

**REPORT NO. 510**

**Non-Pathogens in Food  
Proficiency Testing Program  
Round 1**

**May 2006**

**ACKNOWLEDGMENTS**

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## 1. FOREWORD

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

Proficiency Testing Australia conducted the exercise in February 2006. The aim of the program was to assess laboratories' ability to competently perform the nominated tests.

## 2. FEATURES OF THE PROGRAM

### (a) Participating Laboratories

A total of 53 laboratories participated in the program, all of which returned results for inclusion in the final report. Laboratories from the following states participated:

10	NSW
14	QLD
19	VIC
2	SA
5	WA
3	TAS

### (b) Documentation and Testing Methods

Laboratories were provided with two samples of skim milk powder, labelled PTA 1 and PTA 2. A temperature control bottle, labelled *Temperature*, was included to monitor the sample delivery process. Participants were asked to perform tests for:

- Standard Plate Count
- Coliforms
- *E. coli*
- Thermophiles
- Thermophilic Spores

Laboratories were required to perform all tests for which they hold NATA accreditation, by as many different methods as desired, and were invited to report results for any of the other tests.

Laboratories were requested to perform the tests according to the *Instructions to Participants* provided and to record the results 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, one code number (with letter) could appear several times in the same data set.

**(d) Homogeneity Testing**

One week prior to sample distribution, ten randomly selected samples were analysed for homogeneity by Food Science Australia. Based on the results of this testing, the homogeneity of the samples was established for sample PTA 1. However, homogeneity could not be established for *E. coli* for sample PTA 2. Additionally, the levels of *E. coli* in the samples were much lower than requested, and were considered too low for analysis (see Appendix B).

**(e) Stability Testing**

Three randomly selected samples were analysed for stability by Food Science Australia for standard plate count, coliforms and thermophiles, while ten randomly selected samples were analysed for stability for *E. coli*. Testing occurred one week after sample distribution.

The analysis of the stability testing results showed that the samples were sufficiently stable for standard plate count, coliforms and thermophiles (see Appendix B).

The levels of *E. coli* were quite low initially and it was therefore decided not to calculate summary statistics or z-scores for the *E. coli* results submitted by participants (see Appendix B).

### 3. FORMAT OF THE APPENDICES

- (a) Appendix A is divided into 5 sections (A1–A5). These sections contain the analysis of results reported by laboratories for standard plate count, coliforms, *E. coli*, thermophiles and thermophilic spores.

Sections A1, A2, A4 and A5 contain:

- i) a table of results reported by laboratories for each method, with calculated z-scores;
- ii) a listing of the summary statistics;
- iii) ordered z-score charts.

Section A3 contains:

- i) a table of results reported by laboratories for each method.

- (b) Appendix B contains details of the sample preparation, 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 approximately half a log for standard plate count, coliforms, *E. coli*, thermophiles and thermophilic spores. These samples have not been paired when analysed.

### 5. EXTREME 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 three (ie  $<-3$  or  $>3$ ) 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 (2006)*.

The following summaries were sent to participants shortly after the return of results to provide them with “early information” about the results for the program.

**Table A: Summary Statistics for All Tests**

Test	Method	Summary Statistics	Sample 1	Sample 2
SPC (log <sub>10</sub> )	AS5013.1 / AS1766.2.1	No. of Results Median Normalised IQR	45 4.973 0.087	45 4.531 0.101
	Petrifilm	No. of Results Median Normalised IQR	15 5.004 0.050	15 4.556 0.056
Coliforms (log <sub>10</sub> )	AS5013.3 / AS1766.2.3.7	No. of Results Median Normalised IQR	35 2.663 0.490	35 2.449 0.425
	AS5013.4 / AS1766.2.3.5	No. of Results Median Normalised IQR	19 2.799 0.315	19 2.531 0.317
	Petrifilm	No. of Results Median Normalised IQR	26 2.985 0.270	26 2.607 0.236
Thermophiles (log <sub>10</sub> )	Plate count agar / 55°C / 48 hrs	No. of Results Median Normalised IQR	29 3.602 0.088	29 4.033 0.160
Thermophilic Spores (log <sub>10</sub> )	Heat treatment: 80°C / 10 min	No. of Results Median Normalised IQR	19 3.477 0.178	19 3.845 0.109
	Heat treatment: 100°C / 30 min	No. of Results Median Normalised IQR	7 3.230 0.166	7 3.568 0.310

Notes:

1. The results reported are for log<sub>10</sub>(cfu/g) and log<sub>10</sub>(MPN/g).
2. For SPC, the results for AS5013.1 have been combined with the results for AS1766.2.1.
3. For coliforms, the results for AS5013.3 have been combined with the results for AS1766.2.3.7, while the results for AS5013.4 have been combined with the results for AS1766.2.3.5.
4. The levels of *E. coli* in the samples were considered to be too low to analyse the results.

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

Test	Method	Outliers		False Negatives
		Sample 1	Sample 2	
SPC	AS5013.1 / AS1766.2.1	3	3, 26, 44	-
	Petrifilm	19A	-	-
	Other			-
Coliforms	AS5013.3 / AS1766.2.3.7	-	8	-
	AS5013.4 / AS1766.2.3.5	-	-	-
	Petrifilm	4	4, 30	-
	Other			-
Thermophiles	Plate count agar / 55°C / 48 hrs	13, 14, 22, 24, 29, 42	13, 37	-
	Other			-
Thermophilic Spores	Heat treatment: 80°C / 10 min	-	10	-
	Heat treatment: 100°C / 30 min	-	-	-
	Other			-

## 6. PTA AND TECHNICAL ADVISER'S COMMENTS

The summary statistics, outliers and false results identified for each of the tests/methods analysed are reported in Tables A and B above. Complete details of the statistical analyses and the methods used by laboratories for testing appear in Appendix A.

In statistical analysis, where AS1766 methods have been used by participants, these have been analysed with the corresponding AS 5013 results (as seen in Tables A and B).

## 6.1 Return rate

All of the 53 laboratories that participated in the program submitted results for inclusion in the final report. Twenty four laboratories (45%) submitted results where more than one method was used for a specific test. Twenty four laboratories (45%) provided results for all five tests. The return rate for all tests is as follows:

• Standard Plate Count	53 out of 53	100%
• Coliforms	52 out of 53	98%
• <i>E. coli</i>	49 out of 53	92%
• Thermophiles	30 out of 53	57%
• Thermophilic Spores	26 out of 53	49%

## 6.2 Performance summary

One or more statistical outlier results were reported by 15 laboratories (28%) for this first round of the Non-Pathogens in Food program. There were no false negative results reported. The most recent proficiency testing study to which these results can be compared is round 2 of the NATA Dairy program, conducted in December 2002 (see PTAC Report No 415). For round 2 of the NATA Dairy program, 14 of the 55 participants (25%) reported outlier or false negative results.

A total of 424 results were analysed in this round of the program. Of these results, 18 (4%) were outlier results. For comparison, 20 of the 420 results (5%) analysed in round 2 of the NATA Dairy program were outlier or false negative results (see PTAC Report No 415).

## 6.3 Standard Plate Count

Of the 53 laboratories that tested for standard plate count, 10 laboratories tested using more than one method. Thirty six laboratories tested using AS5013.1, while 9 laboratories tested using AS1766.2.1 and these results were combined for analysis. Thirteen laboratories tested using the Petrifilm method, including two laboratories (19 and 33) that submitted two sets of results. Six laboratories tested using other methods. As there were only a small number of results reported for any of the other methods used, only the results using AS5013.1 / AS1766.2.1 and Petrifilm were analysed.

For the AS5013.1 / AS1766.2.1 combined results, laboratory 3 reported an outlier result for sample PTA 1. Three laboratories (3, 26 and 44) reported outlier results for sample PTA 2.

The robust CVs of 1.8% and 2.2% for the AS methods results for this program are comparable to the values of 2.8% and 2.2%, obtained for the AS1766.2.1 results in round 2 of the NATA Dairy program (see PTAC Report No 415).

For the Petrifilm method, one laboratory (19A) reported an outlier result for sample PTA 1. As the z-scores obtained for laboratory 14 for samples PTA 1 and PTA 2 were both marginally  $< -3.0$ , and the number of participants that submitted Petrifilm results for SPC for analysis was moderately low, this laboratory's results have not be regarded as outliers for this method.

The robust CVs of 1.0% and 1.2%, obtained for the Petrifilm results, were quite low.

The median of the Petrifilm results was slightly higher than the median of the AS5013.1 / AS1766.2.1 combined results for both samples.

#### **6.4 Coliforms**

A total of 52 laboratories submitted results for coliforms. Twenty two of these laboratories used more than one method. Twenty four laboratories tested using AS5013.3, while 11 laboratories tested using AS1766.2.3.7. These results were combined when they were analysed (i.e. AS MPN methods). Sixteen laboratories tested using AS5013.4, while 3 laboratories tested using AS1766.2.3.5. These results were also combined when they were analysed (i.e. AS colony count methods). Twenty four laboratories tested using the Petrifilm method, including two laboratories (19 and 33) that submitted two sets of results. Five laboratories tested using other methods. Because of the small number of results reported for other methods, only the results using AS5013.3 / AS1766.2.3.7, AS5013.4 / AS1766.2.3.5 and Petrifilm were analysed.

For the AS5013.3 / AS1766.2.3.7 combined results, no outlier results were reported for sample PTA 1. Laboratory 8 obtained an outlier result for sample PTA 2.

The robust CVs of 18.4% and 17.4% for the AS5013.3 / AS1766.2.3.7 results for this program are quite high when compared to the values of 14.4% and 10.8%, obtained for the AS1766.2.3.7 results in round 2 of the NATA Dairy program (see PTAC Report No 415).

For the AS5013.4 / AS1766.2.3.5 combined results, no outlier results were reported for either sample PTA 1 or sample PTA 2.

The robust CVs of 11.2% and 12.5% for the AS5013.4 / AS1766.2.3.5 results are much lower than the robust CVs obtained for the AS5013.3 / AS1766.2.3.7 results. These robust CVs compare well to the values of 14.0% and 10.8%, obtained for the AS1766.2.3.5 results in round 2 of the NATA Dairy program (see PTAC Report No 415).

For the Petrifilm method, laboratory 4 reported an outlier result for sample PTA 1. Two laboratories (4 and 30) reported outlier results for sample PTA 2.

The robust CVs for Petrifilm were the lowest for the results reported for coliforms, with values of 9.1% and 9.0% for samples PTA 1 and PTA 2, respectively. These robust CVs are lower than the values of 14.8% and 10.3%, obtained for the Petrifilm results in round 2 of the NATA Dairy program (see PTAC Report No 415).

Of the three methods analysed for coliforms, the Petrifilm method had the highest median results for both samples, while the AS5013.3 / AS1766.2.3.7 results had the lowest median results for both samples.

Two laboratories (5 and 42) reported results as “greater than” results for coliforms, using AS5013.3 / AS1766.2.3.7. These participants should note that maximum coliform levels of 50,000 per gram were indicated in the *Instructions to Participants* sheet. Laboratories were required not to report “greater than” results as such data cannot be statistically analysed.

