WATERS (BIO)

PROFICIENCY TESTING PROGRAM

ROUND 48

NOVEMBER 2013

REPORT NO. 831

ACKNOWLEDGMENTS

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Ms S Mott, Global Proficiency Ltd (New Zealand). This assistance included providing input into the design of the program, technical advice and discussion of the final report. PTA would also like to thank Ms 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 microbiological proficiency testing program on water.

The program was conducted in September 2013 by Proficiency Testing Australia (PTA). The Program Coordinator was Mrs K Weller. This is the forty-eighth round in a series of on-going water proficiency testing programs. This report was authorised by Ms W Fajloun, PTA Quality Coordinator.

The aim of the program was to assess laboratories' ability to competently perform the tests examined.

2. FEATURES OF THE PROGRAM

(a) A total of 9 separate laboratories received samples for the program with 8 laboratories returning results for inclusion in the final report. To ensure confidentiality, each laboratory was allocated a random code number for each sample. Reference to each laboratory in this report is by its code number.

Participants included laboratories from Australia, Indonesia, Korea and Macao.

(b) Two samples of concentrated bacterial mix were supplied to each participant. This was to be re-hydrated according to the instructions supplied (refer to page C2), and would be representative of effluent water samples.

The re-hydrated sample was to be tested as follows:

Escherichia coli (E. coli), Faecal Coliforms, Total Coliforms, Enterococci and 37°C (or 35°C) Plate Count.

Laboratories were requested to perform the tests according to the "Instructions to Participants" and to record their results on the accompanying "Results Sheet", both of which were distributed to participants with the sample.

Copies of the "Instructions to Participants", "Results Sheet" and "Instructions for Re-hydration of Sample" are given in Appendix C of this report.

(c) The results, as reported by participants, are presented in Appendix A, together with calculated z-scores, summary statistics and graphical presentations of the data. As is the convention with microbiological count data, the raw results were transformed (log_{10}) before being analysed statistically.

3. FORMAT OF THE APPENDICES

(a) Appendix A is divided into sections for *E. coli*, Faecal Coliforms, Total Coliforms, Enterococci and 37°C (or 35°C) Plate Count.

For each section the following information is given:

(i) A table of the results and the calculated z-scores.

For plate count, all techniques are tabled and analysed together (pooled).

For the Membrane Filtration (MF), Most Probable Number (MPN) and Colilert technique, each of these tables contains the results returned by each laboratory, including the transformed log values and the two z-scores calculated for each sample.

Outliers are identified in the table by a marker (**§**) next to the relevant score. Please see reference [1] for details on how these z-scores are calculated.

(ii) A listing of the (robust) summary statistics.

The list of summary statistics appears at the bottom of the table of results and consists of:

* the number of results for that test / technique (*No. of Results*);
* the median of laboratories' results – i.e. the middle value (*Median*);
* the normalised interquartile range of the results (*Normalised IQR*);
* the robust coefficient of variation, expressed as a percentage (*Robust CV*) – i.e. 100 x *Normalised IQR* ÷ *Median*;
* the minimum and maximum laboratory results; and
* the range (*Maximum – Minimum*).

The Median is a measure of the centre of the data and the Normalised IQR is a measure of the spread of the results.

(iii) Ordered z-score charts

These charts contain solid lines at +3 and -3, so the outliers are clearly identifiable as those laboratories whose "bar" extends beyond these "cut-off" lines.

Further details of the z-score charts are given in reference [1].

(b) Appendix B contains details of the samples used in the program – including sample source, preparation, and homogeneity and stability testing results.

(c) Appendix C contains a copy of the "Instructions to Participants", "Results Sheet", and "Instructions for Re-hydration of Sample" as supplied to participants.

4. STATISTICAL DESIGN OF THE PROGRAM

For this proficiency testing program a uniform level statistical design, as outlined in reference [1], was used.

5. OUTLIER RESULTS

In order to achieve the program's aim of assessing laboratories' testing performance, use has been made of a robust z-score technique. These scores are used to detect excessively large variation between laboratories.

A result is classified as an outlier if it has an absolute z-score value greater than, or equal to, 3.0 (i.e. $z \leq -3.0$ or $z \geq 3.0$). A table listing all of the statistical outliers for this program is provided on page 5. Also included in this table are the laboratories that reported false results and incomplete results. For further details on the calculation and interpretation of robust z-scores, please see reference [1].

TABLE A – SUMMARY STATISTICS

Test	Technique	Sample (PTA)	No. of Results	Median	Normalised IQR
E. coli orgs/100mL	MF	1	4	4.799	0.094
		2	4	4.363	0.237
	MPN	1	4	4.736	0.243
		2	4	4.544	0.170
	Colilert	1	3	4.964	0.137
		2	3	4.415	0.290
Faecal Coliforms orgs/100mL	MF	1	5	4.875	0.107
		2	5	4.427	0.263
	MPN	1	4	4.732	0.262
		2	4	4.544	0.272
Total Coliforms orgs/100mL	MF	1	2	5.041	0.113
		2	2	4.740	0.103
	MPN	1	3	4.848	0.292
		2	3	4.638	0.478
	Colilert	1	3	5.064	0.122
		2	3	4.693	0.190
Enterococci orgs/100mL	MF	1	3	4.708	0.073
		2	3	4.362	0.097
	MPN	1	1	4.462	0.107
		2	1	4.204	0.116
Plate Count orgs/mL	All	1	4	3.519	0.089
		2	4	3.342	0.130

All statistics are calculated from Global Proficiency Ltd results using same samples.

Notes:

1. Results were transformed to $\log_{10}$ values before they were analysed.
2. Table A does not include open ended, incomplete or approximate results.

TABLE B – SUMMARY OF OUTLIER RESULTS
Outlier Results and False Results

Code numbers of the laboratories whose results have been identified as outliers for single robust z-scores and false results are shown in the table below.

Test	Technique	Outlier Results	False Results	Incomplete Results
E. coli	MF	2, 7	-	-
	MPN	5, 9	-	-
	Colilert	2	-	-
Faecal Coliforms	MF	2, 7	-	-
	MPN	4, 5	-	-
Total Coliforms	MF	-	-	-
	MPN	5	-	-
	Colilert	2, 8	-	-
Enterococci	MF	-	-	-
	MPN	3	-	-
Plate Count	All	5	-	-

Note:

1. A target CV was used to calculate the z-scores for the Faecal Coliforms MF test (sample PTA 1) and also for Total Coliforms MF test (both samples).

6. PTA AND TECHNICAL ADVISER'S COMMENTS

Two samples, representative of effluent water were distributed in this round.

Coliform organisms incorporated in samples PTA 1 and PTA 2 included *E. coli* and *Enterobacter cloacae* (*E. cloacae*) with *Enterococcus faecalis* (*E. faecalis*) included as a member of the Enterococci group. Other mesophilic organisms, which did not interfere with the coliform or enterococci tests, were included in the samples to contribute to the Plate Count at 35^{o}C.

Commentary on performance and comparisons between methods were made for each test and comments are included below.

Total Coliforms:

Between two and three laboratories reported results for the three different Total Coliforms techniques. One participant (laboratory 5) recorded outliers for the MPN technique and two participants (laboratories 2 and 8) recorded outliers for the Colilert technique. Laboratory 5 reported outliers for both samples with results lower than expected, laboratory 2 reported an outlier for sample PTA 2 with a result lower than expected and laboratory 8 reported an outlier for sample PTA 2 with a result higher than expected.

Confidence in the medians can be expressed as the uncertainty of the median, which was calculated for each test and/or method within a test using the following equation: $\sqrt{\frac{\pi}{2}} \times \frac{normIQR}{\sqrt{n}}$

Total Coliforms via:	**Sample PTA 1** Median ± Uncertainty (Log_{10})	**Sample PTA 2** Median ± Uncertainty (Log_{10})
Membrane Filtration	5.041 ± 0.047*	4.740 ± 0.043*
Most Probable Number	4.848 ± 0.149*	4.638 ± 0.244*
Colilert	5.064 ± 0.048*	4.693 ± 0.075*

*Statistics from Global Proficiency Ltd results using same samples.

A t-test (outliers removed, 95% confidence interval) was performed on the result-median for each method, and the Reproducibility Measurement Uncertainty (MU) (95% CI) for the three different Total Coliforms techniques was as follows:

Total Coliforms via:	**Sample PTA 1** **Log_{10}**	**Sample PTA 2** **Log_{10}**
Membrane Filtration	±0.24*	±0.33*
Most Probable Number	±0.49*	±0.68*
Colilert	±0.24*	±0.43*

*Statistics from Global Proficiency Ltd results using same samples.

This corresponds to the spread of data seen in Figures TA-1 and TA-2. Laboratories may use this MU data as a comparison to internal estimations.

Two laboratories reported MU estimations associated with their test results in this round. MU was reported in two different ways i.e. ±cfu/100mL values and a range in cfu/100mL values.

Of the reported MUs for the Total Coliforms methods, some did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median). It is recommended the following participants may need to re-examine their test results or their MU calculations as their results were further from the median than their stated uncertainty.

- **MF:** Laboratory 2 (sample PTA 2)
- **Colilert:** Laboratory 2 (sample PTA 2)

Graphs showing the differentiation of methods used for Total Coliforms testing are included below. These graphs show the distribution of results from the three methods used in this round and include Global Proficiency Ltd and PTA data.

