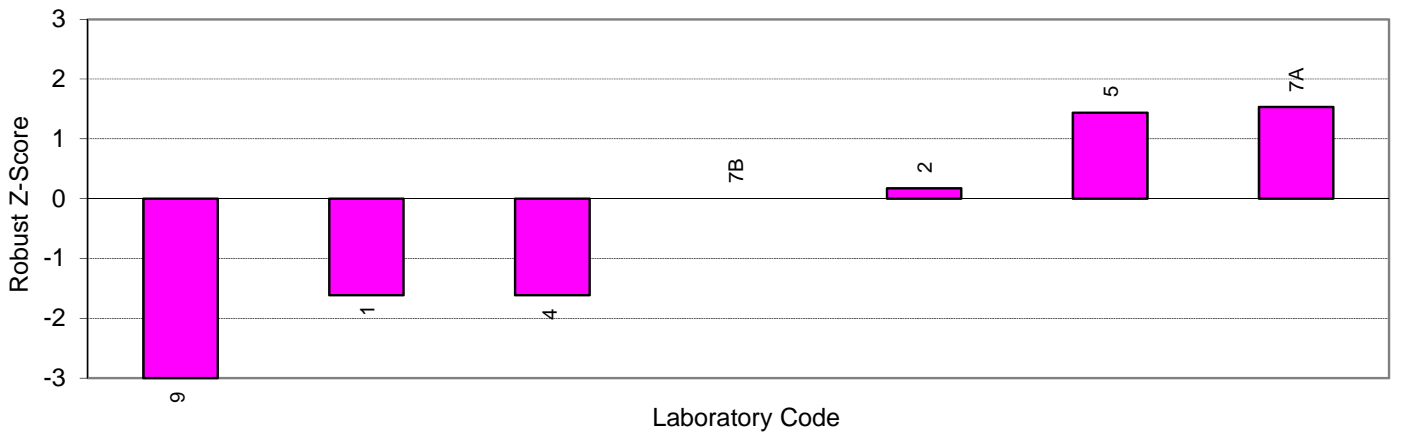
1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. A target CV of 10% was used to calculate the robust z-scores for both samples.
3. The Youden diagram on the following page is provided for information only.

### A3.2

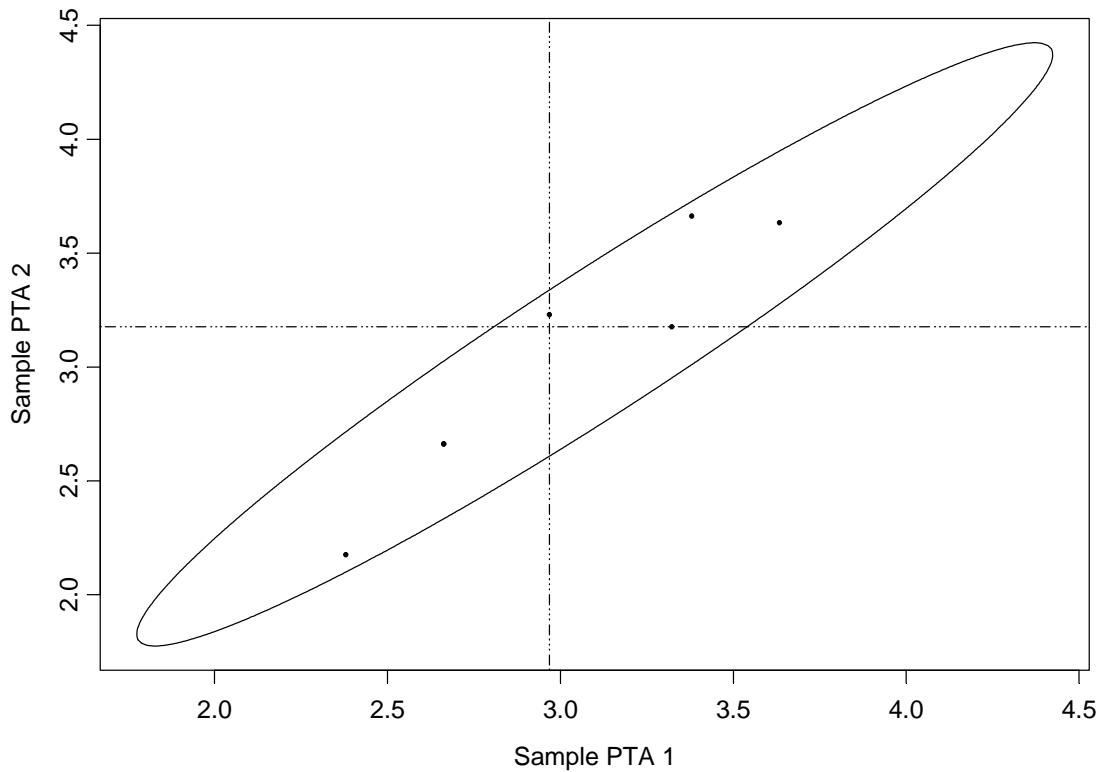
Milk Powder - *E. coli*, MPN [ $\log_{10}(\text{MPN/g})$ ] - PTA 1