## 6.5 *E. coli*

There were 49 laboratories that tested for *E. coli*. Fourteen of these laboratories used more than one method. Twenty seven laboratories tested using AS5013.15, while 10 laboratories tested using AS1766.2.3.7. Twenty three laboratories tested using the Petrifilm method, including two laboratories (19 and 33) that submitted two sets of results. Four laboratories tested using other methods.

Analysis of the *E. coli* results was not performed because the levels of *E. coli* in the samples were considered to be too low to analyse (see Appendix B).

Laboratory 42 reported a result as a “greater than” result for *E. coli*, using AS5013.15. This participant should note that maximum *E. coli* levels of 5,000 per gram were indicated in the *Instructions to Participants* sheet. Laboratories were required not to report “greater than” results as such data cannot be statistically analysed.

## 6.6 Thermophiles

There were 30 laboratories that tested for thermophiles. One of these laboratories used more than one method. A total of 29 of these laboratories tested using plate count agar at 55°C for 48 hours (former AS1095.3.9), while two laboratories used other methods. There were not enough results reported using other methods to analyse.

Six laboratories (13, 14, 22, 24, 29 and 42) reported outlier results for sample PTA 1. Two laboratories (13 and 37) reported outlier results for sample PTA 2.

The robust CVs of 2.4% and 4.0% compare well with the values of 3.7% and 2.6%, obtained in round 2 of the NATA Dairy program (see PTAC Report No 415).

## **6.7 Thermophilic Spores**

Of the 26 laboratories that submitted results for thermophilic spores, 4 laboratories used more than one method. There were 19 laboratories that tested using the 80°C / 10 minute heat treatment. Seven laboratories tested using the 100°C / 30 minute heat treatment. Four laboratories used other methods. There were not enough results reported using other methods to analyse.

For the 80°C / 10 minute heat treatment, there were no outlier results reported for sample PTA 1. Laboratory 10 reported an outlier result for sample PTA 2.

The robust CVs of 5.1% and 2.8% for the 80°C / 10 minute heat treatment are lower than the values of 9.2% and 7.8%, obtained in round 2 of the NATA Dairy program (see PTAC Report No 415).

For the 100°C / 30 minute heat treatment, there were no outlier results reported for either sample.

The robust CVs for the 100°C / 30 minute heat treatment are 5.1% and 8.7%. These values compare well with the robust CVs of 7.4% and 7.2%, obtained in round 2 of the NATA Dairy program (see PTAC Report No 415).

As with round 2 of the NATA Dairy program, the 80°C / 10 minute heat treatment produced higher thermophilic spore counts than the 100°C / 30 minute heat treatment. This is to be expected, as the former treatment is less severe.

## **6.8 General Comments**

Overall, the performance of laboratories in this program was very good. The proportion of laboratories that reported extreme results and the proportion of extreme results reported in this round are similar to those obtained in round 2 of the NATA Dairy program (see PTAC Report No 415).

The Non-Pathogens in Food program will be conducted twice a year. The second round of this program will commence later in 2006.

## **7. REFERENCES**

*Guide to Proficiency Testing Australia (2006).*

# **APPENDIX A**

## **Summary of Results**

## **Section A1**

### **Standard Plate Count**

## A1.1

## Milk Powder – SPC, AS5013.1 / AS1766.2.1 (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
2	94000	32000	4.97	4.51	0.00	-0.26
3	5500000	3450	6.74	3.54	20.36 §	-9.88 §
5	110000	31000	5.04	4.49	0.79	-0.40
6	105000	39500	5.02	4.60	0.55	0.65
7	83000	30000	4.92	4.48	-0.62	-0.54
8	81000	36000	4.91	4.56	-0.74	0.25
9	103000	41000	5.01	4.61	0.46	0.81
10	92000	45000	4.96	4.65	-0.11	1.21
11	110000	22000	5.04	4.34	0.79	-1.88
12	130000	43000	5.11	4.63	1.62	1.01
13	60000	24000	4.78	4.38	-2.25	-1.50
14	70000	23000	4.85	4.36	-1.47	-1.69
15	110000	43000	5.04	4.63	0.79	1.01
16	86000	32000	4.93	4.51	-0.44	-0.26
18	110000	41000	5.04	4.61	0.79	0.81
21	110000	26000	5.04	4.41	0.79	-1.16
22	56000	28000	4.75	4.45	-2.59	-0.84
23	82000	32000	4.91	4.51	-0.68	-0.26
25	100000	40000	5.00	4.60	0.31	0.70
26	110000	440000	5.04	5.64	0.79	11.06 §
27	84000	42000	4.92	4.62	-0.56	0.91
28	83000	39000	4.92	4.59	-0.62	0.59
29	98000	44000	4.99	4.64	0.21	1.11
30	110000	38000	5.04	4.58	0.79	0.48
31	130000	34000	5.11	4.53	1.62	0.00
32	120000	51000	5.08	4.71	1.22	1.75
34	91000	30000	4.96	4.48	-0.16	-0.54
35	94000	33000	4.97	4.52	0.00	-0.13
36	76000	30000	4.88	4.48	-1.06	-0.54
37	120000	49000	5.08	4.69	1.22	1.58
38	95000	31000	4.98	4.49	0.05	-0.40
39	94000	37000	4.97	4.57	0.00	0.37
40	100000	24000	5.00	4.38	0.31	-1.50
41	110000	30000	5.04	4.48	0.79	-0.54
42	77000	25000	4.89	4.40	-1.00	-1.33
43	90000	46000	4.95	4.66	-0.22	1.31
44	128000	14600	5.11	4.16	1.54	-3.65 §
45	88000	48000	4.94	4.68	-0.33	1.49
47	101000	34000	5.00	4.53	0.36	0.00
48	140000	40000	5.15	4.60	1.99	0.70
50	77000	34000	4.89	4.53	-1.00	0.00
51	92000	22000	4.96	4.34	-0.11	-1.88

## A1.2

### Milk Powder – SPC, AS5013.1 / AS1766.2.1 (cfu/g)

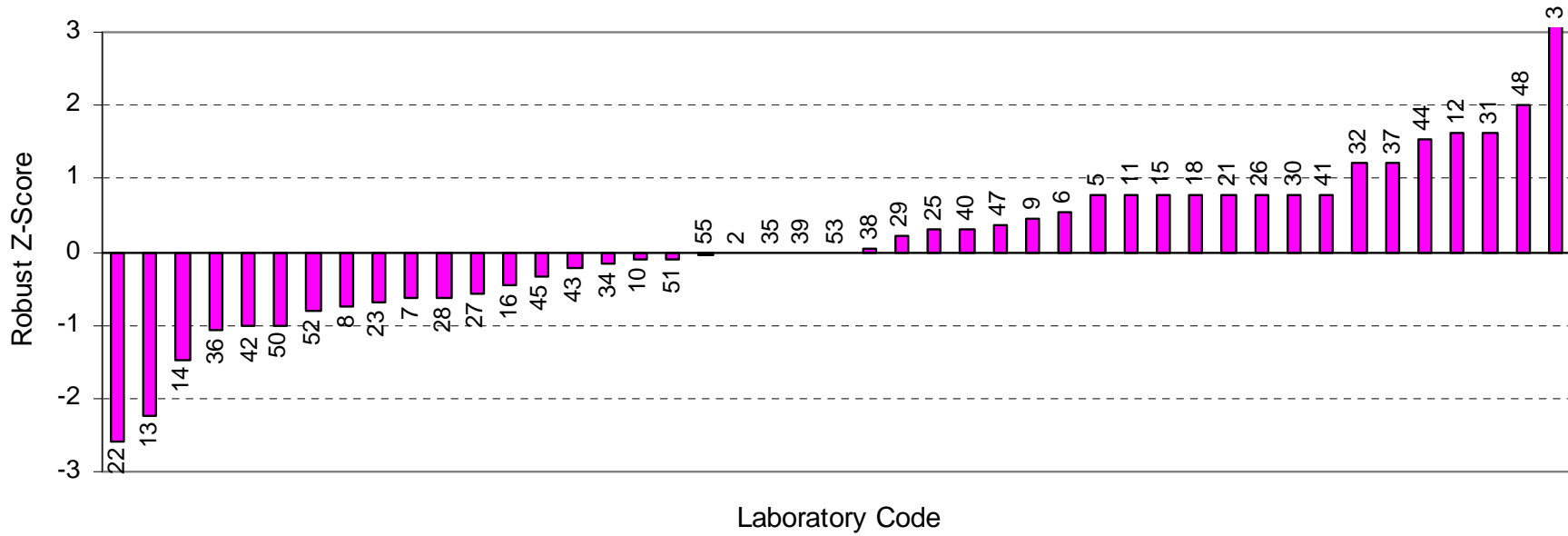
Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
52	80000	36000	4.90	4.56	-0.81	0.25
53	94000	35000	4.97	4.54	0.00	0.13
55	93000	33000	4.97	4.52	-0.05	-0.13

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	45	45
Median	4.973	4.531
Norm IQR	0.087	0.101
Robust CV	1.75%	2.22%
Minimum	4.75	3.54
Maximum	6.74	5.64
Range	1.99	2.11

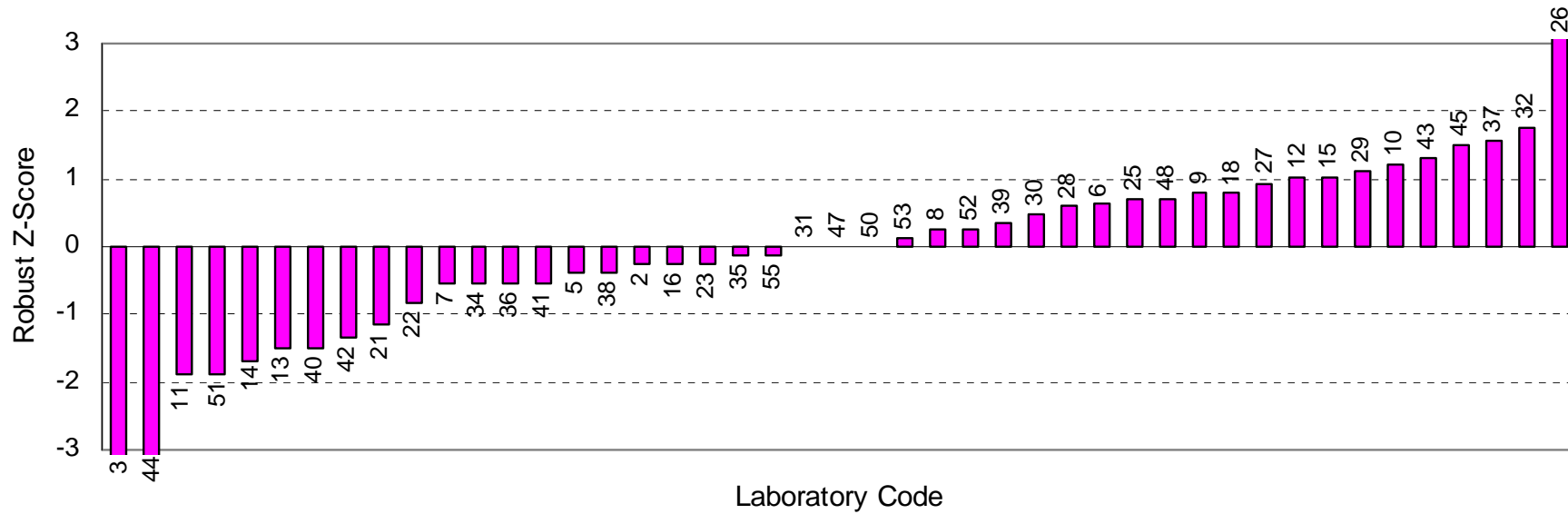
#### Notes:

1. § denotes an outlier (i.e. |z-score| > 3).
2. The results for AS5013.1 have been combined with the results for AS1766.2.1.
3. Laboratories 5, 10, 15, 22, 23, 35, 39, 51 and 52 used AS1766.2.1 for their SPC results.