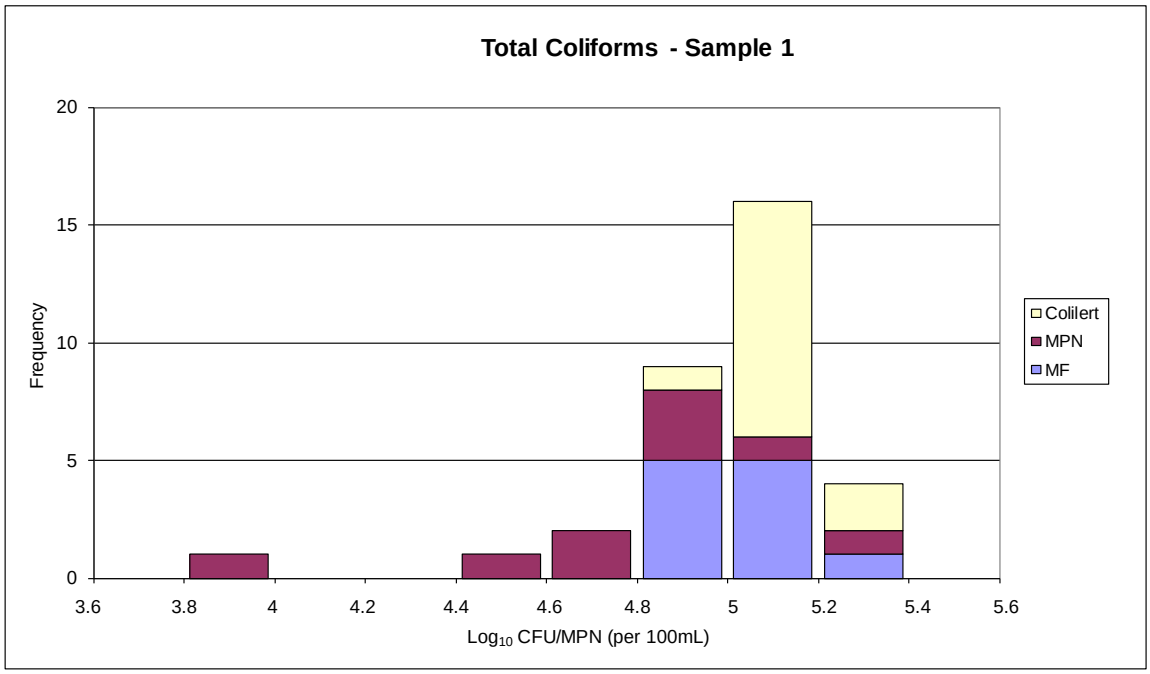


Figure TA-1. Total Coliform results for sample PTA 1

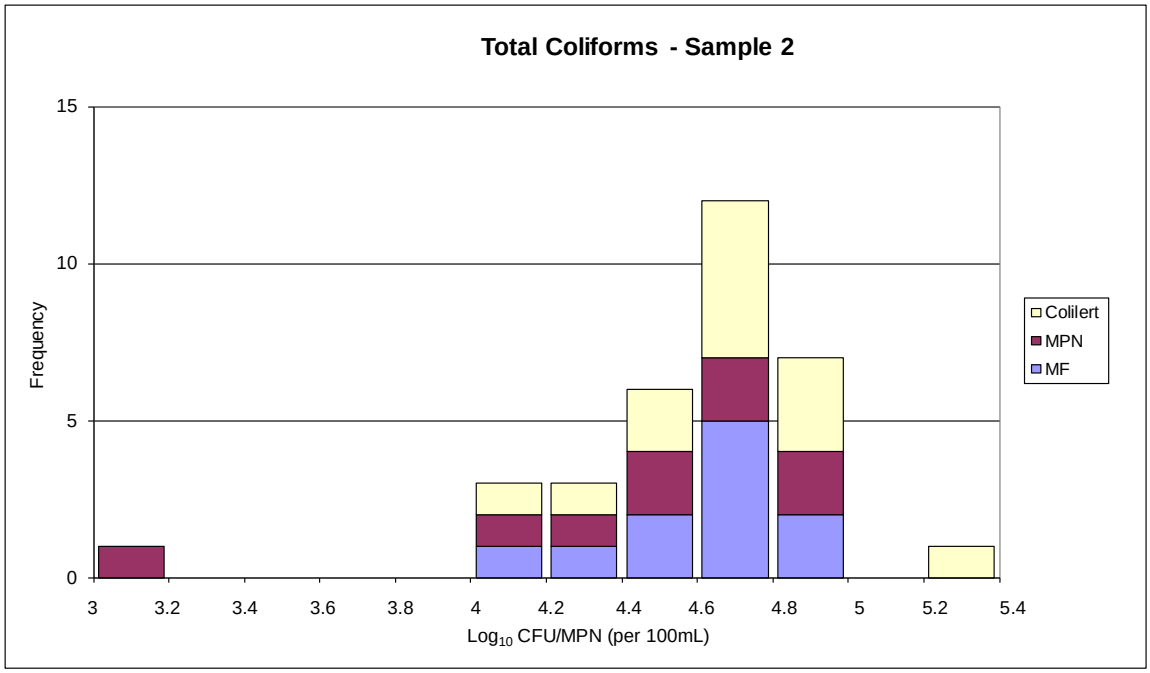


Figure TA-2. Total Coliform results for sample PTA 2

E. coli:

Between three and four laboratories reported results for the three different *E. coli* techniques. Two participants (laboratories 2 and 7) recorded outliers for the MF technique, two participants (laboratories 5 and 9) recorded outliers for the MPN technique and one participant (laboratory 2) recorded an outlier for the Colilert technique. Laboratory 2 reported outliers for sample PTA 2 with results lower than expected for the MF and Colilert technique, laboratory 7 reported an outlier for sample PTA 2 with a result lower than expected and laboratories 5 and 9 reported outliers for both samples with results lower than expected.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 6 of this report), which was calculated for each test and/or method within a test.

***E. coli* via:**	**Sample PTA 1** Median ± Uncertainty (Log_{10})	**Sample PTA 2** Median ± Uncertainty (Log_{10})
Membrane Filtration	4.799 ± 0.030*	4.363 ± 0.074*
Most Probable Number	4.736 ± 0.088*	4.544 ± 0.064*
Colilert	4.964 ± 0.057*	4.415 ± 0.121*

*Statistics from Global Proficiency Ltd results using same samples.

A t-test (outliers removed, 95% confidence interval) was performed on the result-median for each method, and the Reproducibility MU (95% CI) for the three different *E. coli* techniques was as follows:

***E. Coli* via:**	**Sample PTA 1** **Log_{10}**	**Sample PTA 2** **Log_{10}**
Membrane Filtration	±0.22*	±0.59*
Most Probable Number	±0.51*	±0.27*
Colilert	±0.36*	±0.51*

*Statistics from Global Proficiency Ltd results using same samples.

This corresponds to the spread of data seen in Figures TA-3 and TA-4. Laboratories may use this MU data as a comparison to internal estimations.

Four laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways including ±log values, ±cfu/100mL values and a range in cfu/100mL values.

Of the reported MUs for the *E. coli* methods, some did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median). It is recommended the following participants may need to re-examine their test results or their MU calculations as their results were further from the median than their stated uncertainty.

- **MF:** Laboratory 1 (sample PTA 2), laboratory 2 (sample PTA 2) and laboratory 7 (sample PTA 2)
- **MPN:** Laboratory 9 (both samples)
- **Colilert:** Laboratory 1 (sample PTA 2) and laboratory 2 (sample PTA 2)

Graphs showing the differentiation of methods used for *E. coli* testing are included below. These graphs show the distribution of results from the three methods used in this round and include Global Proficiency Ltd and PTA data.

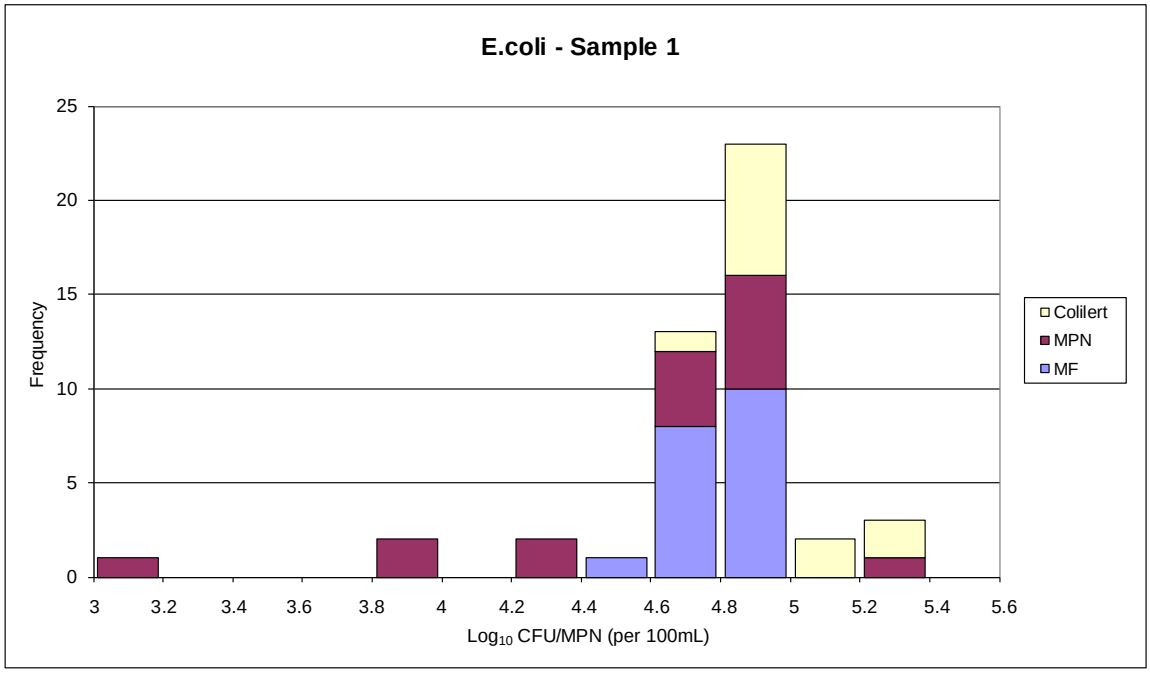


Figure TA-3. *E. coli* results for sample PTA 1

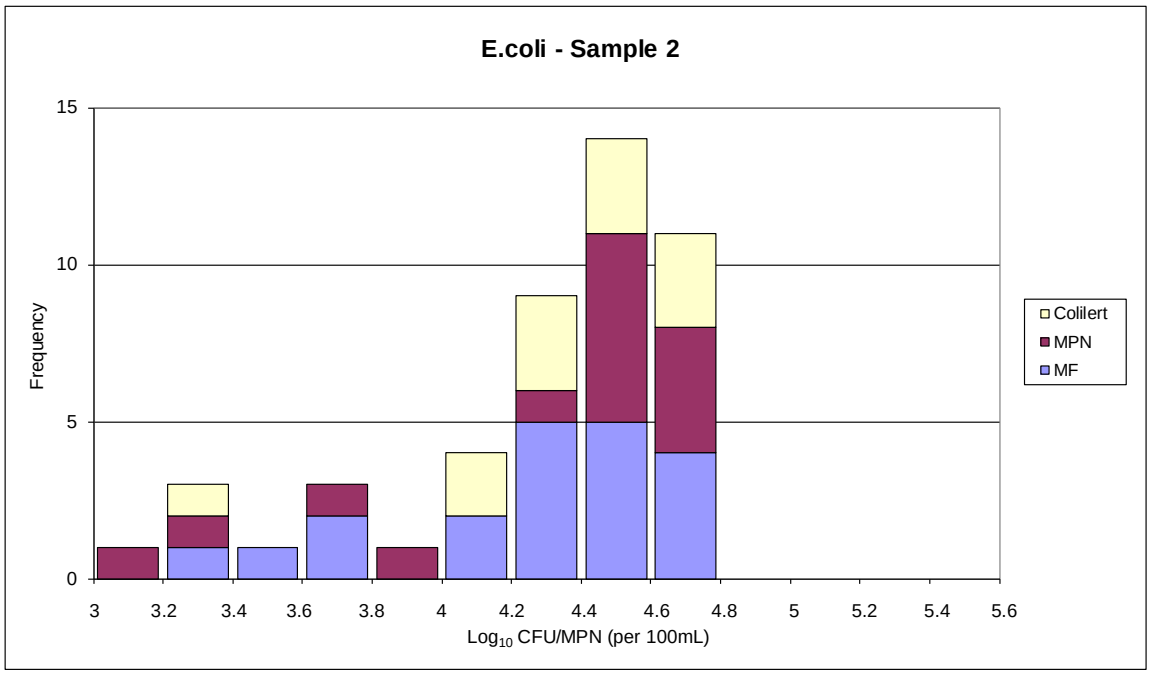


Figure TA-4. *E. coli* results for sample PTA 2

Faecal Coliforms:
Five and four laboratories reported results for the two different Faecal Coliforms techniques; MF and MPN respectively. Two participants (laboratories 2 and 7) recorded outliers for the MF technique and two participants (laboratories 4 and 5) recorded outliers for the MPN technique. Laboratories 2, 5 and 7 reported outliers for sample PTA 2 with results lower than expected and laboratory 4 reported outliers for both samples with results lower than expected.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 6 of this report), which was calculated for each test and/or method within a test.

Faecal Coliforms via:	**Sample PTA 1** Median ± Uncertainty (Log_{10})	**Sample PTA 2** Median ± Uncertainty (Log_{10})
Membrane Filtration	4.875 ± 0.029*	4.427 ± 0.070*
Most Probable Number	4.732 ± 0.091*	4.544 ± 0.098*

*Statistics from Global Proficiency Ltd results using same samples.

A t-test (outliers removed, 95% confidence interval) was performed on the result-median for each method, and the Reproducibility MU (95% CI) for the two different Faecal Coliforms techniques was as follows:

Faecal Coliforms via:	**Sample PTA 1** **Log_{10}**	**Sample PTA 2** **Log_{10}**
Membrane Filtration	±0.22*	±0.57*
Most Probable Number	±0.65*	±0.66*

*Statistics from Global Proficiency Ltd results using same samples.

This corresponds to the spread of data seen in Figures TA-5 and TA-6. Laboratories may use this MU data as a comparison to internal estimations.

Three laboratories reported MU estimations associated with their test results in this round. MU was reported in two different ways i.e. ±log values and ±cfu/100mL values.

Of the reported MUs for the Faecal Coliforms methods, some did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median). It is recommended the following participants may need to re-examine their test results or their MU calculations as their results were further from the median than their stated uncertainty.

- **MF:** Laboratory 2 (sample PTA 2) and laboratory 7 (sample PTA 2)

Graphs showing differentiation of methods used for Faecal Coliforms testing are included below. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data.

