MICROBIOLOGICAL WATERS

PROFICIENCY TESTING PROGRAM

ROUND 62

AUGUST 2018

REPORT NO. 1099

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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CONTENTS PAGE

1. FOREWORD

This report summarises the results of a microbiological proficiency testing program on water. It constitutes the sixty-second round of an ongoing series of programs. This program is accredited to ISO/IEC 17043:2010 "Conformity assessment - General requirements for proficiency testing" by International Accreditation New Zealand (IANZ).

The program was conducted in June 2018 by Proficiency Testing Australia (PTA). The aim of the program was to assess laboratories' abilities to competently perform the prescribed analyses.

The Program Coordinator was Mrs K Weller and the Technical Adviser was Ms S Mott from Global Proficiency Ltd (New Zealand). This report was authorised by Mrs K Cividin, PTA Quality Manager.

2. FEATURES OF THE PROGRAM

(a) A total of six separate laboratories received samples for the program with all six 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 and Sri Lanka.

(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), Thermotolerant (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*, Thermotolerant (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 z-score 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;
* the range (*Maximum* – *Minimum*); and
* the uncertainty of the median; a robust estimate of the standard deviation of the median.

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-score $\leq$ -3.0 or z-score $\geq$ 3.0). 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	26	4.450	0.135
		2	26	4.775	0.145
	MPN	1	11	4.540	0.340
		2	11	4.850	0.311
	Colilert	1	27	4.510	0.119
		2	27	4.790	0.174
Thermotolerant (Faecal) Coliforms orgs/100mL	MF	1	32	4.440	0.100
		2	33	4.720	0.104
	MPN	1	11	4.540	0.259
		2	12	4.790	0.315
Total Coliforms orgs/100mL	MF	1	16	4.595	0.140
		2	17	4.780	0.145
	MPN	1	8	4.710	0.167
		2	9	4.900	0.201
	Colilert	1	24	4.650	0.137
		2	24	4.790	0.163
Enterococci orgs/100mL	MF	1	20	4.490	0.135
		2	21	4.820	0.145
	Enterolert	1	17	4.300	0.059
		2	17	4.610	0.104
Plate Count orgs/mL	All	1	10	3.330	0.111
		2	10	3.565	0.100

All statistics (including No. of Results) are calculated from Global Proficiency Ltd's results from another trial using the 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	-	-	-
	MPN	3	-	-
	Colilert	-	-	-
Faecal Coliforms	MF	-	-	-
	MPN	-	-	-
Total Coliforms	MF	-	-	-
	MPN	3, 6	-	-
	Colilert	2	-	-
Enterococci	MF	-	-	-
	Enterolert	-	-	-
Plate Count	All	3	-	-

Note:

1. Target CVs were used to calculate the z-scores for *E. coli,* Total Coliforms and Enterococci using the MF technique for both samples.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

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

Two coliform organisms were incorporated in Sample PTA 1; *E. coli* and *Citrobacter freundii* (*C. freundii*). *E. coli* was the only coliform organism included in Sample PTA 2. *Enterococcus faecalis* (*E. faecalis*) was included as a member of the enterococci group in both samples. 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.

With regard to Most Probable Number testing, the PTA Water Micro programmes have previously split the assessment of data between Traditional and Colilert methods. As will be discussed further in this report, standard methods do allow for the use of enzyme hydrolysable substrates in the conventional or Traditional format, therefore there is cross-over between the two method types. Some of the options open to laboratories are summarised on the following page:

- Traditional method using the conventional format (e.g. five-tube-three-dilution method) as defined in AS/NZS 4276.6

- Traditional method using the conventional format (e.g. five-tube-three-dilution) with the enzyme hydrolysable substrate as defined in AS/NZS 4276.21; may be via the preparation of the MPN series in-house; via commercially prepared ready-to-use plates such as Colitag™ (MPNPlates)

- Enzyme hydrolysable substrate method as defined in AS/NZS 4276.21 using the Colilert Quanti-Tray (multi-well trays) format

Therefore, in future rounds, the result forms will be examined and may be modified to ensure the range of options discussed above are included.

As there were a small number of participants in this round, participant results were assessed against Global Proficiency Ltd's data using the same samples.

Commentary on performance and comparisons between methods were made for each test and comments are included in the report following.