Milk Powder - SPC, AS5013.1 / AS1766.2.1 [log(cfu/g)] - Sample 1



Milk Powder - SPC, AS5013.1 / AS1766.2.1 [log(cfu/g)] - Sample 2



## A1.4

### Milk Powder – SPC, Petrifilm (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
1	110000	47000	5.04	4.67	0.73	2.05
6	115000	37500	5.06	4.57	1.12	0.31
14	71000	24000	4.85	4.38	-3.03	-3.12
16	100000	36000	5.00	4.56	-0.09	0.00
17	130000	33000	5.11	4.52	2.17	-0.67
19A	195000	40000	5.29	4.60	5.66 §	0.81
19B	119000	36000	5.08	4.56	1.41	0.00
21	92000	35000	4.96	4.54	-0.80	-0.22
28	93000	35000	4.97	4.54	-0.71	-0.22
29	100000	33000	5.00	4.52	-0.09	-0.67
31	100000	37000	5.00	4.57	-0.09	0.21
33A	101000	42500	5.00	4.63	0.00	1.28
33B	128000	26500	5.11	4.42	2.04	-2.36
43	100000	43000	5.00	4.63	-0.09	1.37
47	106000	41000	5.03	4.61	0.42	1.00

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	15	15
Median	5.004	4.556
Norm IQR	0.050	0.056
Robust CV	1.01%	1.24%
Minimum	4.85	4.38
Maximum	5.29	4.67
Range	0.44	0.29

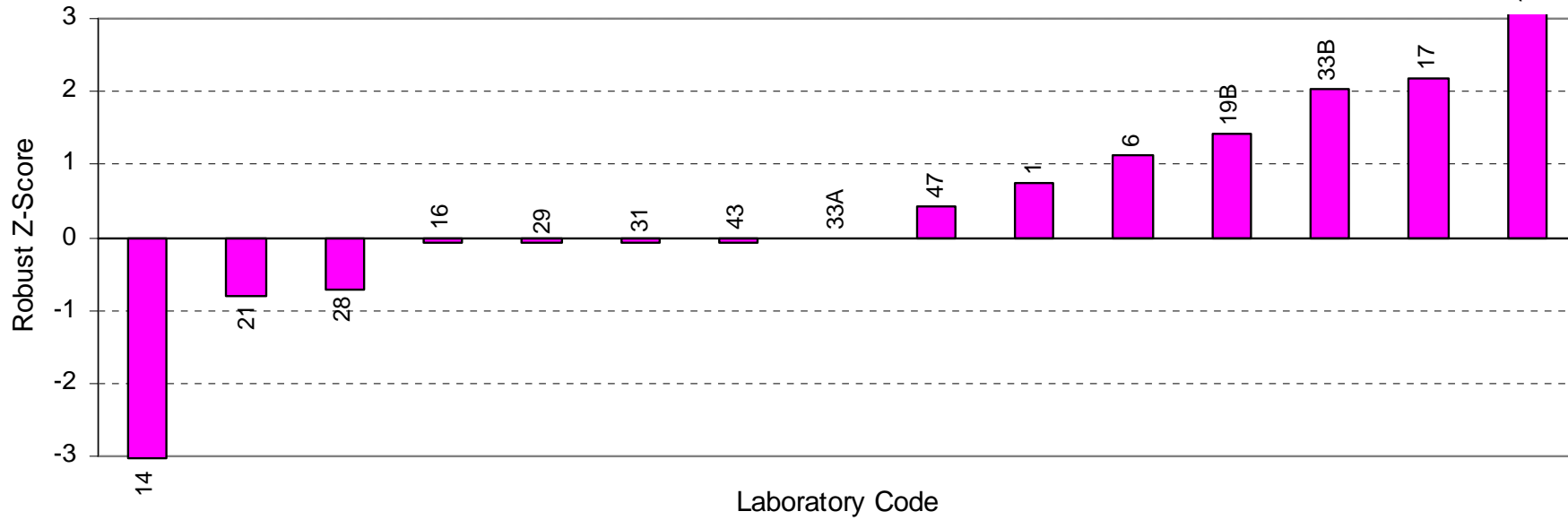
### Milk – SPC, Other Methods (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Method
4	110000	35000	5.04	4.54	Spiral plate
10	140000	50000	5.15	4.70	Spiral plate
24	160000	38000	5.20	4.58	AOAC 977.27
31	120000	37000	5.08	4.57	Spiral plate
46	120000	45000	5.08	4.65	AS1766.1.3
49	120000	42000	5.08	4.62	Spiral plate

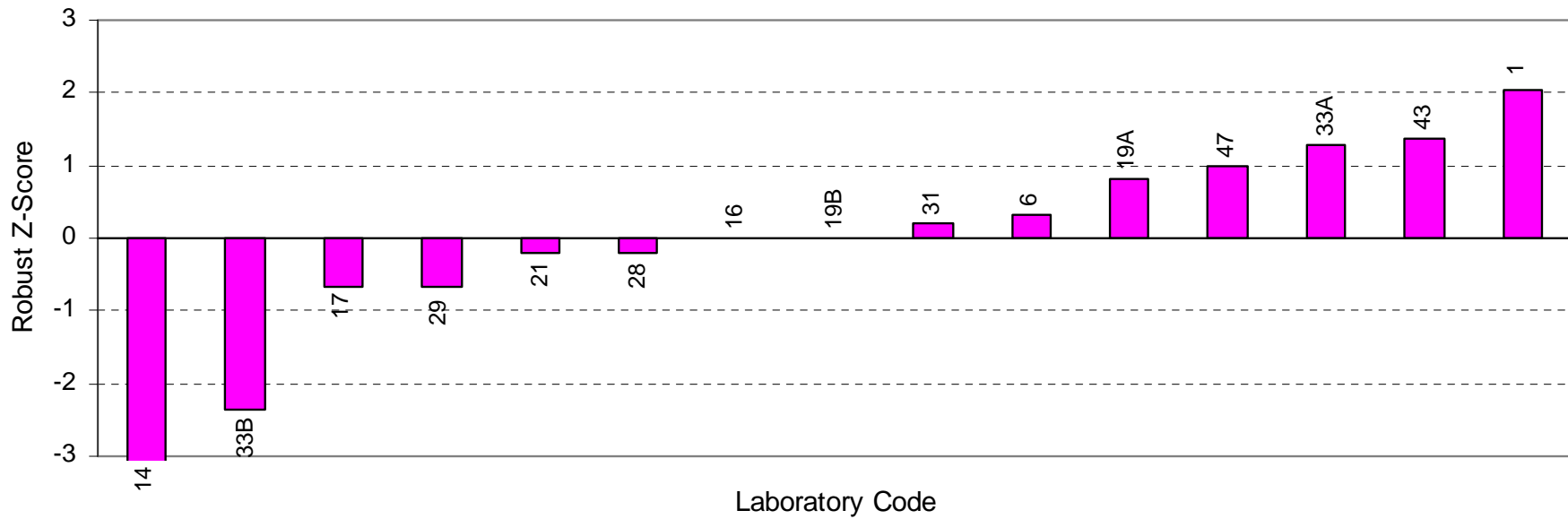
**Notes:**

1. § denotes an outlier (i.e. |z-score| > 3).
2. The results for laboratory were not regarded as outliers.
3. There were not enough SPC results reported using methods other than AS5013.1, AS1766.2.1 or Petrifilm to calculate z-scores.

Milk Powder - SPC, Petrifilm [log(cfu/g)] - Sample 1



Milk Powder - SPC, Petrifilm [log(cfu/g)] - Sample 2



## **Section A2**

### **Coliforms**

## A2.1

## Milk Powder – Coliforms, AS5013.3 / AS1766.2.3.7 (MPN/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
2	460	460	2.66	2.66	0.00	0.50
3	75	93	1.88	1.97	-1.61	-1.13
5	>110	46	-	1.66	-	-1.85
7	75	93	1.88	1.97	-1.61	-1.13
8	38	3.6	1.58	0.56	-2.21	-4.45 §
10	240	240	2.38	2.38	-0.58	-0.16
11	110	110	2.04	2.04	-1.27	-0.96
12	1300	330	3.11	2.52	0.92	0.16
13	930	430	2.97	2.63	0.62	0.43
14	240	93	2.38	1.97	-0.58	-1.13
15	2300	4300	3.36	3.63	1.43	2.78
16	460	460	2.66	2.66	0.00	0.50
22	460	150	2.66	2.18	0.00	-0.64
23	330	330	2.52	2.52	-0.29	0.16
24	350	130	2.54	2.11	-0.24	-0.79
25	230	230	2.36	2.36	-0.61	-0.21
26	1110	240	3.05	2.38	0.78	-0.16
27	46	24	1.66	1.38	-2.04	-2.51
29	2300	420	3.36	2.62	1.43	0.41
30	930	430	2.97	2.63	0.62	0.43
31	3500	700	3.54	2.85	1.80	0.93
35	350	170	2.54	2.23	-0.24	-0.51
37	750	430	2.88	2.63	0.43	0.43
38	430	430	2.63	2.63	-0.06	0.43
39	540	350	2.73	2.54	0.14	0.22
40	430	230	2.63	2.36	-0.06	-0.21
42	> 110	> 110	-	-	-	-
43	1100	460	3.04	2.66	0.77	0.50
44	490	35	2.69	1.54	0.06	-2.13
45	1100	2400	3.04	3.38	0.77	2.19
46	170	79	2.23	1.90	-0.88	-1.30
48	460	540	2.66	2.73	0.00	0.67
51	2400	430	3.38	2.63	1.46	0.43
52	2400	240	3.38	2.38	1.46	-0.16
55	2400	930	3.38	2.97	1.46	1.22