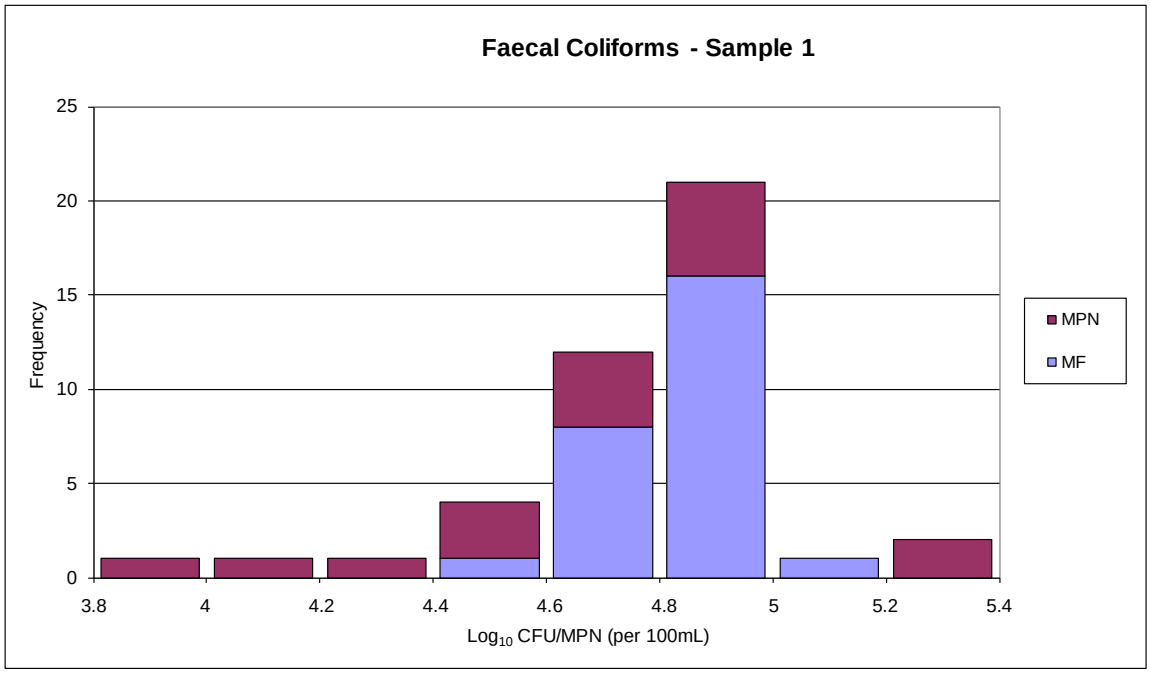


Figure TA-5. Faecal Coliform results for sample PTA 1

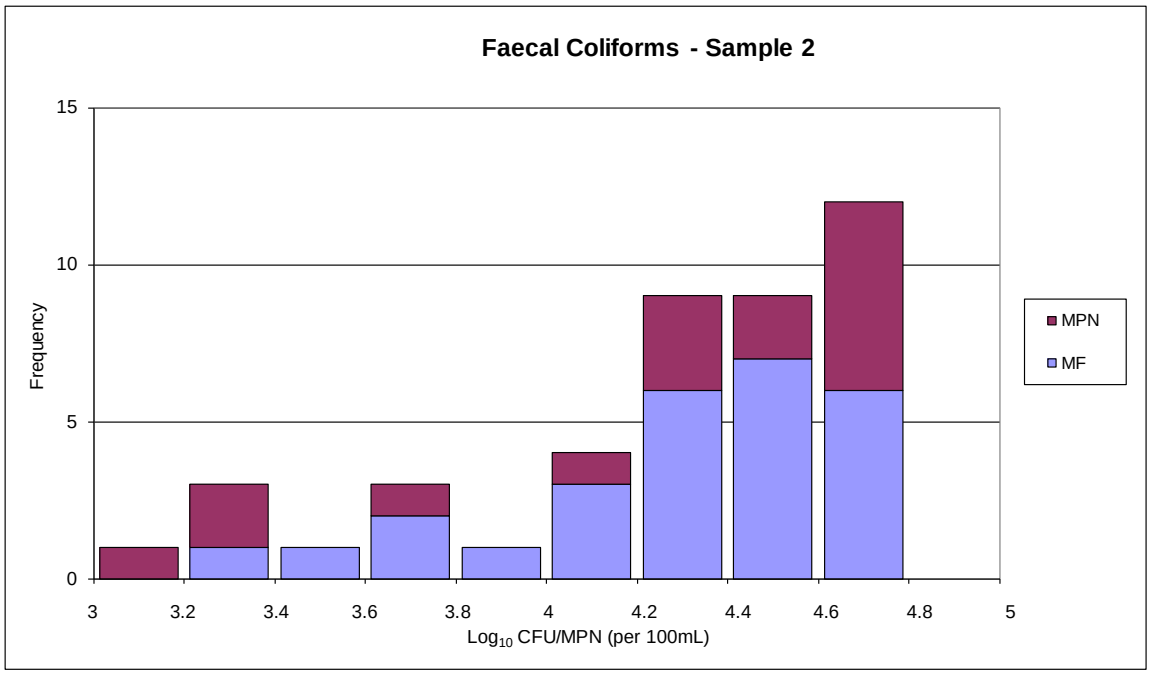


Figure TA-6. Faecal Coliform results for sample PTA 2

Enterococci:
Three and one laboratories reported results for the two different Enterococci methods; MF and MPN respectively. One participant (laboratory 3) recorded outliers using the MPN technique with values higher than expected for both samples.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 6 of this report), which was calculated for each test and/or method within a test.

Enterococci via:	**Sample PTA 1** Median ± Uncertainty (Log_{10})	**Sample PTA 2** Median ± Uncertainty (Log_{10})
Membrane Filtration	4.708 ± 0.025*	4.362 ± 0.033*
Most Probable Number	4.462 ± 0.040*	4.204 ± 0.044*

*Statistics from Global Proficiency Ltd results using same samples.

A t-test (outliers removed, 95% confidence interval) was performed on the result-median for each method, and the Reproducibility MU (95% CI) for the two different Enterococci techniques was as follows:

Enterococci via:	**Sample PTA 1** **Log_{10}**	**Sample PTA 2** **Log_{10}**
Membrane Filtration	±0.14*	±0.25*
Most Probable Number	±0.27*	±0.24*

*Statistics from Global Proficiency Ltd results using same samples.

This corresponds to the spread of data seen in Figures TA-7 and TA-8. Laboratories may use this MU data as a comparison to internal estimations.

Two laboratories reported MU estimations associated with their test results in this round. MU was reported in two different ways i.e. ±log values and ±cfu/100mL values.

Of the reported MUs for the Enterococci methods, some did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median). It is recommended the following participants may need to re-examine their test results or their MU calculations as their results were further from the median than their stated uncertainty.

- **MF:** Laboratory 2 (sample PTA 2)

Graphs showing differentiation of methods used for Enterococci testing are included below. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data.

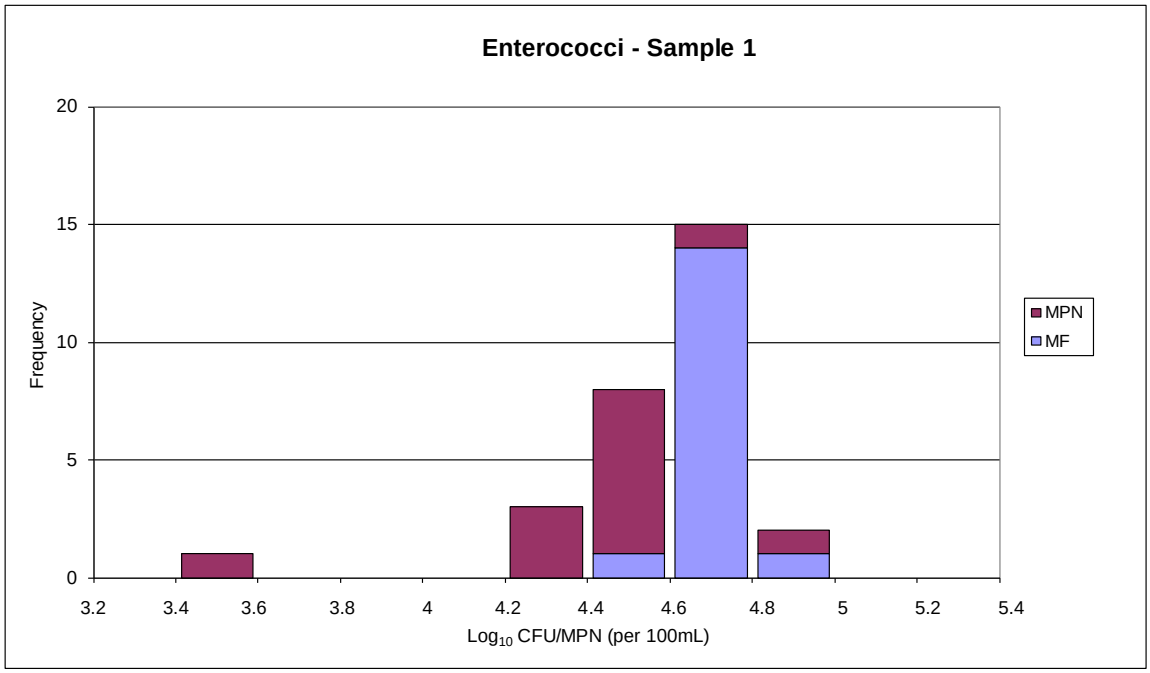


Figure TA-7. Enterococci results for sample PTA 1

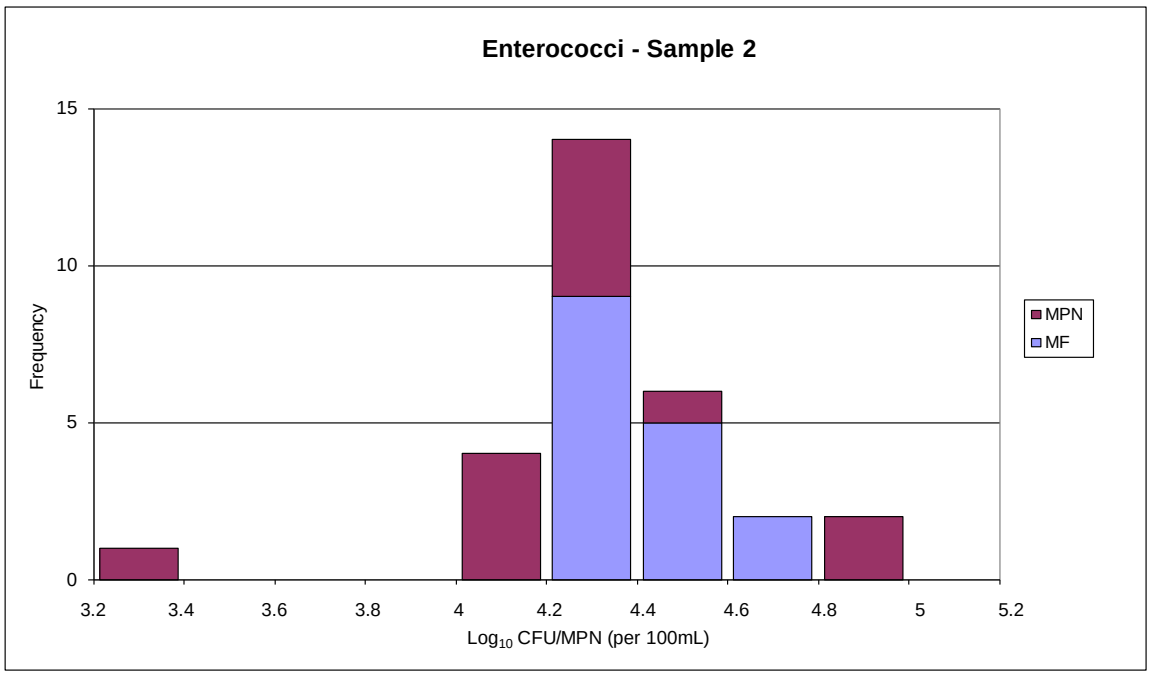


Figure TA-8. Enterococci results for sample PTA 2

Plate Count:
Four sets of results were submitted for the Plate Count test. Of this set, one result was obtained using Australian Standard (AS) method (AS 4276.3.1), two results were obtained using APHA methods and one listed an internal method. Of these methods listed, all reported using the Pour Plate technique. One participant (laboratory 5) recorded outliers for both samples with a result higher than expected for sample PTA 1 and a result lower than expected for sample PTA 2.

	Sample PTA 1 Median ± Uncertainty (Log_{10})	**Sample PTA 2** Median ± Uncertainty (Log_{10})
Plate Count:	3.519 ± 0.042*	3.342 ± 0.062*

*Statistics from Global Proficiency Ltd results using same samples.

A t-test (outliers removed, 95% confidence interval) was performed on the result-median, and the Reproducibility MU (95% CI) was as follows:

	Sample PTA 1 Log_{10}	**Sample PTA 2 Log_{10}**
Plate Count:	±0.16*	±0.26*

*Statistics from Global Proficiency Ltd results using same samples.

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

One laboratory reported MU estimations associated with their test results in this round as ±cfu/mL values.

General Comments

A total of 72 results were submitted for analysis in this round. Of these results 20 (28%) were outlier results. This is much higher than the 2.8% of results which were outlier results in Round 46.

Outlying results, either between laboratories or within a laboratory, are indicative of a problem but are not diagnostic, so further information is usually required to determine the origin of a poor result. As a first step, it is advisable to re-examine the records for the run in question. The following potential problems should be examined:

- Systematic or sporadic mistakes in calculations (are the units correct);
- Incorrect volumes used;
- Out-of-control indications from your routine Internal Quality Control;
- Unusually high blanks;
- Poor recoveries, etc.