Milk Powder - *E. coli*, MPN [ $\log_{10}(\text{MPN/g})$ ] - PTA 2



Milk Powder - *E. coli*, MPN  $\log(\text{MPN/g})$



### A3.3

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

Lab Code	PTA 1			PTA 2			Method
	Result	Log <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	
2	370	2.57	-	710	2.85	-	Pour Plate
8	240	2.38	-	460	2.66	-	Pour Plate

**Note:**

1. There were not enough *E. coli* results reported using methods other than MPN to calculate summary statistics or z-scores.

## **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 <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	PTA 1	PTA 2		
1	1400	3.15	-	600	2.78	-	0.94	0.00	SP	4
2	520	2.72	-	410	2.61	-	-0.70	-0.92	PP	3
3A	1350	3.13	-	600	2.78	-	0.88	0.00	PP	2
3A	1200	3.08	-	750	2.88	-	0.68	0.54	Pfm	2
3B	1100	3.04	-	650	2.81	-	0.54	0.19	PP	2
3B	1050	3.02	-	700	2.85	-	0.46	0.37	Pfm	2
4	1200	3.08	± 0.20 (log)	700	2.85	± 0.20 (log)	0.68	0.37	SP	1
5	700	2.85	-	1000	3.00	-	-0.21	1.23	PP	1
6A	900	2.95	-	700	2.85	-	0.21	0.37	PP	2
6B	700	2.85	-	600	2.78	-	-0.21	0.00	PP	2
7A	500	2.70	-	400	2.60	-	-0.77	-0.98	SP	1
7B	500	2.70	-	400	2.60	-	-0.77	-0.98	SP	1
8	540	2.73	-	290	2.46	-	-0.64	-1.75	PP	3
9	1150	3.06	-	650	2.81	-	0.61	0.19	PP	1
10A	330	2.52	-	320	2.51	-	-1.45	-1.52	PP	3
10B	290	2.46	-	350	2.54	-	-1.67	-1.30	PP	3

Statistic	Log <sub>10</sub> PTA 1	Log <sub>10</sub> PTA 2
Number of Results	16	16
Median	2.900	2.778
Normalised IQR	0.262	0.180
Uncertainty (Median)	0.082	0.056
Robust CV	9.04%	6.49%
Minimum	2.46	2.46
Maximum	3.15	3.00
Range	0.68	0.54

Method	Number of Results
PP = Pour Plate	10
SP = Spread Plate	4
Pfm = Petrifilm™	2