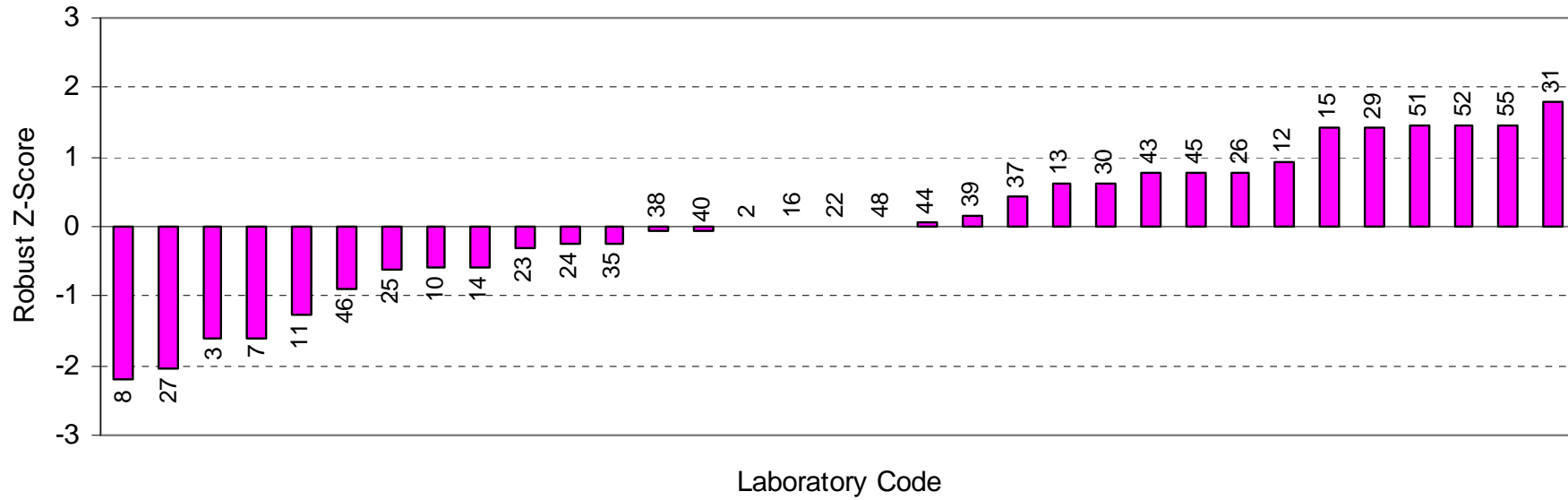
## A2.2

<b>Statistic</b>	<b>Log<sub>10</sub> Sample 1</b>	<b>Log<sub>10</sub> Sample 2</b>
No of Results	33	34
Median	2.663	2.449
Norm IQR	0.490	0.425
Robust CV	18.41%	17.37%
Minimum	1.58	0.56
Maximum	3.54	3.63
Range	1.96	3.08

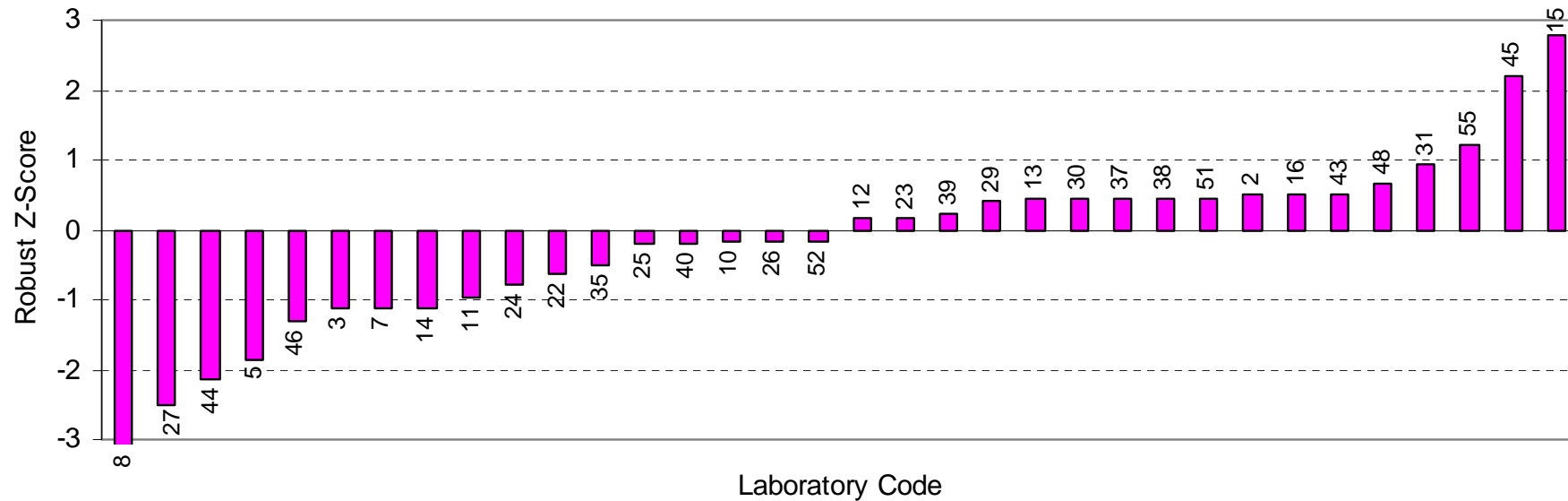
### Notes:

1. § denotes an outlier (i.e.  $|z\text{-score}| > 3$ ).
2. The results for AS5013.3 have been combined with the results for AS1766.2.3.7.
3. Laboratories 5, 10, 11, 15, 22, 23, 35, 39, 46, 51 and 52 used AS1766.2.3.7 for their coliforms results.

Milk Powder - Coliforms, AS5013.3 / AS1766.2.3.7 [log(MPN/g)] - Sample 1



Milk Powder - Coliforms, AS5013.3 / AS1766.2.3.7 [log(MPN/g)] - Sample 2



## A2.4

### Milk Powder – Coliforms, AS5013.4 / AS1766.2.3.5 (cfu/g)

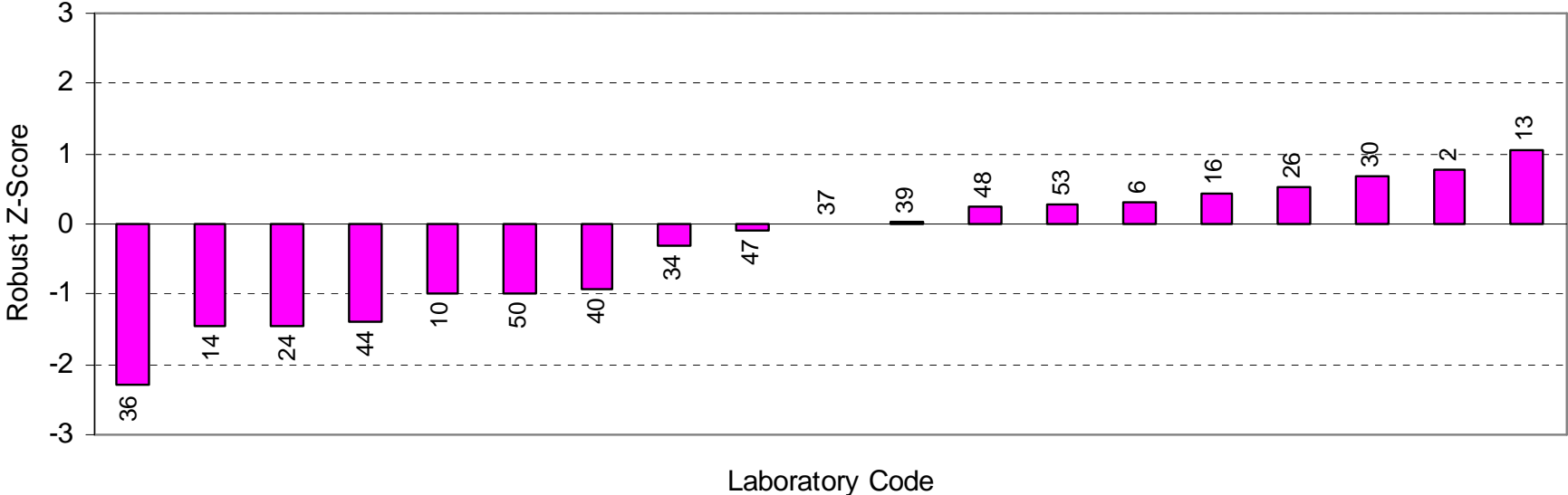
Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
2	1100	610	3.04	2.79	0.77	0.80
6	780	585	2.89	2.77	0.29	0.74
10	310	180	2.49	2.26	-0.98	-0.87
13	1340	600	3.13	2.78	1.04	0.78
14	220	160	2.34	2.20	-1.45	-1.03
16	870	460	2.94	2.66	0.45	0.41
24	220	260	2.34	2.41	-1.45	-0.37
26	925	515	2.97	2.71	0.53	0.57
30	1040	1230	3.02	3.09	0.69	1.76
34	500	200	2.70	2.30	-0.32	-0.73
36	120	120	2.08	2.08	-2.29	-1.43
37	630	340	2.80	2.53	0.00	0.00
39	640	400	2.81	2.60	0.02	0.22
40	325	130	2.51	2.11	-0.91	-1.32
44	230	180	2.36	2.26	-1.39	-0.87
47	590	280	2.77	2.45	-0.09	-0.27
48	760	500	2.88	2.70	0.26	0.53
50	310	240	2.49	2.38	-0.98	-0.48
53	770	450	2.89	2.65	0.28	0.38

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	19	19
Median	2.799	2.531
Norm IQR	0.315	0.317
Robust CV	11.24%	12.51%
Minimum	2.08	2.08
Maximum	3.13	3.09
Range	1.05	1.01

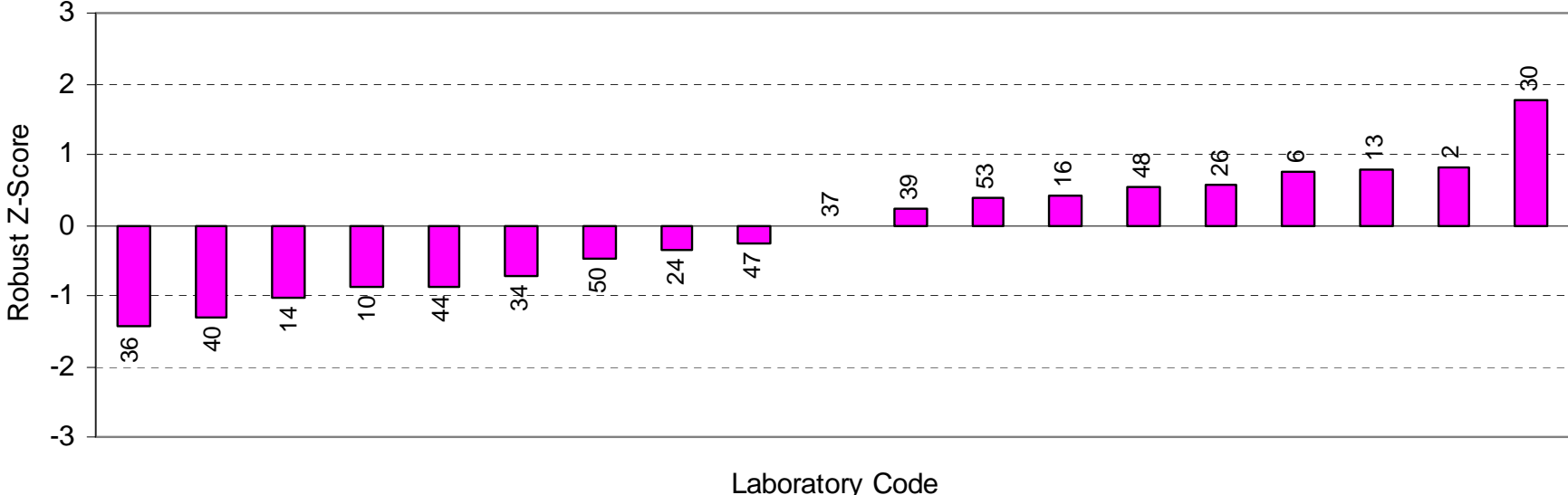
**Notes:**

1. The results for AS5013.4 have been combined with the results for AS1766.2.3.5.
2. Laboratories 10, 16 and 39 used AS1766.2.3.5 for their coliforms results.