If these actions yield no insight, then further measurements, such as carrying out a re-test of the proficiency sample, may be required. If the poor result persists, a more extensive investigation may be required. Consideration should also be given to reviewing performance in previous rounds to detect apparent trends.

Metrological Traceability

Consensus values (median) derived from participants' results are used in this program. These values are not metrologically traceable to an external reference.

Samples were prepared using cultures sourced from internationally recognised culture collections. Culture maintenance and subsequent batch preparation was undertaken according to Global Proficiency Ltd's Standard Operating Procedures to ensure samples were fit-for-purpose, homogeneous and stable.

7. REFERENCES

[1] *Guide to Proficiency Testing Australia* (2012). (This document can be found on the PTA website, www.pta.asn.au)

[2] AS/NZS 4276.1-2007 *Water microbiology - General information and procedures (ISO 8199-2005 MOD)*

[3] AS 4276.2-1995 *Water microbiology - Culture media diluents and reagents*

[4] AS 4276.3.1-2007: *Water microbiology - Heterotrophic colony count methods - Pour plate method using yeast extract agar*

[5] AS/NZS 4276.5-2007 *Water microbiology - Coliforms - Membrane filtration method*

[6] AS/NZS 4276.6-2007 *Water microbiology – Coliforms, Escherichia coli and thermotolerant coliforms - Determination of Most Probable Number (MPN)*

[7] AS 4276.7-2007 *Water microbiology - Thermotolerant coliforms and Escherichia coli - Membrane filtration method*

[8] AS 4276.9-2007 *Water microbiology - Faecal streptococci - Membrane filtration method*

[9] AS 4276.21-2005 *Water microbiology - Examination for coliforms and Escherichia coli - Determination of Most Probable Number (MPN) using enzyme hydrolysable substrates*

[10] APHA 9230C – *Fecal Enterococcus/Streptococcus Groups – Membrane Filtration techniques. American Public Health Association: Standard methods for the examination of water and wastewater, 22nd Edition (2012)*

[11] APHA 9230D – *Fecal Enterococcus/Streptococcus Groups – Fluorogenic Substrate Enterococcus test. American Public Health Association: Standard methods for the examination of water and wastewater, 22nd Edition (2012)*

APPENDIX A

Tables of Results and Z-Scores,

Summary Statistics

and

Graphical Displays

SECTIONS A1 to A3

E. coli

A1.1

E. coli (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	81000	±0.16 log10	41000	±0.16 log10	4.91	4.61	1.17	1.05
2	63000	±11113	3600	±635	4.80	3.56	0.00	-3.40 **§**
3	67000		15000		4.83	4.18	0.29	-0.79
7	79500	±24000	2150	±650	4.90	3.33	1.08	-4.34 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	15
Median	4.799
Norm IQR	0.094
Robust CV	1.9%
Minimum	4.57
Maximum	4.98
Range	0.41

Sample - PTA 2

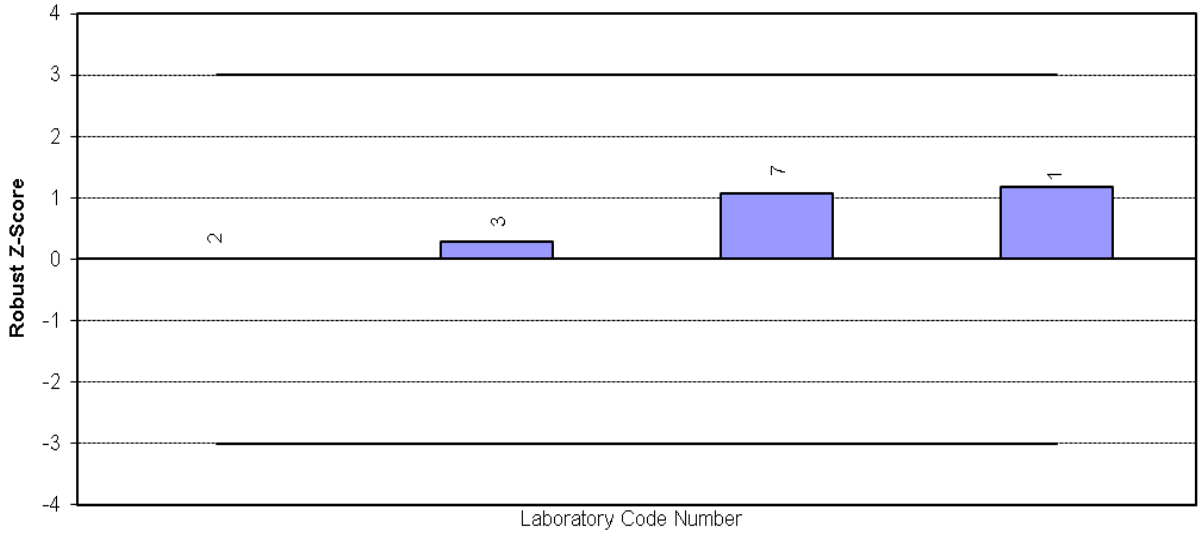
No. of Results	16
Median	4.363
Norm IQR	0.237
Robust CV	5.4%
Minimum	3.73
Maximum	4.65
Range	0.92

Note:

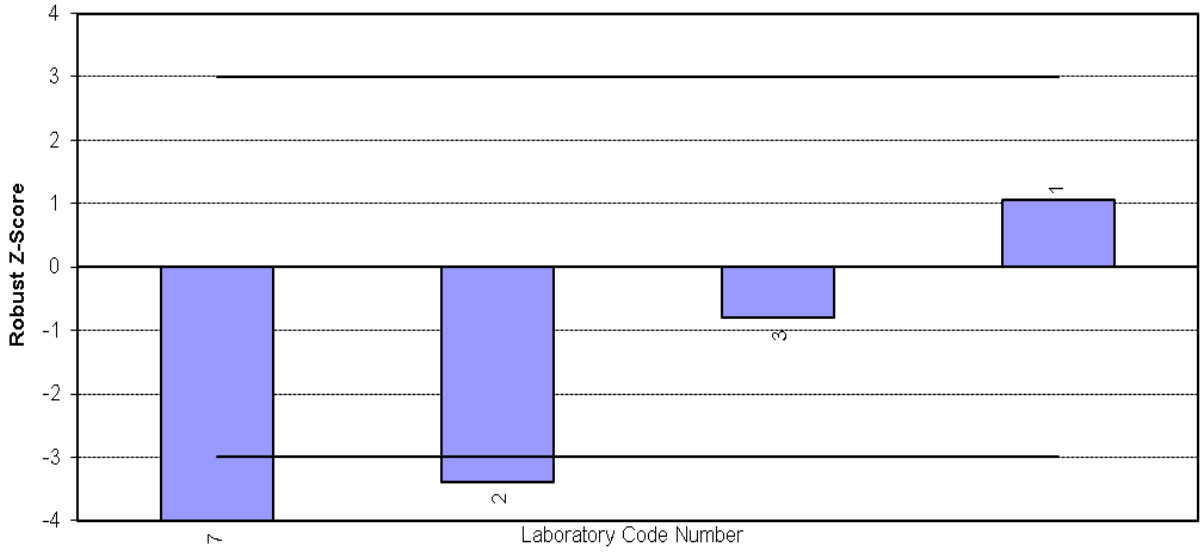
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

E. coli (orgs/100mL) – MF Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



E. coli (orgs/100mL) – MPN Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
2	70000	±17500	25000	±6250	4.85	4.40	0.45	-0.86
3	70000		54000		4.85	4.73	0.45	1.11
5	9200		11		3.96	1.04	-3.18 **§**	-20.60 **§**
9	542	180 - 1405	1600	640 - 5800	2.73	3.20	-8.25 **§**	-7.88 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	12
Median	4.736
Norm IQR	0.243
Robust CV	5.1%
Minimum	3.85
Maximum	5.20
Range	1.36

Sample - PTA 2

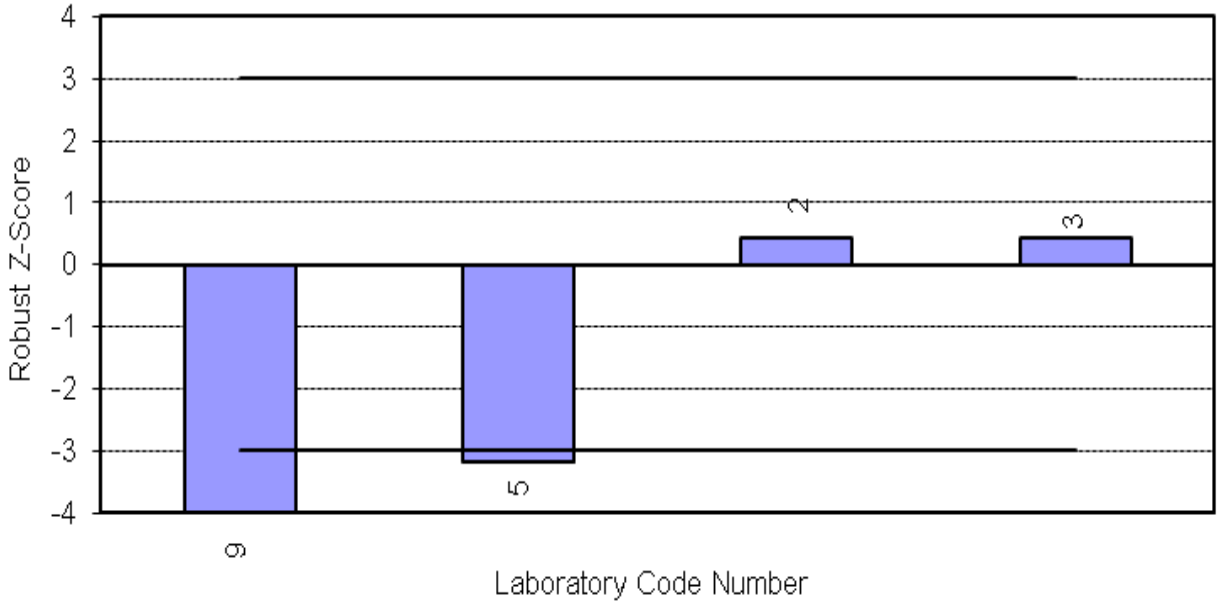
No. of Results	11
Median	4.544
Norm IQR	0.170
Robust CV	3.7%
Minimum	3.73
Maximum	4.66
Range	0.93

Note:

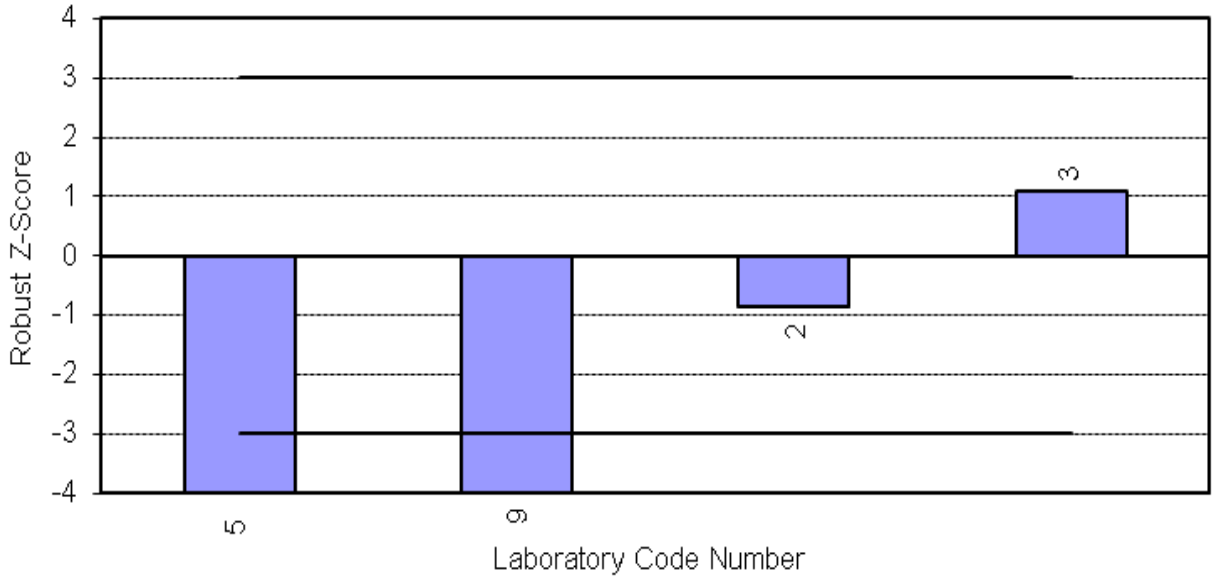
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