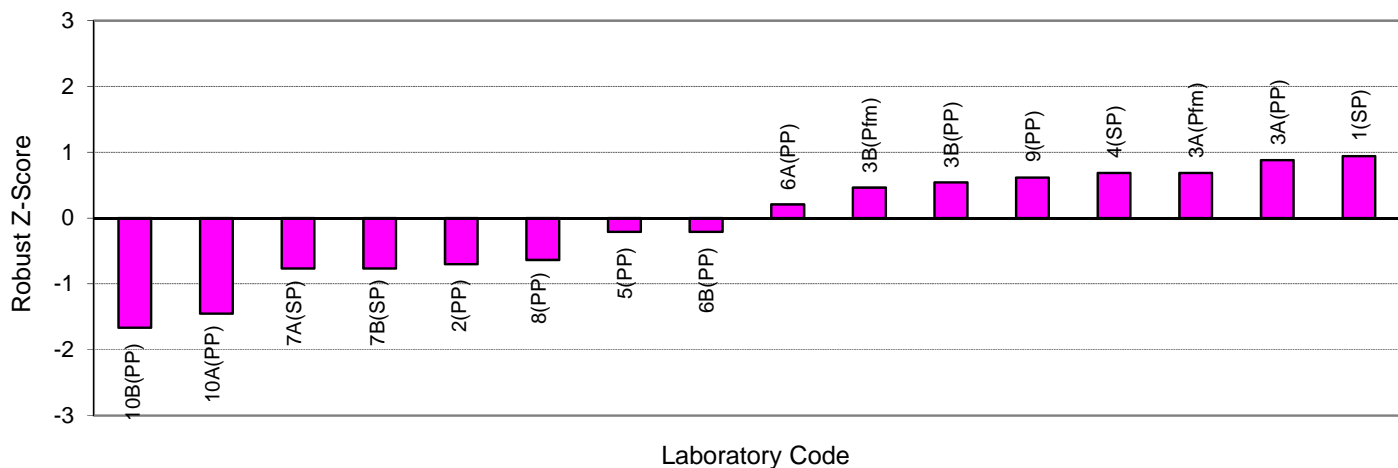
AS / ISO / Other Code	No. of Laboratories
1 = AS 5013.29-2009	4
2 = ISO 6611-2004	2
3 = Other	3
4 = Unspecified	1

#### Notes:

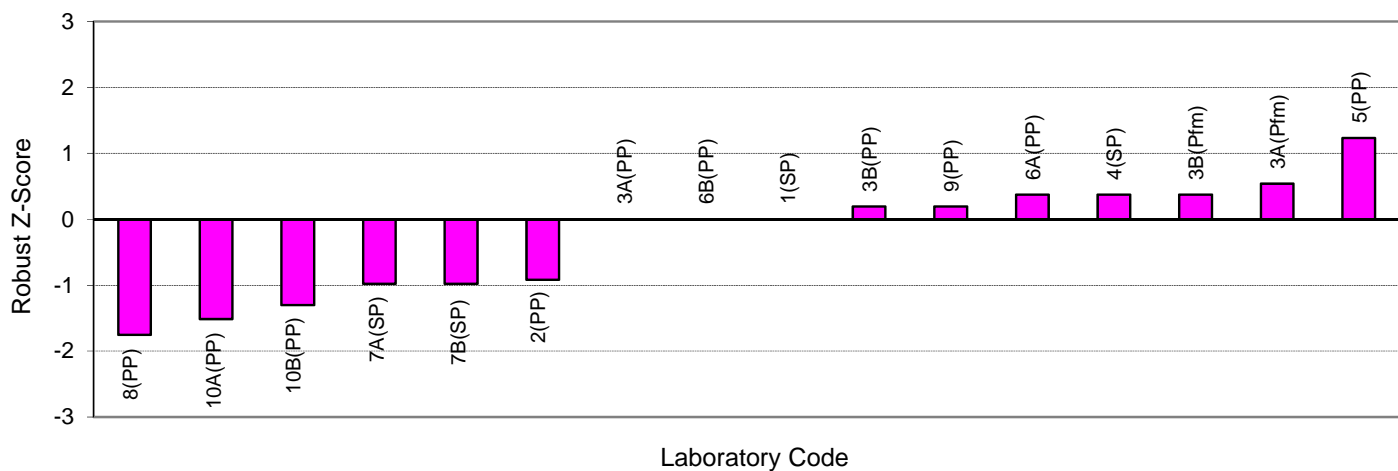
1. The sample PTA 2 result for laboratory 4 is an estimate.
2. All the methods were pooled when analysing the Yeasts results.
3. The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
4. The Youden diagram on the following page is provided for information only.