Total Coliforms:
Between three and four laboratories reported results for the three different Total Coliforms techniques. Two participants (laboratories 3 and 6) recorded outliers for the MPN technique with laboratory 3 reporting results lower than expected for both samples and laboratory 6 reporting a result lower than expected for Sample PTA 1. One participant (laboratory 2) recorded an outlier for the Colilert technique with a result higher than expected for Sample PTA 2.

All laboratories reported using AS or APHA methods. One laboratory reported results for the Colilert test where they had used Colitag, referencing AS 4276.21. The assumption is that a Traditional (conventional) MPN set up was used, with the enzyme hydrolysable substrate present in Colitag used as a more rapid test – this option is covered as an option in this standard.

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} cfu/100mL)	**Sample PTA 2** Median ± Uncertainty (Log_{10} cfu/100mL)
Membrane Filtration	4.595 ± 0.044	4.780 ± 0.044
Most Probable Number	4.710 ± 0.074	4.900 ± 0.084
Colilert	4.650 ± 0.035	4.790 ± 0.042

Statistics from Global Proficiency Ltd's results using the same samples were used for all methods.

Measurement Uncertainty: Total Coliforms

Five laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported in three different ways; i.e. ± log values, a range of cfu/100mL values and % relative expanded uncertainty.

Of the reported MUs for the Total Coliforms methods, two did not accurately reflect the difference between the laboratory result and the median (taking into consideration the uncertainty associated with the median), details as follows:

- Laboratory 2 may need to re-examine their test results or their MU calculations for the Colilert method as their results for PTA 2 and the stated uncertainty were outside the expected range of the median and its associated uncertainty.
- Laboratory 5 may also need to re-examine their MU calculations for the Membrane Filtration method as their result and stated uncertainty for sample PTA 1 were outside the expected range of the median and its associated uncertainty.

Graphs showing the differentiation of methods used for Total Coliform 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 for the methods listed above.