E. coli (orgs/100mL) – MPN Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



E. coli (orgs/100mL) – Colilert Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	92000	62000-130000	58000	38000-85000	4.96	4.76	0.00	1.20
2	73000	±9899	2200	±298	4.86	3.34	-0.74	-3.70 **§**
8	209200		16800		5.32	4.23	2.61	-0.66

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	9
Median	4.964
Norm IQR	0.137
Robust CV	2.8%
Minimum	4.77
Maximum	5.20
Range	0.43

Sample - PTA 2

No. of Results	9
Median	4.415
Norm IQR	0.290
Robust CV	6.6%
Minimum	4.15
Maximum	4.73
Range	0.58

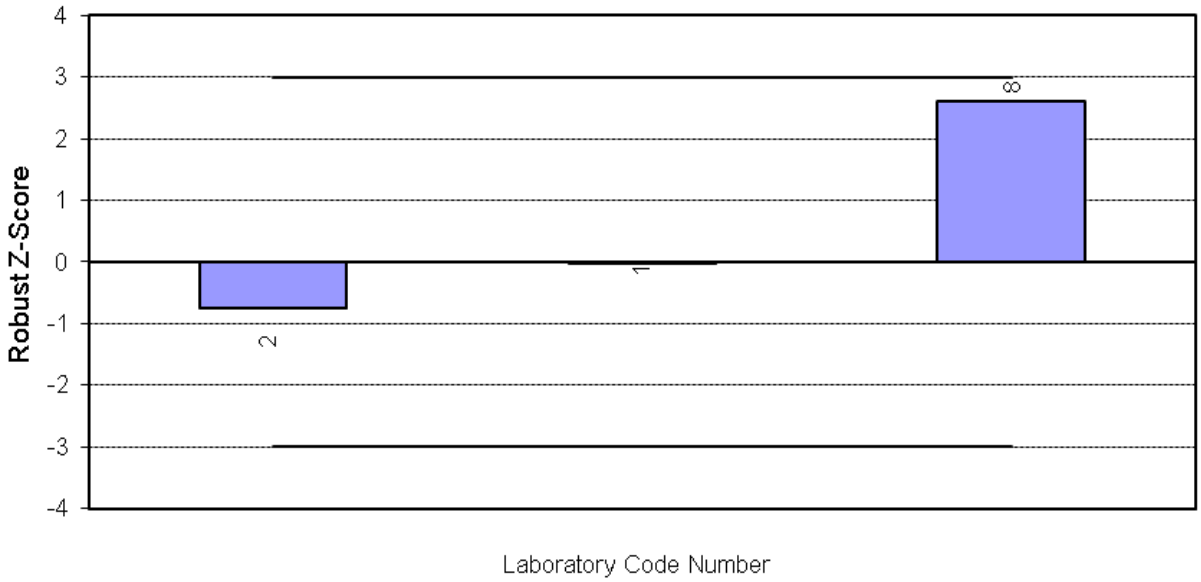
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

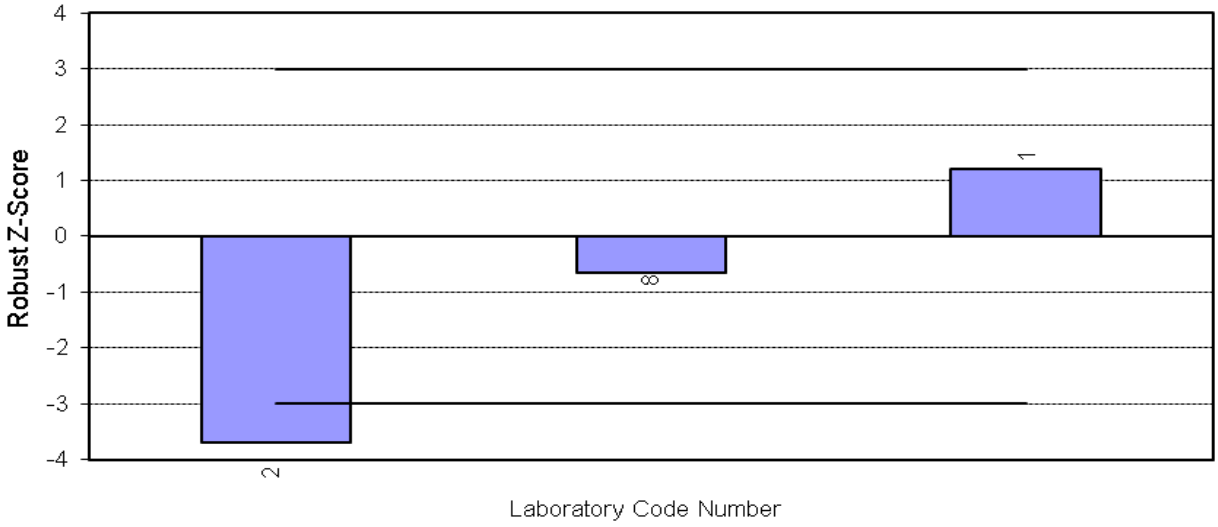
A3.2

E. coli (orgs/100mL) – Colilert Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



SECTIONS A4 to A5

Faecal Coliforms

Faecal Coliforms (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 $\log_{10}$ Result	PTA 2 $\log_{10}$ Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	81000	±0.16 log10	41000	±0.16 log10	4.91	4.61	0.23	0.71
2	63000	±11113	3600	±635	4.80	3.56	-0.52	-3.31 **§**
3	67000		15000		4.83	4.18	-0.34	-0.95
4	33000		5600		4.52	3.75	-2.44	-2.58
7	80000	±24000	2150	±650	4.90	3.33	0.19	-4.16 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	21
Median	4.875
Norm IQR	0.107
Robust CV	2.2%
Minimum	4.65
Maximum	5.18
Range	0.52

*The robust CV achieved for Sample PTA 1 was low (robust CV = 2.2%) so in this case a target robust CV = 3.0% was considered more appropriate for this sample and was used to determine z-scores. For more information on calculating z-scores using target CVs refer to reference [1].

Sample - PTA 2

No. of Results	22
Median	4.427
Norm IQR	0.263
Robust CV	5.9%
Minimum	3.73
Maximum	4.71
Range	0.98

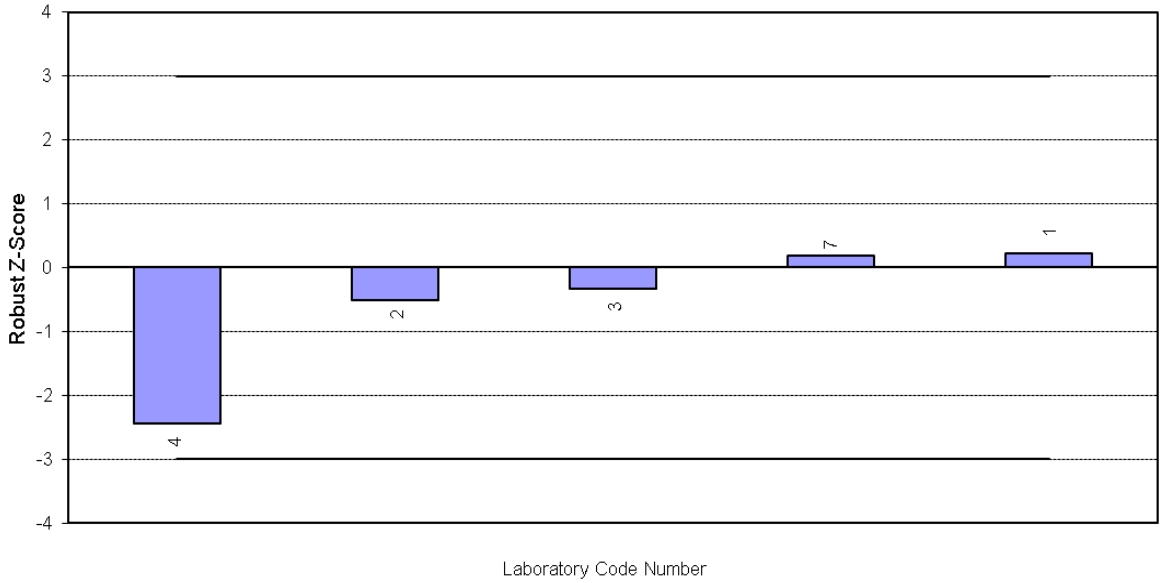
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

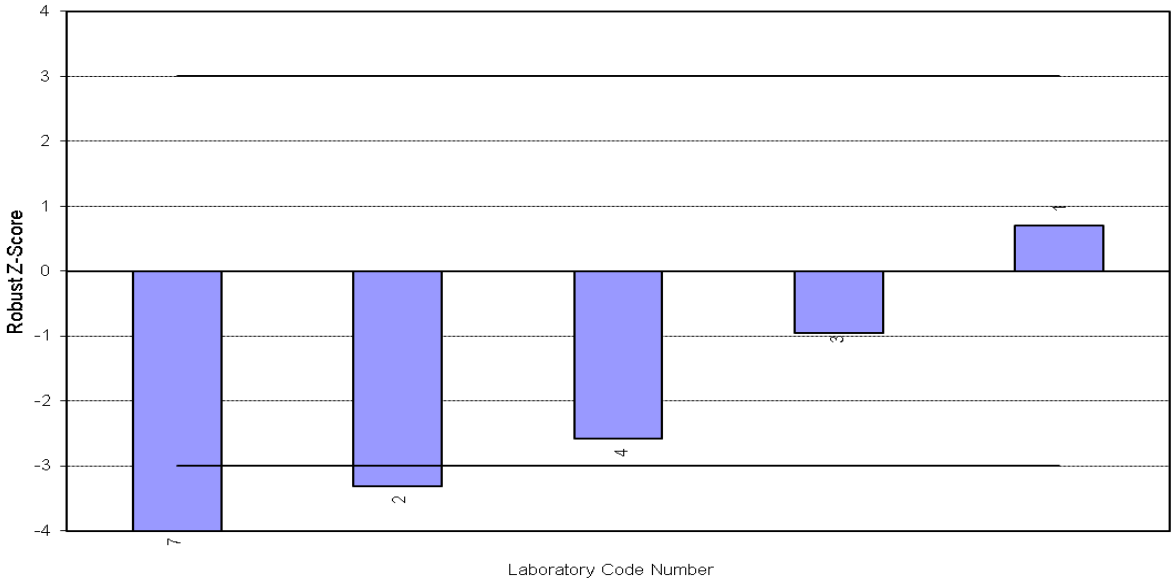
A4.2

Faecal Coliforms (orgs/100mL) – MF Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



A5.1

Faecal Coliforms (orgs/100mL) – MPN Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 $\log_{10}$ Result	PTA 2 $\log_{10}$ Result	PTA 1 Robust z-score	PTA 2 Robust z-score
2	70000	±17500	25000	±6250	4.85	4.40	0.43	-0.54
3	70000		54000		4.85	4.73	0.43	0.69
4	4900		1700		3.69	3.23	-3.98 **§**	-4.83 **§**
5	9200		11		3.96	1.04	-2.93	-12.89 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	13
Median	4.732
Norm IQR	0.262
Robust CV	5.5%
Minimum	4.34
Maximum	5.20
Range	0.86

Sample - PTA 2

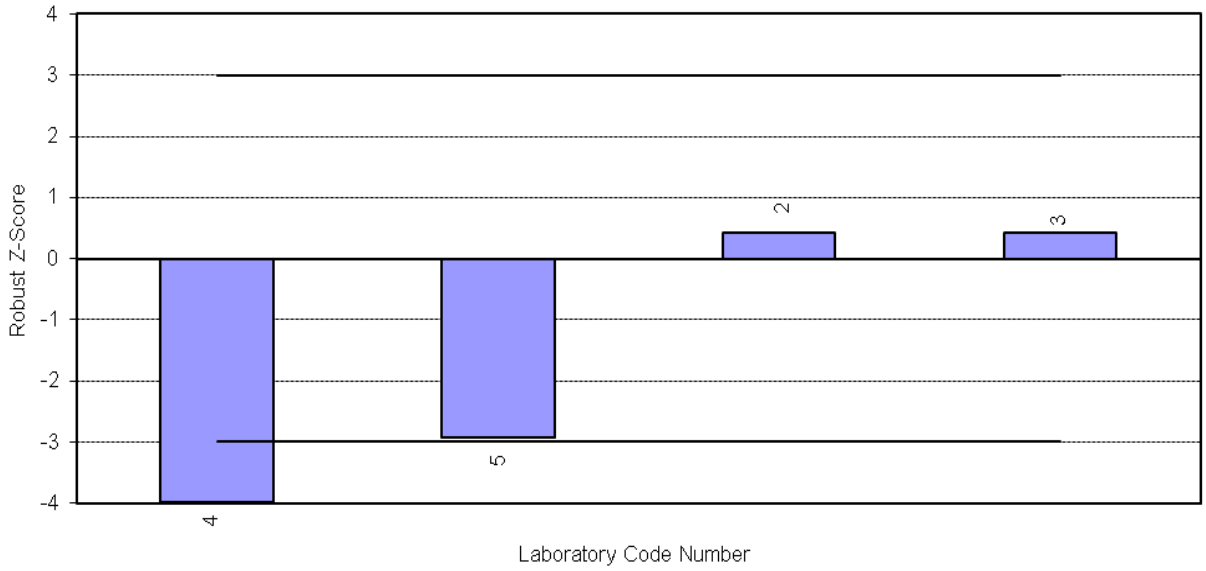
No. of Results	12
Median	4.544
Norm IQR	0.272
Robust CV	6.0%
Minimum	3.23
Maximum	4.80
Range	1.57