## A4.2

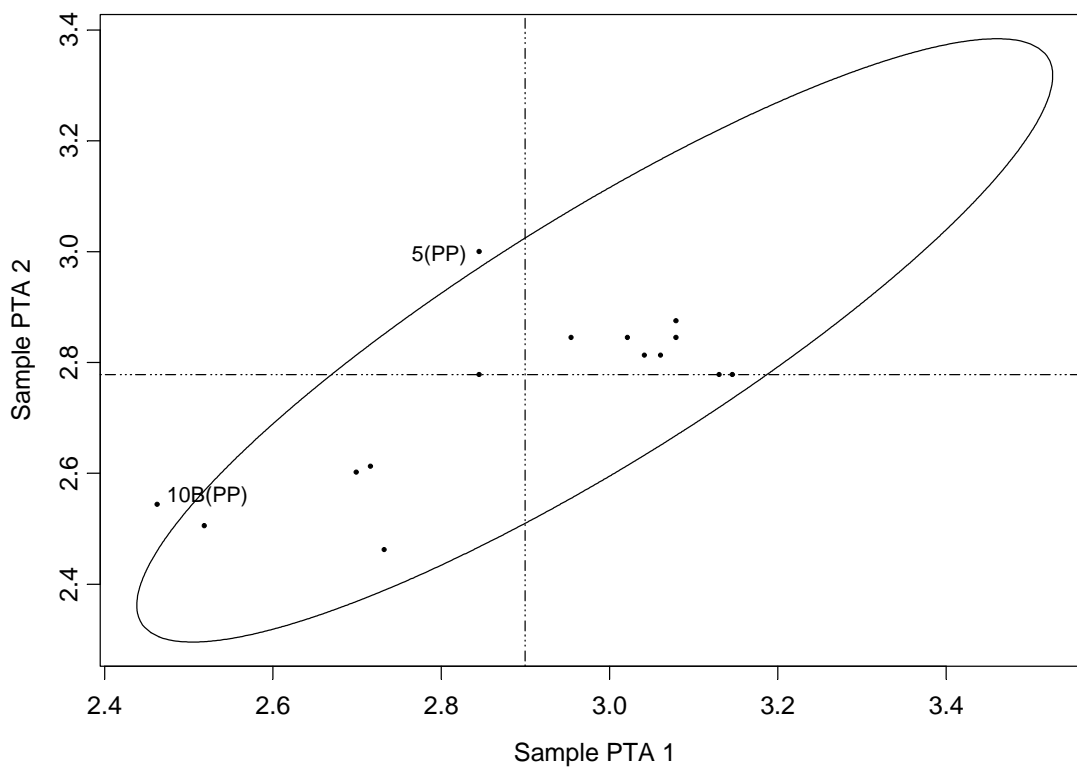
Milk Powder - Yeasts, All Methods Pooled [ $\log_{10}(\text{cfu/g})$ ] - PTA 1



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



Milk Powder - Yeasts, All Methods Pooled  $\log(\text{cfu/g})$





## **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 <sub>10</sub>	MU	Result	Log <sub>10</sub>	MU	PTA 1	PTA 2		
1	7300	3.86	-	3500	3.54	-	3.29 §	0.29	SP	4
2	3400	3.53	-	3100	3.49	-	-0.34	-0.23	PP	3
3A	4100	3.61	-	5000	3.70	-	0.55	1.83	PP	2
3A	4000	3.60	-	4550	3.66	-	0.44	1.42	Pfm	2
3B	3850	3.59	-	5000	3.70	-	0.25	1.83	PP	2
3B	5600	3.75	-	4750	3.68	-	2.03	1.61	Pfm	2
4	3300	3.52	± 0.26 (log)	3500	3.54	± 0.26 (log)	-0.48	0.29	SP	1
5	3600	3.56	-	2900	3.46	-	-0.07	-0.52	PP	1
6A	3700	3.57	-	2900	3.46	-	0.07	-0.52	PP	2
6B	3200	3.51	-	2600	3.41	-	-0.62	-0.99	PP	2
7A	2100	3.32	-	2700	3.43	-	-2.62	-0.83	SP	1
7B	3500	3.54	-	3700	3.57	-	-0.20	0.53	SP	1
8	5300	3.72	-	2900	3.46	-	1.77	-0.52	PP	3
9	5200	3.72	-	3450	3.54	-	1.68	0.23	PP	1
10A	1400	3.15	-	2300	3.36	-	-4.55 §	-1.52	PP	3
10B	1400	3.15	-	2100	3.32	-	-4.55 §	-1.91	PP	3