Milk Powder - Coliforms, AS5013.4 / AS1766.2.3.5 [log(cfu/g)] - Sample 1



Milk Powder - Coliforms, AS5013.4 / AS1766.2.3.5 [log(cfu/g)] - Sample 2



**A2.6**

**Milk Powder – Coliforms, Petrifilm (cfu/g)**

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
1	940	660	2.97	2.82	-0.05	0.90
2	1200	570	3.08	2.76	0.35	0.63
4	120	58	2.08	1.76	-3.36 §	-3.58 §
6	370	525	2.57	2.72	-1.54	0.48
9	1400	600	3.15	2.78	0.59	0.72
10	440	230	2.64	2.36	-1.27	-1.04
13	1000	400	3.00	2.60	0.05	-0.02
14	400	220	2.60	2.34	-1.42	-1.12
16	1300	810	3.11	2.91	0.48	1.28
17	600	430	2.78	2.63	-0.77	0.11
18	1600	1000	3.20	3.00	0.81	1.67
19A	690	300	2.84	2.48	-0.54	-0.55
19B	505	255	2.70	2.41	-1.04	-0.85
21	1220	850	3.09	2.93	0.37	1.37
28	400	300	2.60	2.48	-1.42	-0.55
29	1400	300	3.15	2.48	0.59	-0.55
30	1650	2150	3.22	3.33	0.86	3.08 §
31	1400	1100	3.15	3.04	0.59	1.84
33A	995	275	3.00	2.44	0.05	-0.71
33B	1045	265	3.02	2.42	0.12	-0.78
34	1100	300	3.04	2.48	0.21	-0.55
37	400	280	2.60	2.45	-1.42	-0.68
43	1000	410	3.00	2.61	0.05	0.02
47	690	310	2.84	2.49	-0.54	-0.49
48	590	570	2.77	2.76	-0.79	0.63
49	750	410	2.88	2.61	-0.41	0.02

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	26	26
Median	2.985	2.607
Norm IQR	0.270	0.236
Robust CV	9.05%	9.04%
Minimum	2.08	1.76
Maximum	3.22	3.33
Range	1.14	1.57

## A2.7

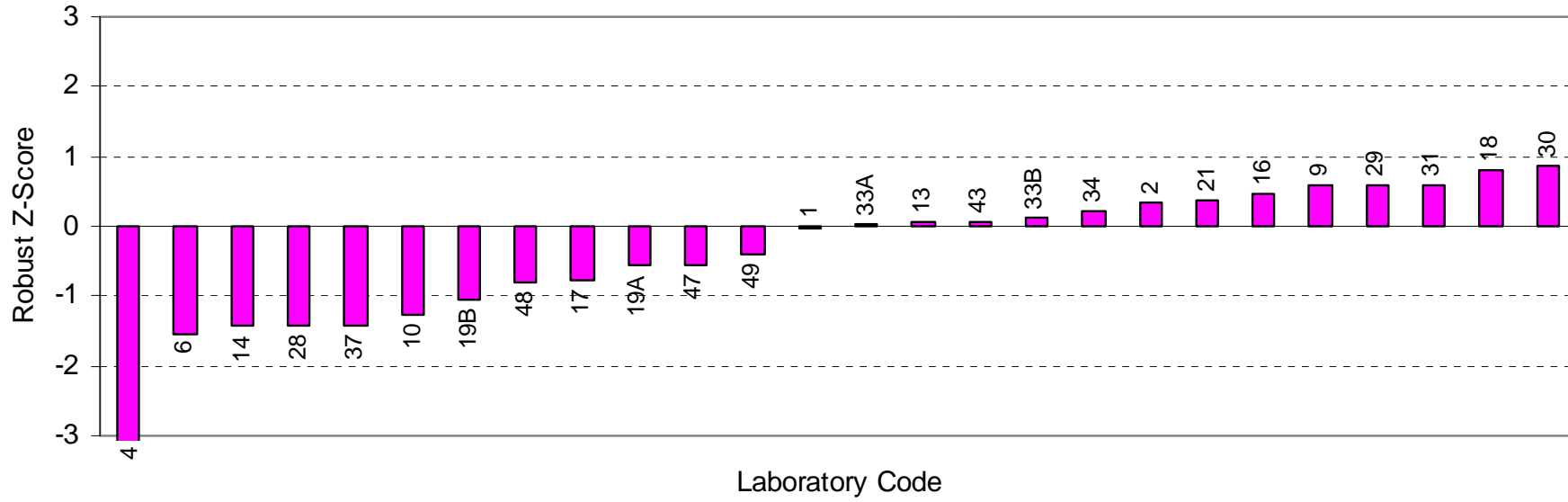
### Milk Powder – Coliforms, Other Methods (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Method
21	460	240	2.66	2.38	In house
31	1100	980	3.04	2.99	Coli ID
38	670	360	2.83	2.56	Coli ID
41	620	420	2.79	2.62	Coli ID
42	530	330	2.72	2.52	VRBA (MCOSO BFL)

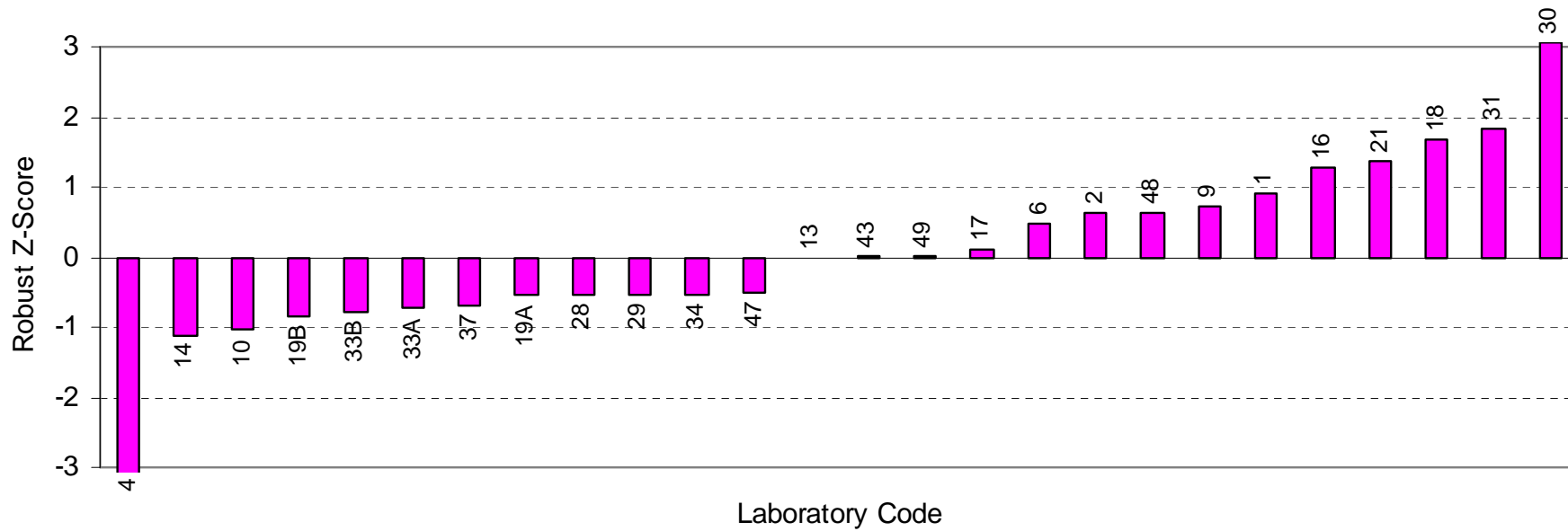
#### Notes:

1. § denotes an outlier (i.e.  $|z\text{-score}| > 3$ ).
2. There were not enough coliforms results reported using methods other than AS5013.3, AS5013.4, AS1766.2.3.5, AS1766.2.3.7 or Petrifilm to calculate z-scores.

Milk Powder - Coliforms, Petrifilm [log(cfu/g)] - Sample 1



Milk Powder - Coliforms, Petrifilm [log(cfu/g)] - Sample 2



## **Section A3**

***E. coli***

## A3.1

Milk Powder – *E. coli*, AS5013.15 / AS1766.2.3.7 (MPN/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
2	110	240	2.04	2.38
3	15	93	1.18	1.97
5	7.5	15	0.88	1.18
7	4	4	0.60	0.60
8	< 3	< 3	-	-
10	23	93	1.36	1.97
11	110	110	2.04	2.04
12	140	46	2.15	1.66
13	93	23	1.97	1.36
14	9	23	0.95	1.36
16	93	120	1.97	2.08
22	23	9	1.36	0.95
23	49	33	1.69	1.52
24	33	49	1.52	1.69
25	230	93	2.36	1.97
26	23	150	1.36	2.18
27	0.92	2.3	-0.04	0.36
28	23	23	1.36	1.36
29	92	92	1.96	1.96
30	930	230	2.97	2.36
31	33	110	1.52	2.04
35	20	14	1.30	1.15
37	93	230	1.97	2.36
39	49	17	1.69	1.23
40	15	43	1.18	1.63
42	110	> 110	2.04	-
43	100	300	2.00	2.48
44	11	11	1.04	1.04
45	150	1100	2.18	3.04
46	11	14	1.04	1.15
47	9	4	0.95	0.60
48	110	170	2.04	2.23
50	23	43	1.36	1.63
51	23	43	1.36	1.63
52	240	93	2.38	1.97
53	23	150	1.36	2.18
55	240	150	2.38	2.18

**A3.2**

**Milk Powder – *E. coli*, Petrifilm (cfu/g)**

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
2	< 10	10	-	1.00
4	6	10	0.78	1.00
6	0	80	-	1.90
9	90	100	1.95	2.00
10	20	60	1.30	1.78
13	110	60	2.04	1.78
14	10	22	1.00	1.34
16	20	50	1.30	1.70
17	40	90	1.60	1.95
18	130	200	2.11	2.30
19A	5	10	0.70	1.00
19B	5	10	0.70	1.00
21	50	90	1.70	1.95
28	100	100	2.00	2.00
29	300	100	2.48	2.00
30	105	170	2.02	2.23
31	35	45	1.54	1.65
32	10	10	1.00	1.00
33A	30	25	1.48	1.40
33B	35	25	1.54	1.40
37	40	70	1.60	1.85
43	110	240	2.04	2.38
47	15	-	1.18	-
48	40	80	1.60	1.90
49	30	70	1.48	1.85

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

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Method
21	43	93	1.63	1.97	In house
31	95	190	1.98	2.28	Coli ID
38	50	100	1.70	2.00	Coli ID
41	70	100	1.85	2.00	Coli ID

### A3.3

#### Notes:

1. Z-scores and summary statistics have not been calculated for the *E. coli* results because the levels of *E. coli* in the samples were too low to analyse.
2. The results for laboratory 8, using AS5013.15, were not considered to be false negative results because the levels of *E. coli* in the samples were low.
3. The results for laboratories 2 and 6 for sample 1 and the result for laboratory 47 for sample 2, using Petrifilm, were not considered to be false negative results because the levels of *E. coli* in the samples were low.
4. Laboratories 5, 10, 11, 22, 23, 35, 39, 46, 51 and 52 used AS1766.2.3.7 for their *E. coli* MPN results.
5. Laboratory 38 had a control failure for *E. coli*, using AS5013.15, and was unable to report results for this method.
6. The results for laboratory 49, using Petrifilm, are estimates only.