Note:

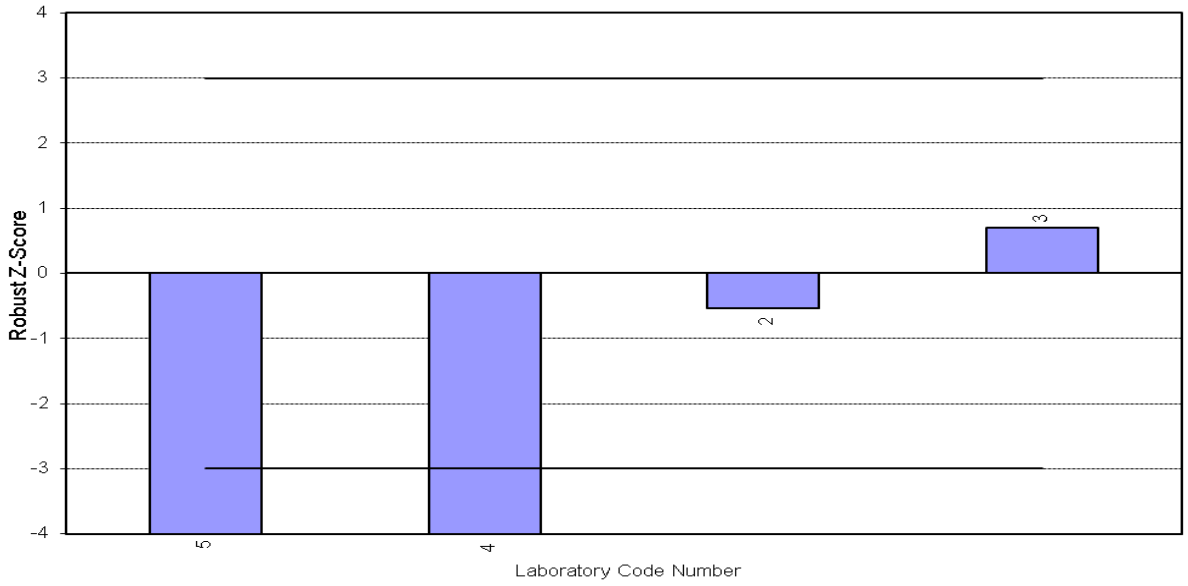
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

Faecal Coliforms (orgs/100mL) – MPN Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



SECTIONS A6 to A8

Total Coliforms

Total Coliforms (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 $\log_{10}$ Result	PTA 2 $\log_{10}$ Result	PTA 1 Robust z-score	PTA 2 Robust z-score
2	100000	±15540	21000	±3263	5.00	4.32	-0.19	-2.08
3	78000		14000		4.89	4.15	-0.69	-2.95

Summary Statistics
Sample - PTA 1

No. of Results	9
Median	5.041
Norm IQR	0.113
Robust CV	2.3%
Minimum	4.81
Maximum	5.20
Range	0.39

Sample - PTA 2

No. of Results	9
Median	4.740
Norm IQR	0.103
Robust CV	2.2%
Minimum	4.46
Maximum	4.93
Range	0.47

*The robust CV achieved for both samples were low (robust CV = 2.3% and 2.2%) so in this case a target robust CV = 4.3% was considered more appropriate for both samples and was used to determine z-scores. For more information on calculating z-scores using target CVs refer to reference [1].

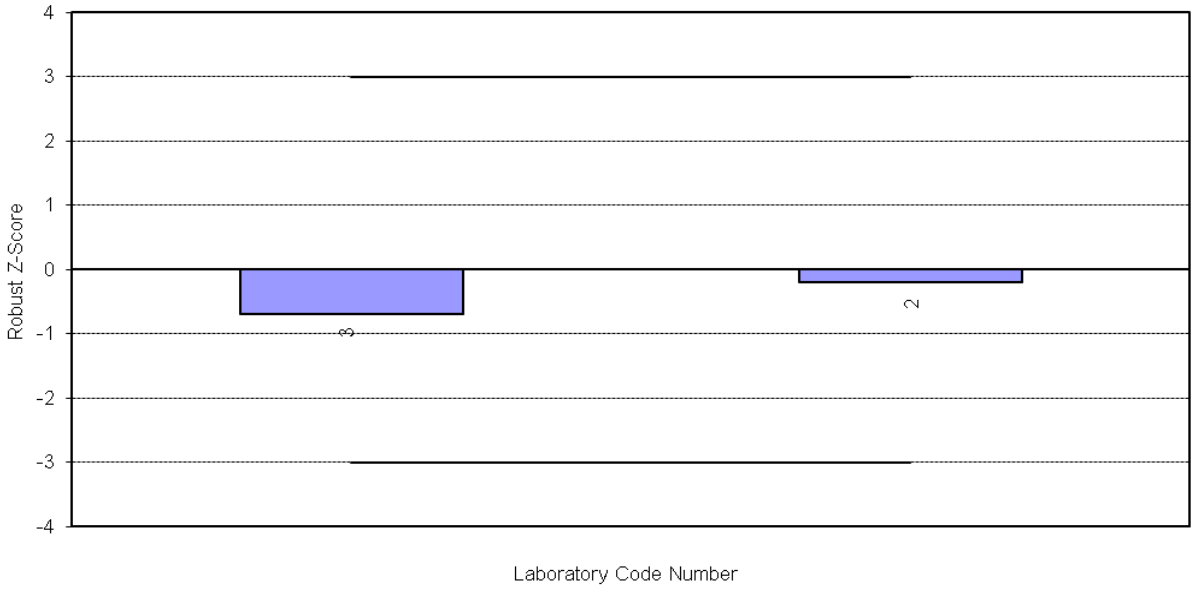
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

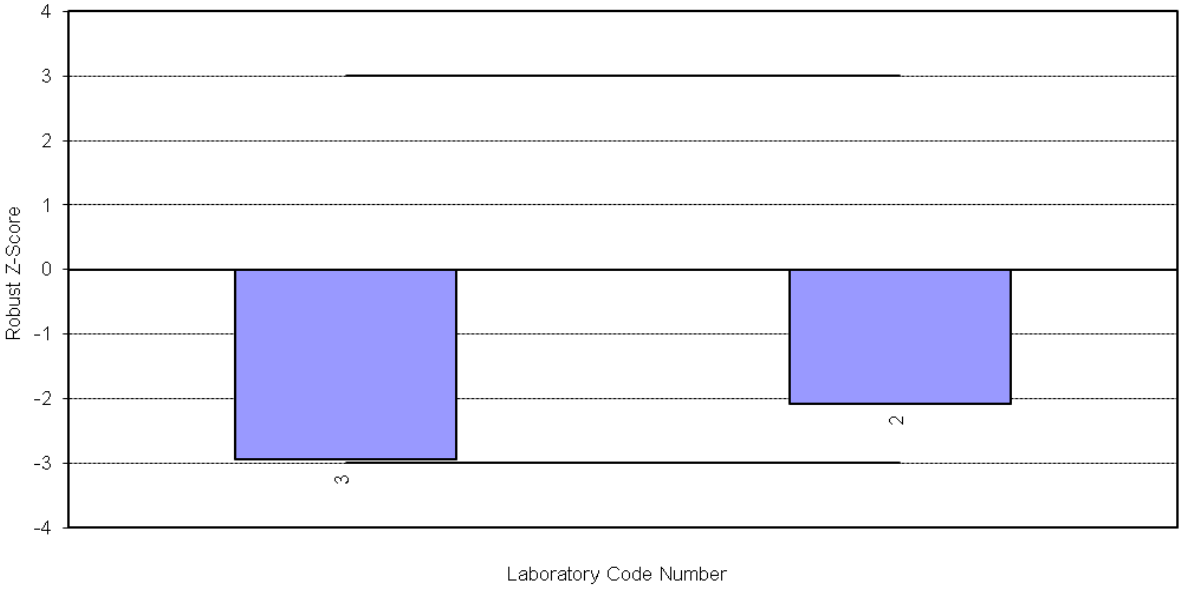
A6.2

Total Coliforms (orgs/100mL) – MF Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



Total Coliforms (orgs/100mL) – MPN Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
2	70000	±17500	35000	±8750	4.85	4.54	-0.01	-0.20
3	70000		54000		4.85	4.73	-0.01	0.20
5	9200		11		3.96	1.04	-3.03 **§**	-7.53 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	6
Median	4.848
Norm IQR	0.292
Robust CV	6.0%
Minimum	4.54
Maximum	5.20
Range	0.66

Sample - PTA 2

No. of Results	6
Median	4.638
Norm IQR	0.478
Robust CV	10.3%
Minimum	4.15
Maximum	4.96
Range	0.82

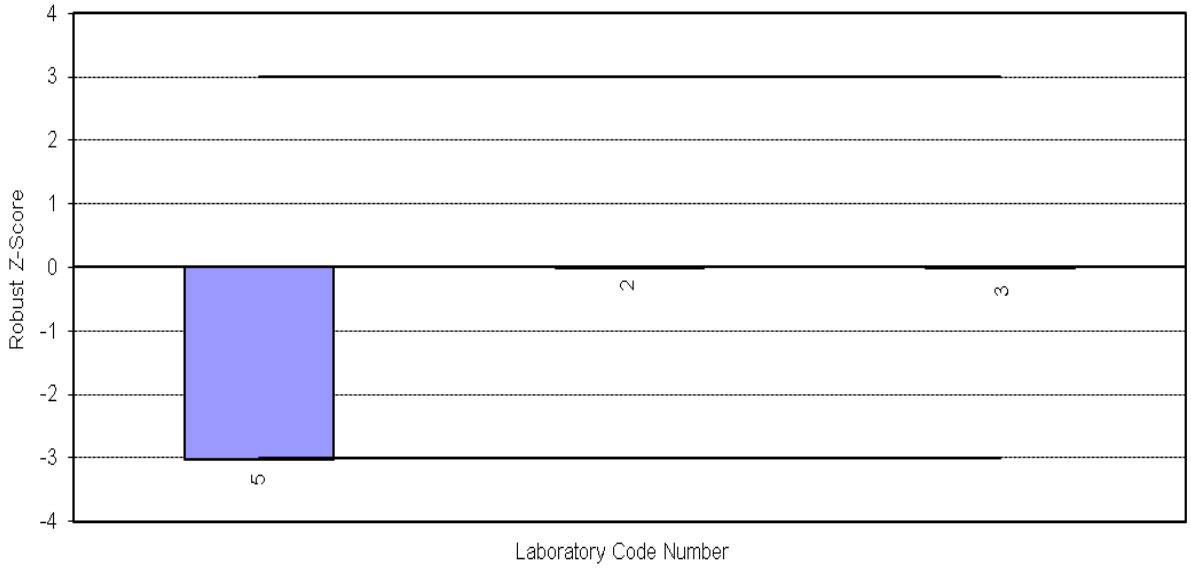
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