Statistic	Log <sub>10</sub> PTA 1	Log <sub>10</sub> PTA 2
Number of Results	16	16
Median	3.562	3.515
Normalised IQR	0.091	0.101
Uncertainty (Median)	0.029	0.032
Robust CV	2.57%	2.87%
Minimum	3.15	3.32
Maximum	3.86	3.70
Range	0.72	0.38

Method	Number of Results
PP = Pour Plate	10
SP = Spread Plate	4
Pfm = Petrifilm™	2

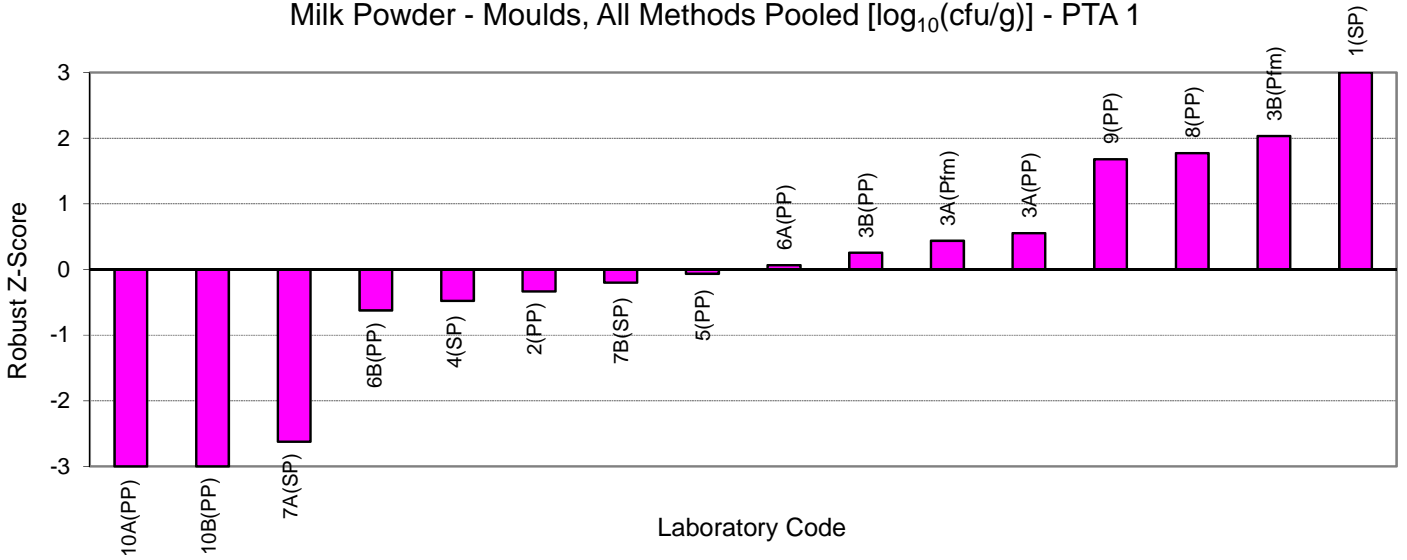
AS / ISO / Other Code	No. of Laboratories
1 = AS 5013.29-2009	4
2 = ISO 6611-2004	2
3 = Other	3
4 = Unspecified	1