**Section A4**  
**Thermophiles**

### A4.1

#### Milk Powder – Thermophiles, Plate Count Agar / 55°C / 48 hrs (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
1	4000	10800	3.60	4.03	0.00	0.00
2	4000	13000	3.60	4.11	0.00	0.50
5	3400	10000	3.53	4.00	-0.80	-0.21
8	3500	8800	3.54	3.94	-0.66	-0.55
10	4100	7900	3.61	3.90	0.12	-0.85
12	5300	13000	3.72	4.11	1.39	0.50
13	1300	1900	3.11	3.28	-5.56 §	-4.71 §
14	58000	22000	4.76	4.34	13.23 §	1.93
15	6800	16000	3.83	4.20	2.63	1.06
16	3200	7400	3.51	3.87	-1.10	-1.02
21	2400	7500	3.38	3.88	-2.53	-0.99
22	2100	6700	3.32	3.83	-3.19 §	-1.29
24	15000	14000	4.18	4.15	6.54 §	0.70
25	4000	6900	3.60	3.84	0.00	-1.21
29	1400	4400	3.15	3.64	-5.19 §	-2.43
34	3200	8500	3.51	3.93	-1.10	-0.65
35	4200	11000	3.62	4.04	0.24	0.05
36	3700	11000	3.57	4.04	-0.39	0.05
37	3900	35000	3.59	4.54	-0.13	3.18 §
39	4200	12000	3.62	4.08	0.24	0.29
42	2000	8000	3.30	3.90	-3.43 §	-0.81
44	3750	6100	3.57	3.79	-0.32	-1.55
45	4600	17000	3.66	4.23	0.69	1.23
46	5000	12000	3.70	4.08	1.10	0.29
47	3350	11000	3.53	4.04	-0.88	0.05
48	4200	11000	3.62	4.04	0.24	0.05
50	4400	8800	3.64	3.94	0.47	-0.55
51	3800	9200	3.58	3.96	-0.25	-0.43
53	5300	15000	3.72	4.18	1.39	0.89

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	29	29
Median	3.602	4.033
Norm IQR	0.088	0.160
Robust CV	2.44%	3.98%
Minimum	3.11	3.28
Maximum	4.76	4.54
Range	1.65	1.27

## A4.2

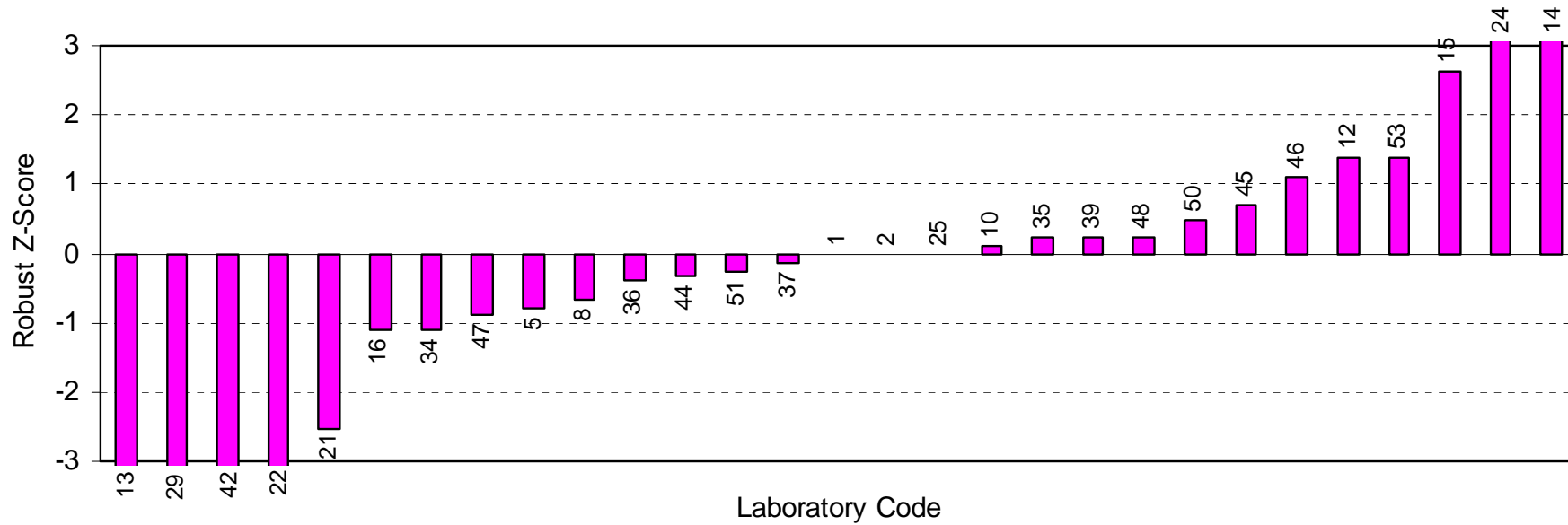
### Milk Powder – Thermophiles, Other Methods (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Method
21	2900	7600	3.46	3.88	In house
40	5000	5600	3.70	3.75	Tryptone soya agar

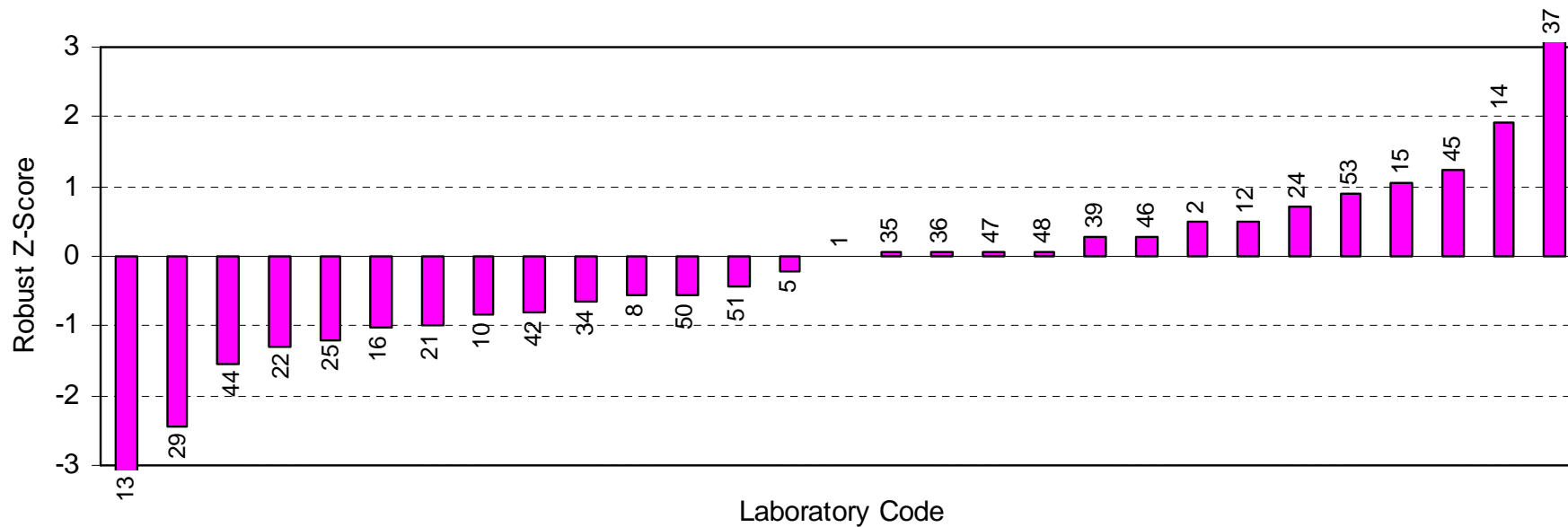
#### Notes:

1. § denotes an outlier (i.e.  $|z\text{-score}| > 3$ ).
2. There were not enough thermophiles results reported using other methods to calculate z-scores.

Milk Powder – Thermophiles, Plate Count Agar / 55°C / 48 hrs [log(cfu/g)] - Sample 1



Milk Powder – Thermophiles, Plate Count Agar / 55°C / 48 hrs [log(cfu/g)] - Sample 2



## **Section A5**

# **Thermophilic Spores**

## A5.1

### Milk Powder – Thermophilic Spores, Heat Treatment: 80°C / 10 min (cfu/g)

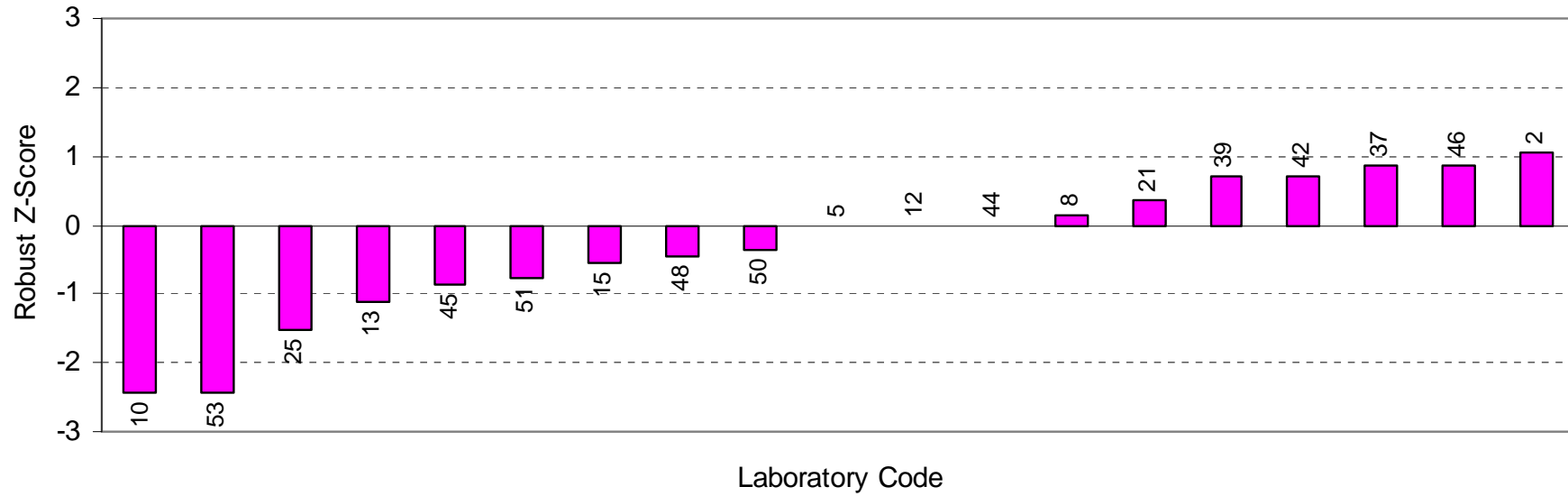
Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
2	4600	8800	3.66	3.94	1.04	0.91
5	3000	6700	3.48	3.83	0.00	-0.18
8	3200	8400	3.51	3.92	0.16	0.73
10	1100	2000	3.04	3.30	-2.44	-5.01 §
12	3000	8700	3.48	3.94	0.00	0.87
13	1900	4000	3.28	3.60	-1.11	-2.24
15	2400	4400	3.38	3.64	-0.54	-1.86
21	3500	8400	3.54	3.92	0.38	0.73
25	1600	3400	3.20	3.53	-1.53	-2.89
37	4300	11000	3.63	4.04	0.88	1.81
39	4000	9000	3.60	3.95	0.70	1.00
42	4000	7000	3.60	3.85	0.70	0.00
44	3000	7450	3.48	3.87	0.00	0.25
45	2100	6000	3.32	3.78	-0.87	-0.62
46	4300	9400	3.63	3.97	0.88	1.18
48	2500	6700	3.40	3.83	-0.44	-0.18
50	2600	6200	3.41	3.79	-0.35	-0.48
51	2200	6600	3.34	3.82	-0.75	-0.24
53	1100	7000	3.04	3.85	-2.44	0.00