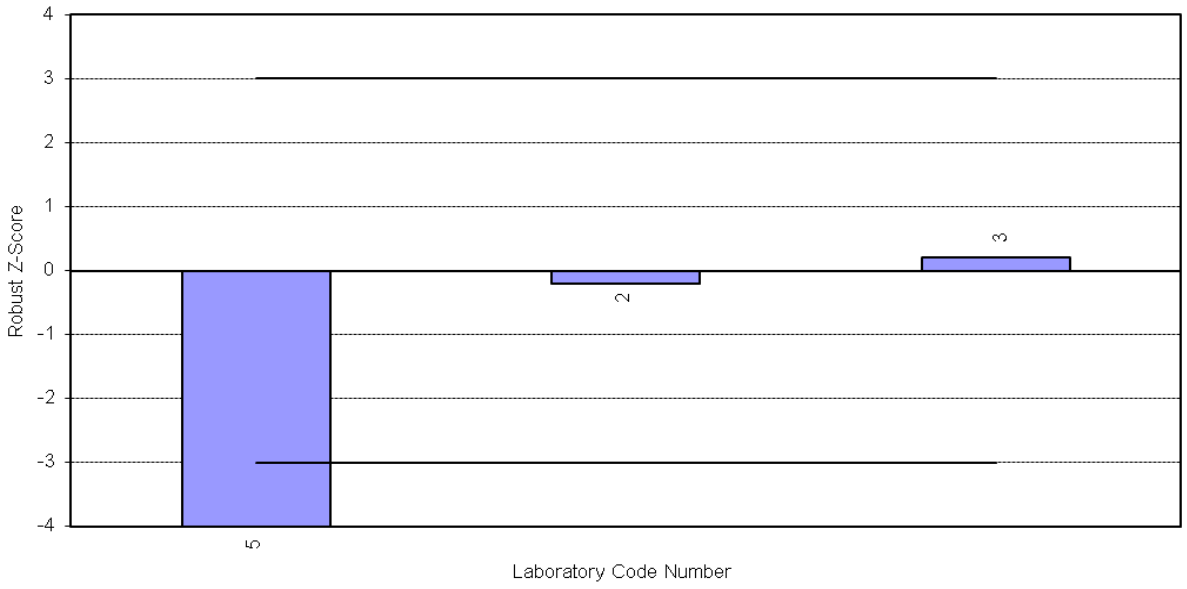
A7.2

Total Coliforms (orgs/100mL) – MPN Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



Total Coliforms (orgs/100mL) – Colilert Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	160000	110000-230000	77000	55000-110000	5.20	4.89	1.15	1.02
2	130000	±17628	11000	±1492	5.11	4.04	0.41	-3.44 **§**
8	224000		224000		5.35	5.35	2.35	3.47 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample – PTA 1

No. of Results	10
Median	5.064
Norm IQR	0.122
Robust CV	2.4%
Minimum	4.96
Maximum	5.19
Range	0.23

Sample – PTA 2

No. of Results	10
Median	4.693
Norm IQR	0.190
Robust CV	4.0%
Minimum	4.30
Maximum	4.89
Range	0.59

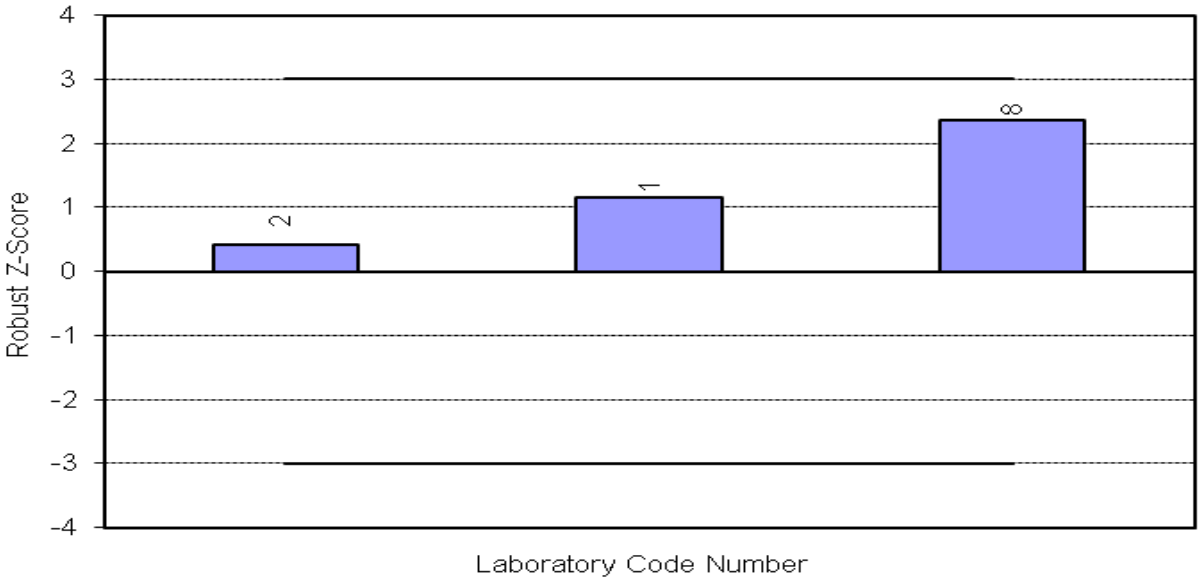
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

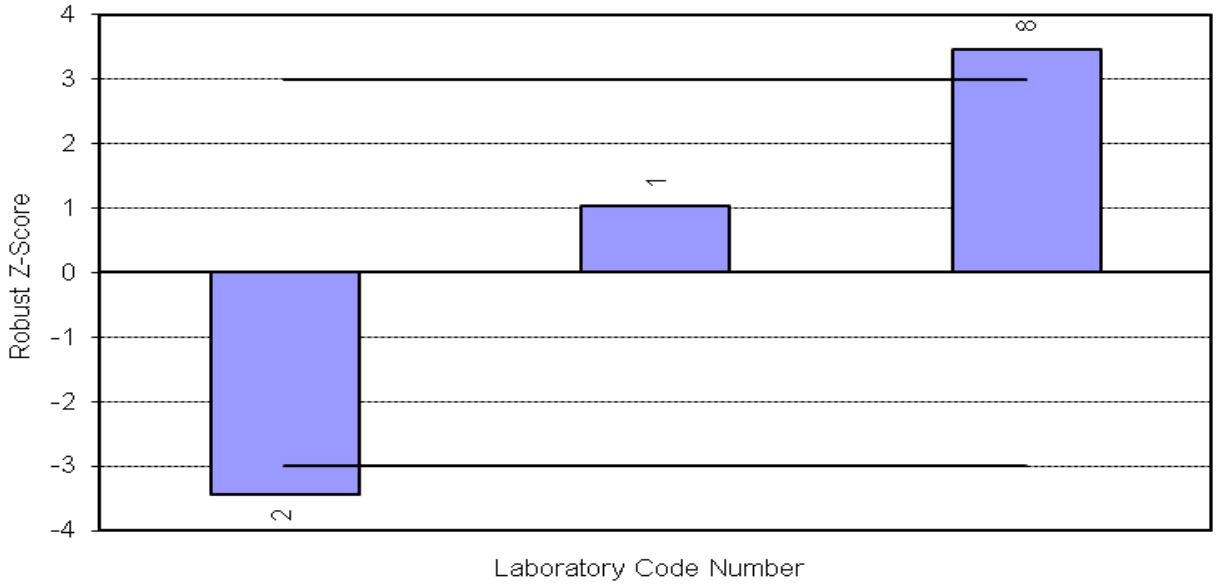
A8.2

Total Coliforms (orgs/100mL) – Colilert Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



SECTIONS A9 to A10

Enterococci

A9.1

Enterococci (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	40000	±0.24 log10	28000	±0.24 log10	4.60	4.45	-1.44	0.88
2	48000	±6307	40000	±5256	4.68	4.60	-0.36	2.49
3	46000		24000		4.66	4.38	-0.61	0.19

Summary Statistics
Sample - PTA 1

No. of Results	13
Median	4.708
Norm IQR	0.073
Robust CV	1.6%
Minimum	4.60
Maximum	4.84
Range	0.24

Sample - PTA 2

No. of Results	13
Median	4.362
Norm IQR	0.097
Robust CV	2.2%
Minimum	4.21
Maximum	4.61
Range	0.40

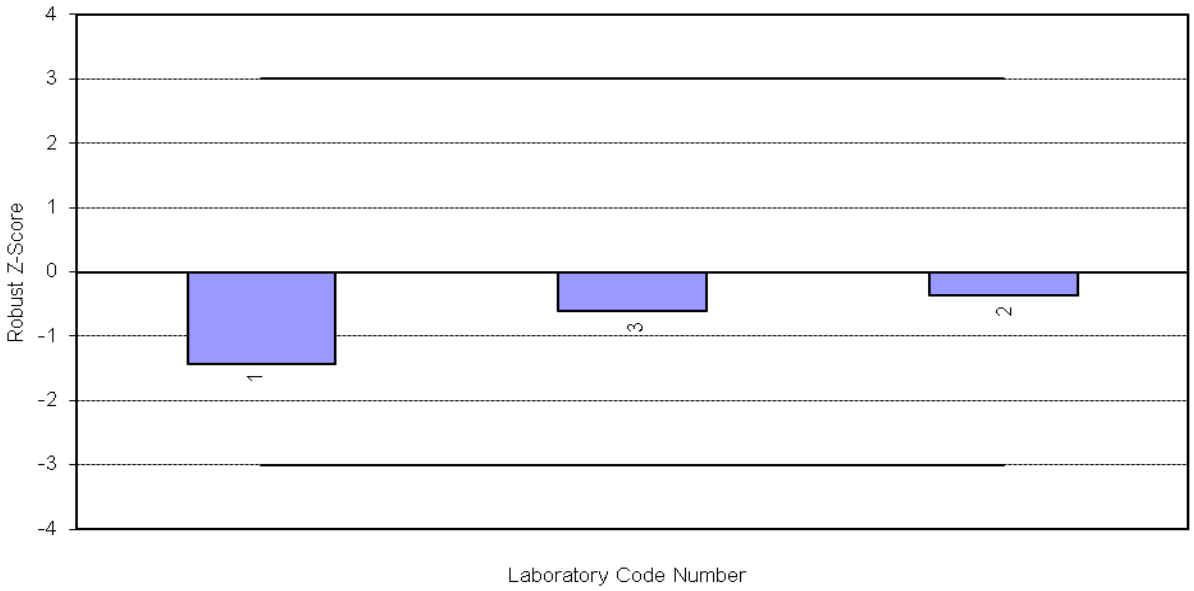
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

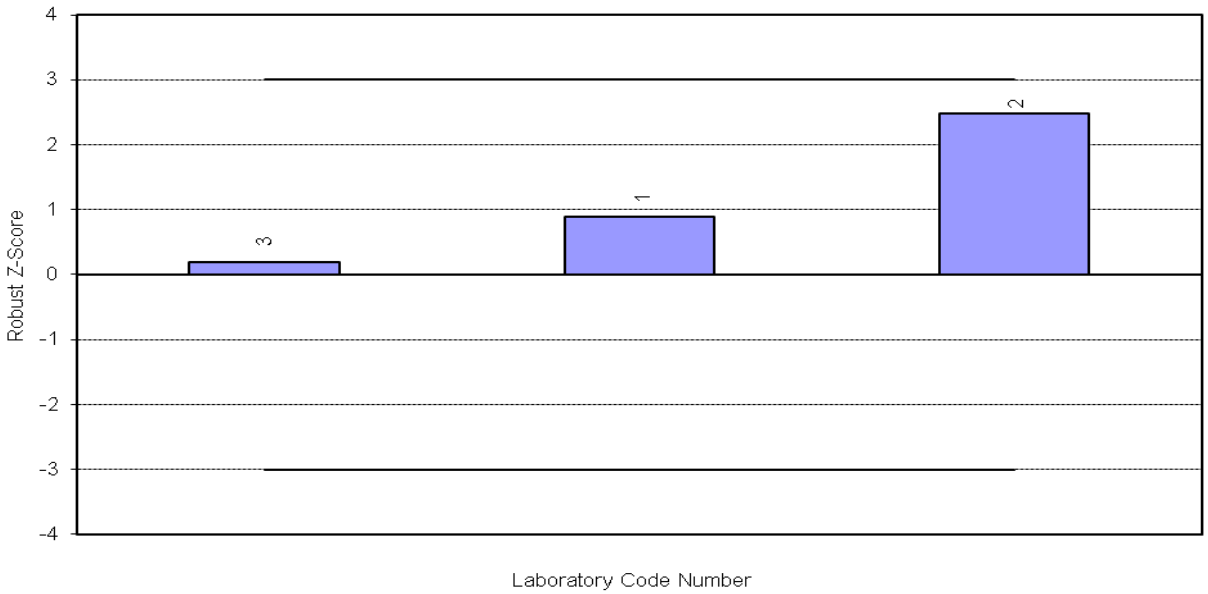
A9.2

Enterococci (orgs/100mL) – MF Technique
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



Enterococci (orgs/100mL) – MPN Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log_{10} Result	PTA 2 log_{10} Result	PTA 1 Robust z-score	PTA 2 Robust z-score
3	79000		92000		4.90	4.96	4.08 **§**	6.57 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	11
Median	4.462
Norm IQR	0.107
Robust CV	2.4%
Minimum	4.23
Maximum	4.61
Range	0.38

Sample - PTA 2

No. of Results	11
Median	4.204
Norm IQR	0.116
Robust CV	2.7%
Minimum	4.08
Maximum	4.85
Range	0.77