#### Notes:

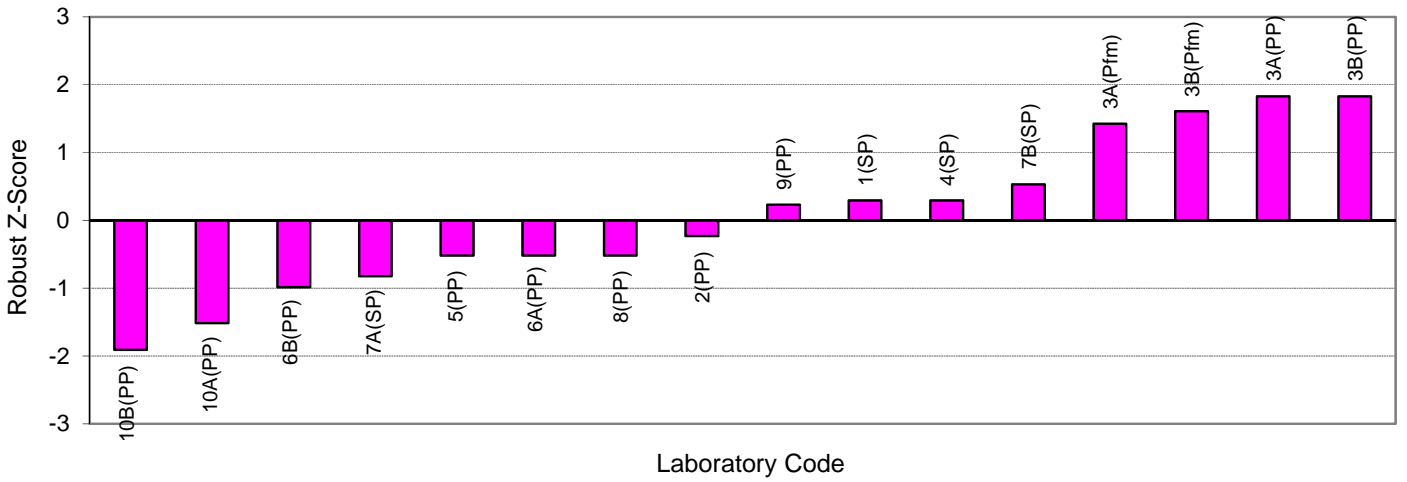
- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- All the methods were pooled when analysing the Moulds results.
- The method used has been appended to the laboratory code on the ordered z-score charts and Youden diagram on the following page.
- The Youden diagram on the following page is provided for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

## A5.2

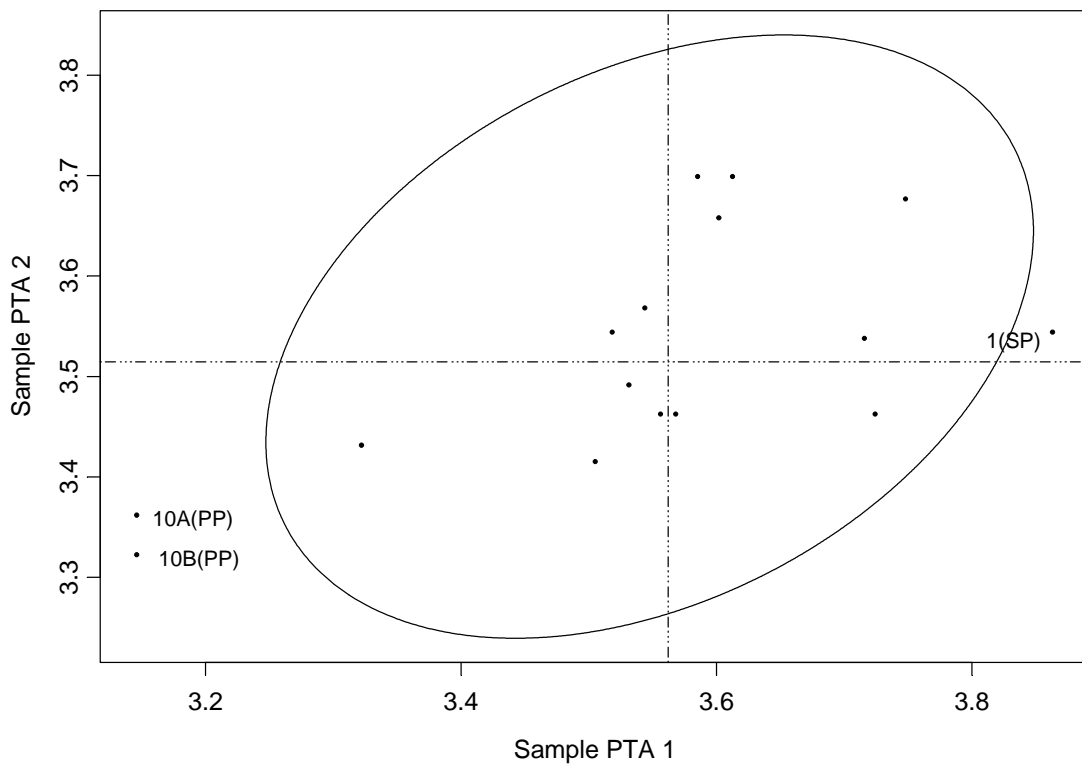
Milk Powder - Moulds, All Methods Pooled [ $\log_{10}(\text{cfu/g})$ ] - PTA 1