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	19	19
Median	3.477	3.845
Norm IQR	0.178	0.109
Robust CV	5.13%	2.83%
Minimum	3.04	3.30
Maximum	3.66	4.04
Range	0.62	0.74

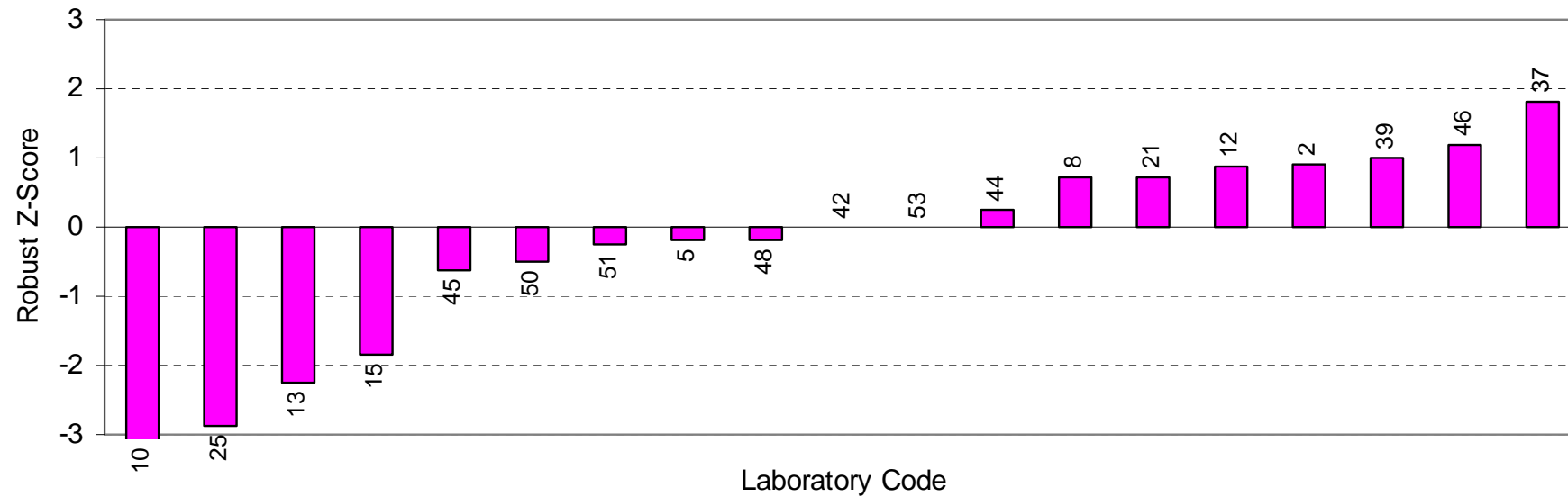
**Notes:**

- § denotes an outlier (i.e. |z-score| > 3).

Milk Powder – Thermophilic Spores, Heat Treatment: 80°C / 10 min [log(cfu/g)] - Sample 1



Milk Powder – Thermophilic Spores, Heat Treatment: 80°C / 10 min [log(cfu/g)] - Sample 2



### A5.3

#### Milk Powder – Thermophilic Spores, Heat Treatment: 100°C / 30 min (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Robust Z-Score Sample 1	Robust Z-Score Sample 2
14	700	1300	2.85	3.11	-2.33	-1.47
15	1700	11000	3.23	4.04	0.00	1.53
21	3000	8000	3.48	3.90	1.49	1.08
22	1100	2800	3.04	3.45	-1.14	-0.39
29	1300	2500	3.11	3.40	-0.70	-0.55
42	2000	3700	3.30	3.57	0.43	0.00
48	2000	6000	3.30	3.78	0.43	0.68

Statistic	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2
No of Results	7	7
Median	3.230	3.568
Norm IQR	0.166	0.310
Robust CV	5.13%	8.69%
Minimum	2.85	3.11
Maximum	3.48	4.04
Range	0.63	0.93

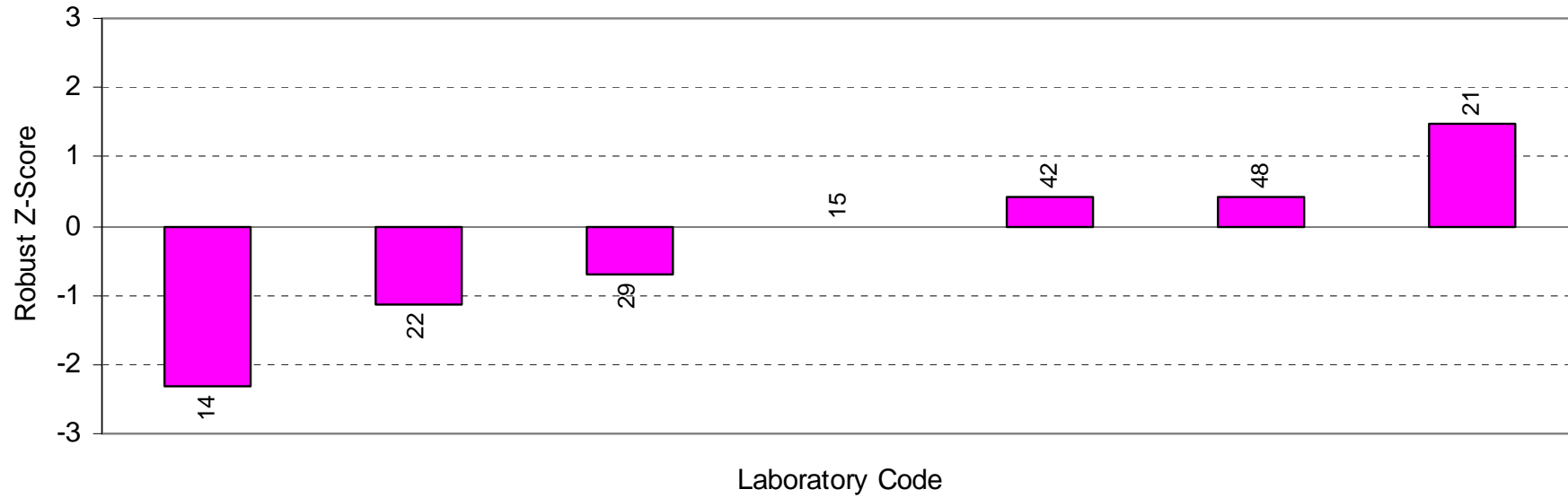
#### Milk Powder – Thermophilic Spores, Other Methods (cfu/g)

Lab Code	Sample 1	Sample 2	Log <sub>10</sub> Sample 1	Log <sub>10</sub> Sample 2	Method
24	1200	2600	3.08	3.41	ICUMSA 652/3-49
35	1500	7000	3.18	3.85	80°C/10 min, PCA
36	5200	7000	3.72	3.85	100°C / 10 mins, MPC 55°C / 48 hrs
40	350	814	2.54	2.91	80°C / 10 min including skim milk 1.0% PCA / 55°C / 48 hrs

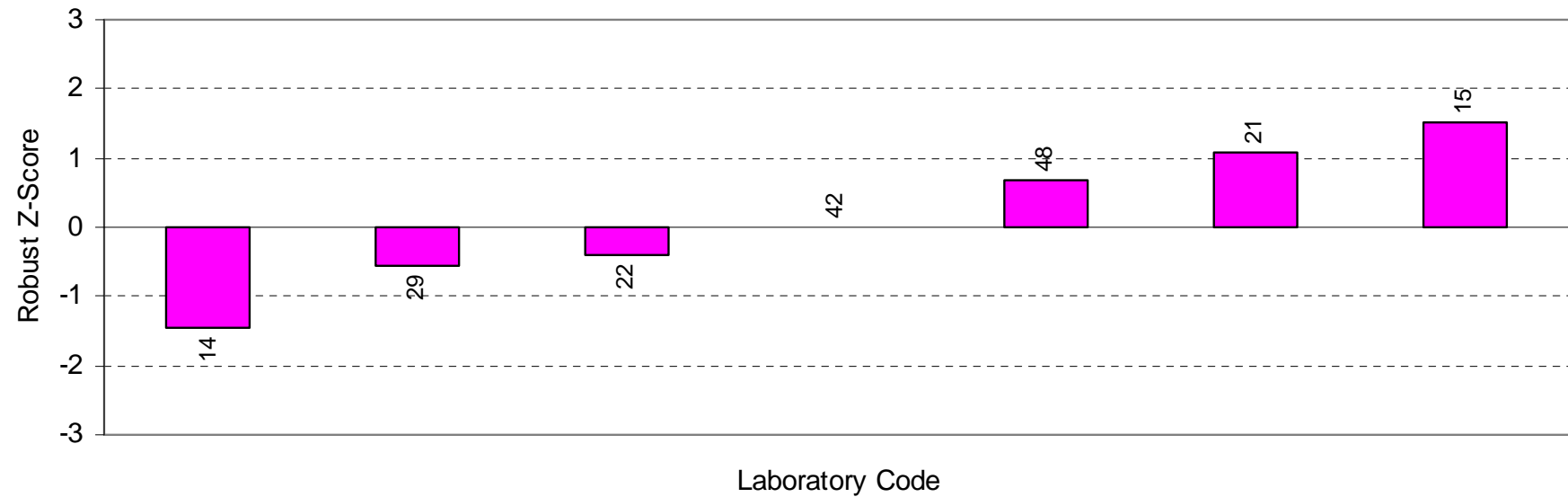
#### Notes:

1. There were not enough thermophilic spores results reported using other methods to calculate z-scores.

Milk Powder – Thermophilic Spores, Heat Treatment: 100°C / 30 min [log(cfu/g)] - Sample 1



Milk Powder – Thermophilic Spores, Heat Treatment: 100°C / 30 min [log(cfu/g)] - Sample 2



# **APPENDIX B**

## **Homogeneity and Stability Testing**

## B1.1

### SAMPLE PREPARATION

Two batches of skim milk powder were prepared by Food Science Australia. One batch was to contain approximately 80,000 cfu/g aerobic mesophiles (for the standard plate count test), 5,000 cfu/g coliforms, 500 cfu/g *E. coli*, 10,000 cfu/g thermophiles and 3,000 cfu/g thermophilic spores. The other batch was to contain approximately 30,000 cfu/g aerobic mesophiles, 2,000 cfu/g coliforms, 1,000 cfu/g *E. coli*, 30,000 cfu/g thermophiles and 10,000 cfu/g thermophilic spores.