Notes:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

2. Robust z-score charts have not been included as there is only one PTA result.

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SECTION A11

Plate Count
All Techniques

Plate Count (orgs/mL) – All Techniques

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 $\log_{10}$ Result	PTA 2 $\log_{10}$ Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	4200		2200		3.62	3.34	1.17	0.00
2	3400	±690	2400	±487	3.53	3.38	0.15	0.29
3	3800		1700		3.58	3.23	0.69	-0.86
5	110000		180		5.04	2.26	17.08 **§**	-8.35 **§**

Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

Summary Statistics
Sample - PTA 1

No. of Results	7
Median	3.519
Norm IQR	0.089
Robust CV	2.5%
Minimum	3.41
Maximum	3.62
Range	0.21

Sample - PTA 2

No. of Results	7
Median	3.342
Norm IQR	0.130
Robust CV	3.9%
Minimum	3.15
Maximum	3.54
Range	0.40

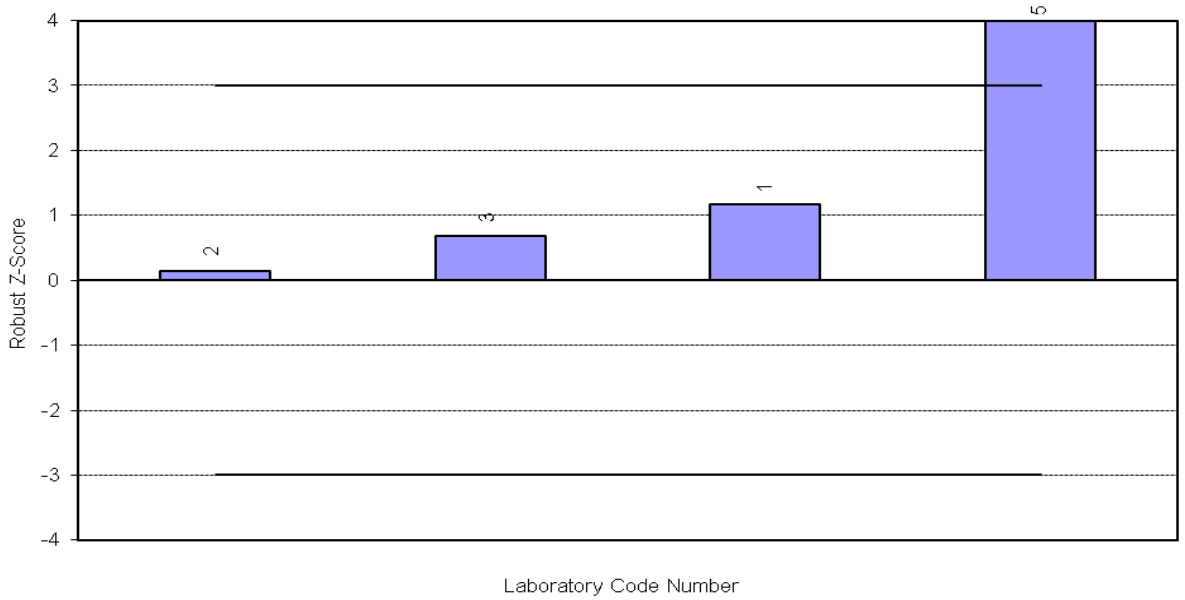
Note:

1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd results (from another trial using the same samples).

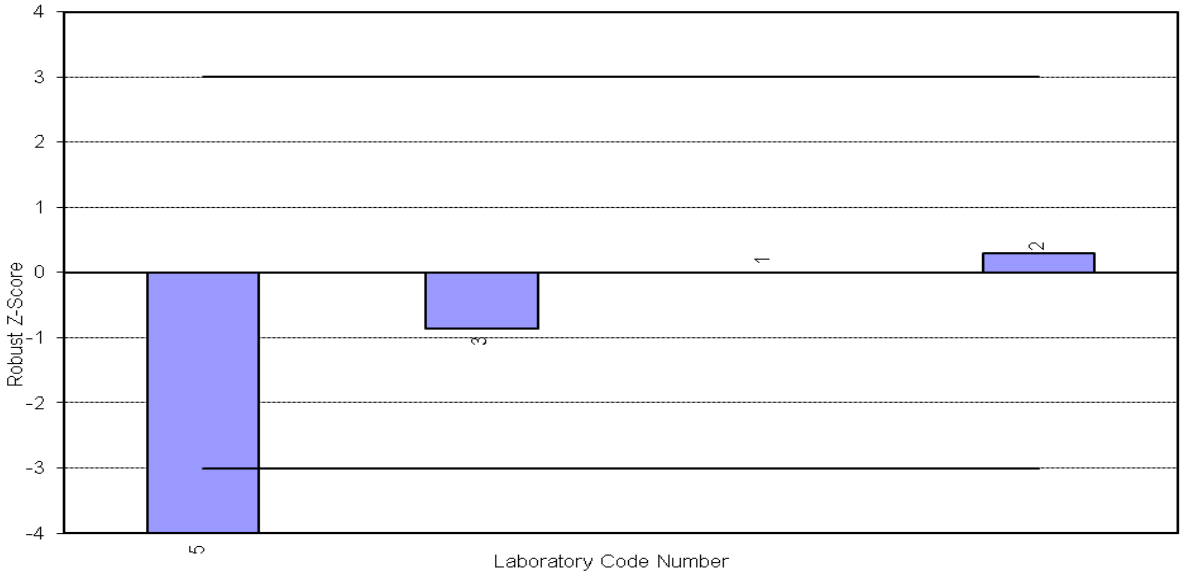
A11.2

Plate Count (orgs/mL) – All Techniques
Ordered Robust Z-Score Charts

Sample - PTA 1



Sample - PTA 2



APPENDIX B

Sample Preparation,

Homogeneity and Stability Testing

SAMPLE PREPARATION

The samples used for this program were prepared by Global Proficiency Ltd (New Zealand).

The samples were dispatched to all laboratories on 2 September 2013. When reconstituted and added to the specified volume of sterile water, each sample was representative of an effluent sample.

HOMOGENEITY AND STABILITY TESTING

A number of samples were selected for quality control sample analyses, to ensure that sample variability was not a contributing factor to the performance of the participants.

During sample preparation for this program, ten randomly selected samples from PTA 2 were set aside for homogeneity testing and three other randomly selected samples were set aside for stability testing.

Samples were tested for homogeneity and stability using the following media and techniques:

1. Faecal Coliforms: Spread plate using mFC agar.
2. Enterococci: Spread plate using mEnterococcus agar.

Faecal Coliforms

The samples were tested for homogeneity and stability, in duplicate, on mFC agar at 44.5ºC for 22 hours. The results of this testing appear in the following table.

Faecal Coliforms (cfu/100mL equivalent)							
Homogeneity Testing				Stability Testing			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
49000	4.6902	49000	4.6902	41000	4.6128	44000	4.6435
58000	4.7634	57000	4.7559	44000	4.6435	47000	4.6721
52000	4.7160	46000	4.6628	35000	4.5441	37000	4.5682
53000	4.7243	53000	4.7243				
45000	4.6532	54000	4.7324				
45000	4.6532	47000	4.6721				
52000	4.7160	58000	4.7634				
48000	4.6812	49000	4.6902				
48000	4.6812	43000	4.6335				
52000	4.7160	55000	4.7404				

Enterococci

The samples were tested for homogeneity and stability, in duplicate, via mEnterococcus agar with incubation at 37ºC for 48 hours. The results of this testing appear in the following table.

Enterococci (cfu/100mL equivalent)							
Homogeneity Testing				**Stability Testing**			
Result A	Log A	Result B	Log B	Result A	Log A	Result B	Log B
34000	4.5315	36000	4.5563	29000	4.4624	32000	4.5051
30000	4.4771	25000	4.3979	34000	4.5315	35000	4.5441
36000	4.5563	36000	4.5563	28000	4.4472	36000	4.5563
33000	4.5185	34000	4.5315				
30000	4.4771	23000	4.3617				
36000	4.5563	30000	4.4771				
39000	4.5911	34000	4.5315				
35000	4.5441	31000	4.4914				
38000	4.5798	33000	4.5185				
35000	4.5441	27000	4.4314				

From the analysis of these results, it was concluded that the samples were sufficiently homogeneous.

Stability testing was undertaken where samples were exposed to ambient temperatures for a period of four days. It was concluded that samples were stable for the period of the trial.

APPENDIX C

Instructions to Participants

Instructions for Re-hydration of Sample

Results Sheet

PROFICIENCY TESTING AUSTRALIA

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

INSTRUCTIONS TO PARTICIPANTS

ROUND 48 – SEPTEMBER 2013

Please read instructions carefully **BEFORE** commencing testing.

To ensure that the results of this program can be analysed properly, participants are asked to adhere carefully to the following instructions.

1. For this round each participant will be supplied with two freeze-dried samples, labelled PTA 1 and PTA 2, which are to be re-hydrated as outlined in the instructions below. When re-hydrated both samples will be representative of effluent water samples.

2. Commence testing as soon as possible after receipt. Please store all samples at <4^{o}C until testing commences.

3. To aid us with the statistical analyses of the results we ask that all laboratories set up methods such that you can report actual numerical results.

4. The re-hydrated samples are to be examined as follows:

   Analyse for *E. coli*, faecal coliforms, total coliforms, enterococci and 37°C (or 35°C) plate count.

5. These tests are to be conducted by the methods used routinely in your laboratory.

6. On the *Results Sheet* provided, please report results for each test performed for each sample. Please indicate the technique used for plate count in the blank entry of the *Technique* column for plate count on the results sheet. Please also complete the column *Method Source/ Year.*

7. Laboratories are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of MU must be given as a 95% confidence interval (coverage factor k ≈ 2). For microbiological testing, you may submit MU information as either a range of results if reporting in standard form (e.g. 6.2 x 10^{1} cfu/100mL) or confidence limits from MPN tables if MPN methods are used, or as a Log_{10} value if reporting a +/- value (please follow the procedure you use in your laboratory). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.

8. All laboratories are to return their results **by Monday 16 September 2013 to**:

   Kathy Weller
   Kathy.Weller@pta.asn.au
   Telephone: +61 7 3721 7373
   Fax: +61 7 3217 1844

9. To allow for the confidential treatment of your results in the final report, you have been allocated a code number which appears on your results sheet.

PROFICIENCY TESTING AUSTRALIA

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

ROUND 48 – SEPTEMBER 2013

INSTRUCTIONS FOR RE-HYDRATION OF SAMPLE

1. **For EACH sample, re-hydrate the freeze-dried vial by adding 3.0mL of sterile diluent eg (0.1% (w/v) peptone or 0.85% (w/v) NaCl (ISO 6887-1) at room temperature.**

2. **Allow to stand at room temperature for 10 minutes.**

3. **Mix the vial contents using a vortex mixer or shake 25 times in about 7 seconds.**

4. **Aseptically transfer 2.0mL of vial contents to 1000mL sterile deionised (or distilled) water. This will leave 1.0mL remaining in the vial, which may be used to prepare samples for intra-laboratory comparison purposes, if required by the laboratory.**

5. **Shake the sample bottle 25 times to produce the simulated water sample.**

6. **Examine the sample using your routine test methods.**

7. **Repeat steps 1 through 6 for the second sample.**

C3

PROFICIENCY TESTING AUSTRALIA

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

ROUND 48 SEPTEMBER 2013

RESULTS SHEET

Laboratory Code: _____

Test	Technique	PTA 1	MU	PTA 2	MU	Method Source/ Year/Technique
E. coli (orgs/100mL)	MF					☐ AS/NZS 4276.7-2007 ☐ Other:
	MPN					☐ AS/NZS 4276.6-2007 ☐ Other:
	Colilert					☐ AS 4276.21-2005 ☐ Other:
Faecal Coliforms (orgs/100mL)	MF					☐ AS/NZS 4276.7-2007 ☐ Other:
	MPN					☐ AS/NZS 4276.6-2007 ☐ Other:
Total Coliforms (orgs/100mL)	MF					☐ AS/NZS 4276.5-2007 ☐ Other:
	MPN					☐ AS/NZS 4276.6-2007 ☐ Other:
	Colilert					☐ AS 4276.21-2005 ☐ Other:
Enterococci (orgs/100mL)	MF					☐ AS NZS 4276.9-2007 ☐ Other:
	MPN					☐ APHA 9230B or 9230D ☐ Other:
Plate Count 37°C (or 35°C) (orgs/mL)	Pour Plate					☐ AS/NZS 4276.3.1-2007 ☐ Other:
	Other					

Date Sample Received: ….....……

Temperature of samples on arrival: ……

Date Sample Processed: .....……..…....

Comments .....

……

Signature: _____ Date: _____

-- End of Report --