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



Milk Powder - Moulds, All Methods Pooled  $\log(\text{cfu/g})$



# **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 Standard Plate Count, Yeasts and Moulds on 22 May 2013.

#### Standard Plate Count

The samples from PTA 2 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 2				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
10	10000	4.00	11000	4.04
17	9200	3.96	9100	3.96
52	12000	4.08	12000	4.08
60	11000	4.04	10000	4.00
61	9900	4.00	11000	4.04
85	11000	4.04	12000	4.08
88	10000	4.00	8800	3.94
89	11000	4.04	12000	4.08
100	11000	4.04	10000	4.00
107	9000	3.95	7300	3.86

#### Yeasts

The samples from PTA 2 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 2				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
10	1200	3.08	1300	3.11
17	1200	3.08	950	2.98
52	1700	3.23	1200	3.08
60	1300	3.11	1200	3.08
61	800	2.90	1200	3.08
85	1500	3.18	1400	3.15
88	850	2.93	1500	3.18
89	1600	3.20	1300	3.11
100	900	2.95	950	2.98
107	1600	3.20	1200	3.08

## B1.2

### Moulds

The samples from PTA 2 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.

<b>Moulds (cfu/g)</b>				
<b>PTA 2</b>				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
10	4100	3.61	3600	3.56
17	4100	3.61	4000	3.60
52	4200	3.62	3600	3.56
60	4100	3.61	3700	3.57
61	3400	3.53	4500	3.65
85	4100	3.61	4100	3.61
88	3500	3.54	4700	3.67
89	5000	3.70	5300	3.72
100	4100	3.61	4300	3.63
107	3400	3.53	3500	3.54

### 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.

## B1.3

### STABILITY TESTING

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

#### Standard Plate Count

The samples from PTA 2 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 2				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
6	8600	3.93	8000	3.90
44	6800	3.83	6600	3.82
58	7600	3.88	9200	3.96

#### Yeasts

The samples from PTA 2 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 2				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
6	1200	3.08	1000	3.00
44	950	2.98	900	2.95
58	550	2.74	900	2.95

#### Moulds

The samples from PTA 2 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 2				
Sample	Result A	Log <sub>10</sub> A	Result B	Log <sub>10</sub> B
6	3100	3.49	4500	3.65
44	4600	3.66	3400	3.53
58	3200	3.51	3500	3.54

## **B1.4**

### **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**

**Non-Pathogens in Food  
Proficiency Testing Program  
Round 14, June 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**.

## 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), a range if reported in standard form, e.g.  $7.5 \times 10^3$  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
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All results should arrive at the above address by no later than **Friday 12 July 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.

**PROFICIENCY TESTING AUSTRALIA**  
**Non-Pathogens in Food Proficiency Testing Program**  
**Round 14, June 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 &amp; Date: \_\_\_\_\_

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