Each batch of milk powder was split into 80 sub-samples of approximately 25g and added to sterile plastic bags. The sub-samples from the respective batches of milk powder were labelled PTA 1 and PTA 2. One set of each of the two sub-samples was packed into a plastic bag, sealed and sent to each of the participating laboratories by overnight courier in eskies with freeze bricks. A bottle of water was included in each esky so that the temperature of the samples upon arrival could be monitored. Sample dispatch occurred on 20 February 2006.

### HOMOGENEITY TESTING

Ten sets of sub-samples, chosen at random, were retained for homogeneity testing by Food Science Australia. These ten sets of sub-samples were tested on 14 February 2006.

#### Standard Plate Count

The ten samples from PTA 1 and PTA 2 were tested in duplicate for standard plate count using AS5013.1. The results of this homogeneity testing appear in the following table.

Standard Plate Count (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
100000	5.00	*		40000	4.60	33000	4.52
76000	4.88	83000	4.92	34000	4.53	35000	4.54
160000	5.20	82000	4.91	36000	4.56	37000	4.57
110000	5.04	110000	5.04	31000	4.49	25000	4.40
85000	4.93	83000	4.92	53000	4.72	29000	4.46
97000	4.99	80000	4.90	37000	4.57	48000	4.68
75000	4.88	120000	5.08	30000	4.48	23000	4.36
160000	5.20	94000	4.97	27000	4.43	42000	4.62
80000	4.90	95000	4.98	27000	4.43	30000	4.48
130000	5.11	92000	4.96	24000	4.38	37000	4.57

\* Results unavailable (plate contaminated).

## B1.2

### Coliforms

The ten samples from PTA 1 and PTA 2 were tested in duplicate for coliforms using VRBA pour plates. The results of this homogeneity testing appear in the following table.

Coliforms (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
1000	3.00	980	2.99	570	2.76	710	2.85
1100	3.04	900	2.95	640	2.81	620	2.79
1100	3.04	1100	3.04	530	2.72	770	2.89
1400	3.15	1100	3.04	560	2.75	430	2.63
1200	3.08	1200	3.08	650	2.81	430	2.63
1100	3.04	1200	3.08	490	2.69	530	2.72
1200	3.08	1100	3.04	570	2.76	410	2.61
1100	3.04	980	2.99	560	2.75	510	2.71
1100	3.04	1100	3.04	450	2.65	530	2.72
1000	3.00	1000	3.00	500	2.70	520	2.72

### *E. coli*

The ten samples from PTA 1 and PTA 2 were tested in duplicate for *E. coli* using Petrifilm. The results of this homogeneity testing appear in the following table.

<i>E. coli</i> (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
85	1.93	70	1.85	130	2.11	95	1.98
95	1.98	100	2.00	120	2.08	100	2.00
65	1.81	80	1.90	120	2.08	95	1.98
85	1.93	80	1.90	40	1.60	60	1.78
75	1.88	60	1.78	100	2.00	90	1.95
95	1.98	60	1.78	95	1.98	110	2.04
70	1.85	60	1.78	70	1.85	85	1.93
55	1.74	55	1.74	40	1.60	130	2.11
90	1.95	80	1.90	90	1.95	65	1.81
50	1.70	55	1.74	70	1.85	85	1.93

## B1.3

### Thermophiles

The ten samples from PTA 1 and PTA 2 were tested in duplicate for thermophiles using a plate count agar / 55°C / 48 hour method. The results of this homogeneity testing appear in the following table.

Thermophiles (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
4500	3.65	5200	3.72	11000	4.04	12000	4.08
4000	3.60	3500	3.54	10000	4.00	11000	4.04
4300	3.63	4400	3.64	*		*	
4800	3.68	4200	3.62	11000	4.04	12000	4.08
3900	3.59	4500	3.65	15000	4.18	11000	4.04
3900	3.59	4000	3.60	9100	3.96	13000	4.11
4800	3.68	5500	3.74	12000	4.08	9300	3.97
5300	3.72	4000	3.60	11000	4.04	14000	4.15
4000	3.60	4800	3.68	10000	4.00	12000	4.08
3700	3.57	4300	3.63	*		12000	4.08

\* Results unavailable (sample incorrectly incubated).

### Comments on the Homogeneity Testing

The analysis of the homogeneity data indicated that sample PTA 1 was sufficiently homogeneous for use in the program. Sample PTA 2, however, was not homogeneous for *E. coli*. The levels of *E. coli* in both samples were considered to be too low.

### STABILITY TESTING

Three sets of sub-samples, chosen at random, were retained for stability testing by Food Science Australia for standard plate count, coliforms and thermophiles. Due to concern about the low levels of *E. coli* in the samples, ten sets of sub-samples, chosen at random, were retained for the *E. coli* stability testing by Food Science Australia. These sets of sub-samples were tested on 27 February 2006, one week after sample dispatch.

### Standard Plate Count

The three samples from PTA 1 and PTA 2 were tested in duplicate for standard plate count using AS5013.1. The results of this stability testing appear in the following table.

## B1.4

Standard Plate Count (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
140000	5.15	91000	4.96	24000	4.38	48000	4.68
120000	5.08	82000	4.91	40000	4.60	35000	4.54
89000	4.95	91000	4.96	35000	4.54	54000	4.73

### Coliforms

The three samples from PTA 1 and PTA 2 were tested in duplicate for coliforms using VRBA pour plates. The results of this stability testing appear in the following table.

Coliforms (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
530	2.72	510	2.71	290	2.46	340	2.53
850	2.93	600	2.78	350	2.54	320	2.51
590	2.77	460	2.66	340	2.53	370	2.57

### *E. coli*

The ten samples from PTA 1 and PTA 2 were tested in duplicate for *E. coli* using Petrifilm. The results of this stability testing appear in the following table.

<i>E. coli</i> (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
40	1.60	55	1.74	110	2.04	110	2.04
95	1.98	55	1.74	120	2.08	55	1.74
40	1.60	25	1.40	75	1.88	85	1.93
25	1.40	45	1.65	35	1.54	25	1.40
50	1.70	45	1.65	45	1.65	95	1.98
20	1.30	30	1.48	55	1.74	55	1.74
35	1.54	35	1.54	55	1.74	70	1.85
25	1.40	45	1.65	55	1.74	100	2.00
45	1.65	10	1.00	65	1.81	65	1.81
50	1.70	45	1.65	55	1.74	80	1.90

## B1.5

### Thermophiles

The three samples from PTA 1 and PTA 2 were tested in duplicate for thermophiles using a plate count agar / 55°C / 48 hour method. The results of this stability testing appear in the following table.

Thermophiles (cfu/g)							
PTA 1				PTA 2			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
3800	3.58	4300	3.63	9800	3.99	14000	4.15
3900	3.59	5000	3.70	11000	4.04	12000	4.08
4000	3.60	3500	3.54	11000	4.04	15000	4.18

### Comments on the Stability Testing

For standard plate count and thermophiles, the results showed no loss of viability of the test organisms in the samples in the two weeks between homogeneity testing and stability testing. Therefore, the samples were assessed as stable.

For coliforms the results dropped by approximately 0.3 log between the time of homogeneity testing and the time of stability testing (approximately 2 weeks apart). However, the average result of the stability testing for each sample was very close to the median of the participants' results for each sample. On this basis, the samples were assessed as sufficiently stable for coliforms.

Because the levels of *E. coli* in the samples were low at the time of homogeneity testing and there had been a further drop in the levels, it was decided not to analyse any of the *E. coli* results for this round of the program.

# **APPENDIX C**

## **Instructions to Participants and Results Sheet**

## Non-Pathogens in Food Proficiency Testing Program

### Round 1, February 2006

#### INSTRUCTIONS TO PARTICIPANTS

To ensure that the results of this program can be analysed correctly, participants are asked to note carefully:

- 1) Immediately upon opening the esky the temperature of the temperature control bottle should be taken and recorded on the attached **Results Sheet**.
- 2) The samples available for testing in this program are as follows:

Two approx. 25g skim milk powder samples, labelled PTA 1 and PTA 2, for microbiological analysis. These samples are provided in sealed plastic bags and should be stored below 5°C prior to testing. These samples may be tested for some, or all of the following tests, according to each laboratory's requirements.

- 3) The tests to be performed in this program are:
  - Standard plate count;
  - Coliforms;
  - *E. coli*;
  - Thermophiles; and
  - Thermophilic spores.
- 4) *Testing is to commence on Monday 20 February 2006 or as soon as possible after this date. Testing must not commence after Friday 24 February 2006.*
- 5) Tests performed by participants should be conducted under repeatability conditions. That is, the same operator, using the same equipment, within as short a time interval as possible, should conduct tests on the two separate samples using a particular method. This is necessary to allow for the valid statistical evaluation of results.
- 6) In order for results to be analysed, laboratories are requested to report quantitative results. Samples may contain up to 50,000 coliforms, 5,000 *E. coli*, 50,000 thermophiles, 50,000 thermophilic spores and 250,000 aerobic mesophilic organisms per gram. **Results should not be reported as “greater than ....” or “less than ....”, as such data cannot be statistically analysed.**
- 7) Report results on the attached **Results Sheet** to the specified number of decimal places. Laboratories should report their results in the row corresponding to the method used for each particular test.

## C1.2

- 8) Laboratories are requested to perform all tests listed for which NATA accreditation is held. Laboratories are welcome to report results for any other tests for which NATA accreditation is not held, however, please note this on the **Results Sheet**.
- 9) 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.
- 10) 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**. If AS1766 is used, please ensure that the method number is fully described in the **Method** column of the **Results Sheet** (eg. AS1766.2.1 for SPC, AS1766.2.3.5 (pour plate) or AS1766.2.3.7 (MPN) for coliforms, etc).
- 11) Participants are advised that their results for a particular test, using a particular method, may not be analysed if the number of participants who report results using that method is small.
- 12) Foam eskies and contents need not be returned.
- 13) Return *Results Sheets*, either by mail or facsimile 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
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All results should arrive at the above address by no later than **Friday 3 March 2006**. Results reported later than this date may not be analysed in the final report.

## Non-Pathogens in Food Proficiency Testing Program

Round 1, February 2006

## RESULTS SHEET

Laboratory Code: 

Date Samples Received: \_\_\_\_\_

Temperature of control sample: \_\_\_\_\_ °C

Determination	Report results to nearest	Results		Method (see Note)
		Sample 1	Sample 2	
SPC	2 significant figures (cfu/g)			AS5013.1
				Petrifilm
Coliforms	Report actual count (coliforms/g)			AS5013.3 (MPN)
				AS5013.4 (colony count)
				Petrifilm
<i>E. coli</i>	Report actual count ( <i>E. coli</i> /g)			AS5013.15 (MPN)
				Petrifilm
Thermophiles	2 significant figures (cfu/g)			Plate count agar/55°C/48hrs (former AS1095.3.9)
Thermophilic Spores	Report actual count (cfu/g)			Heat treatment: 80°C/10min. Inc'n: starch nutrient agar/55°C/48hrs (former AS1095.3.7)
				Heat treatment: 100°C/30min. Incubation: starch nutrient agar/55°C/48hrs

**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: \_\_\_\_\_