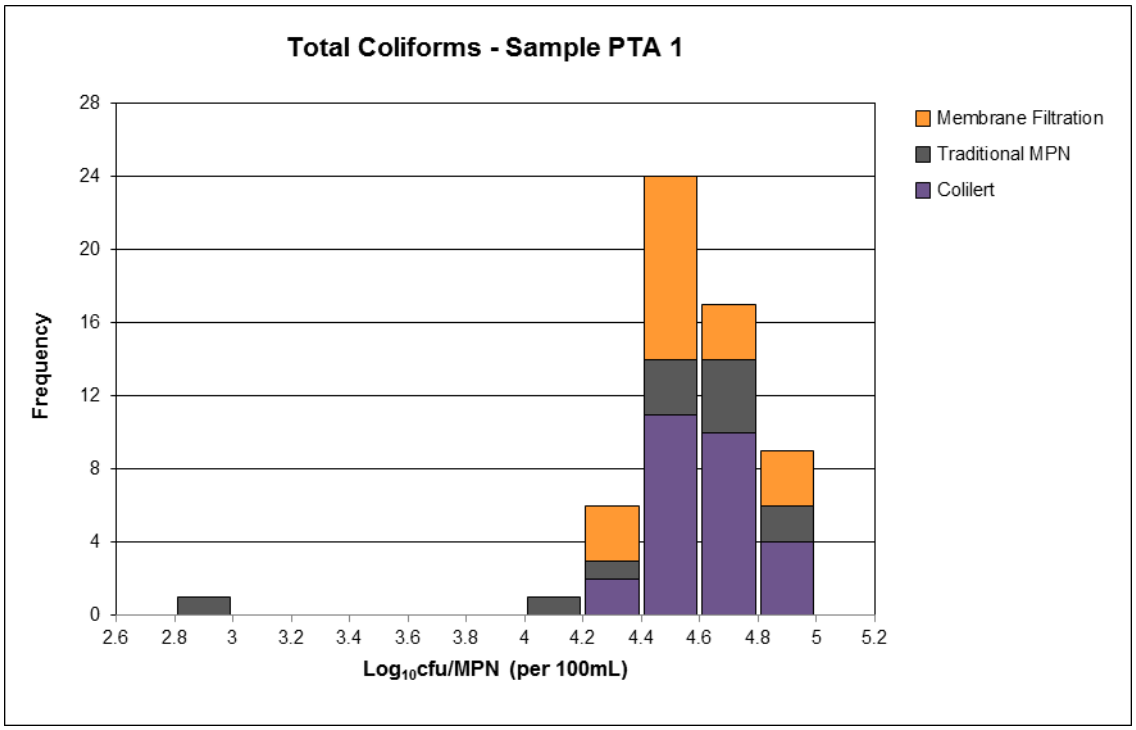


Figure TA-1. Total Coliform results for Sample PTA 1

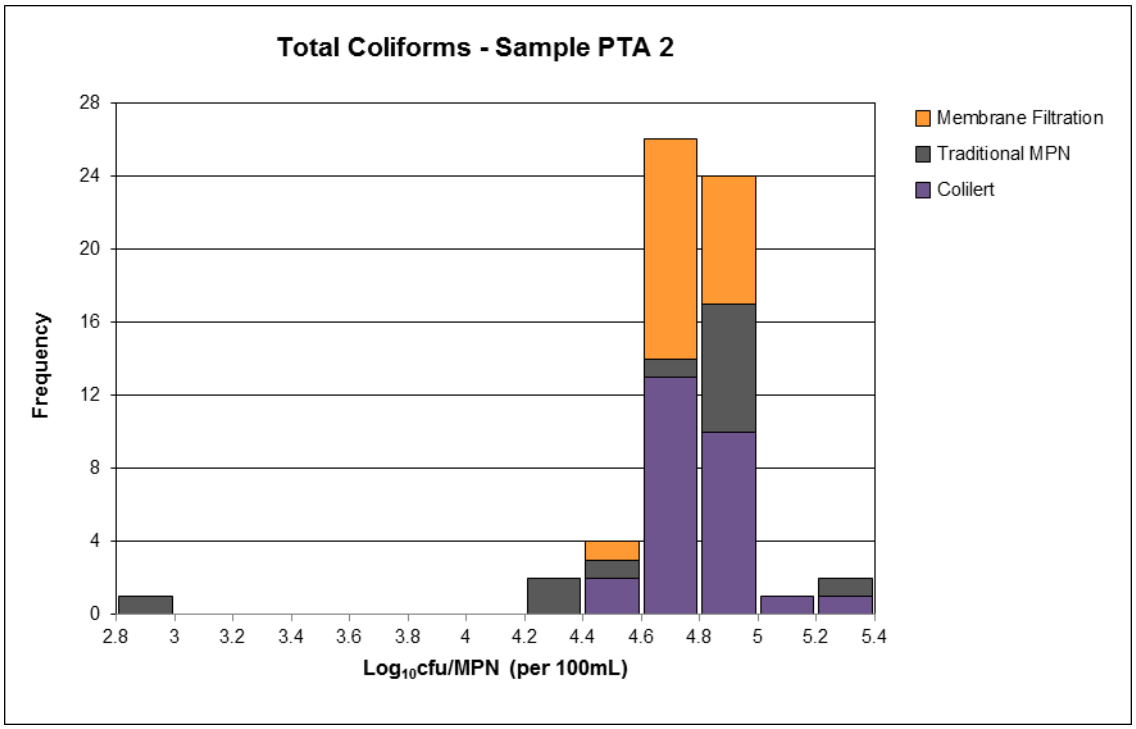


Figure TA-2. Total Coliform results for Sample PTA 2

E. coli:
Between three and four laboratories reported results for the three *E. coli* techniques. One participant (laboratory 3) recorded outliers for the MPN technique with reported results lower than expected for both samples.

All laboratories reported using AS or APHA methods. One laboratory reported results for the Colilert test where they had used Colitag, referencing AS 4276.21. The assumption is that a Traditional (conventional) MPN set up was used, with the enzyme hydrolysable substrate present in Colitag used as a more rapid test – this option is covered as an option in this standard.

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} cfu/100mL)	**Sample PTA 2** Median ± Uncertainty (Log_{10} cfu/100mL)
Membrane Filtration	4.450 ± 0.033	4.775 ± 0.036
Most Probable Number	4.540 ± 0.128	4.850 ± 0.118
Colilert	4.510 ± 0.029	4.790 ± 0.042

Statistics from Global Proficiency Ltd's results using the same samples were used for all methods.

Measurement Uncertainty: *E. coli*
Five laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways; i.e. ± log values, a range of cfu/100mL values and % relative expanded uncertainty.

- Laboratory 2 may need to re-examine their test results or their MU calculations for the Colilert method as their results for PTA 2 and the stated uncertainty were outside the expected range of the median and its associated uncertainty.
- Laboratory 5 may also need to re-examine their MU calculations for the Membrane Filtration method as their result and stated uncertainty for sample PTA 1 were outside the expected range of the median and its associated uncertainty.

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 for methods indicated above.

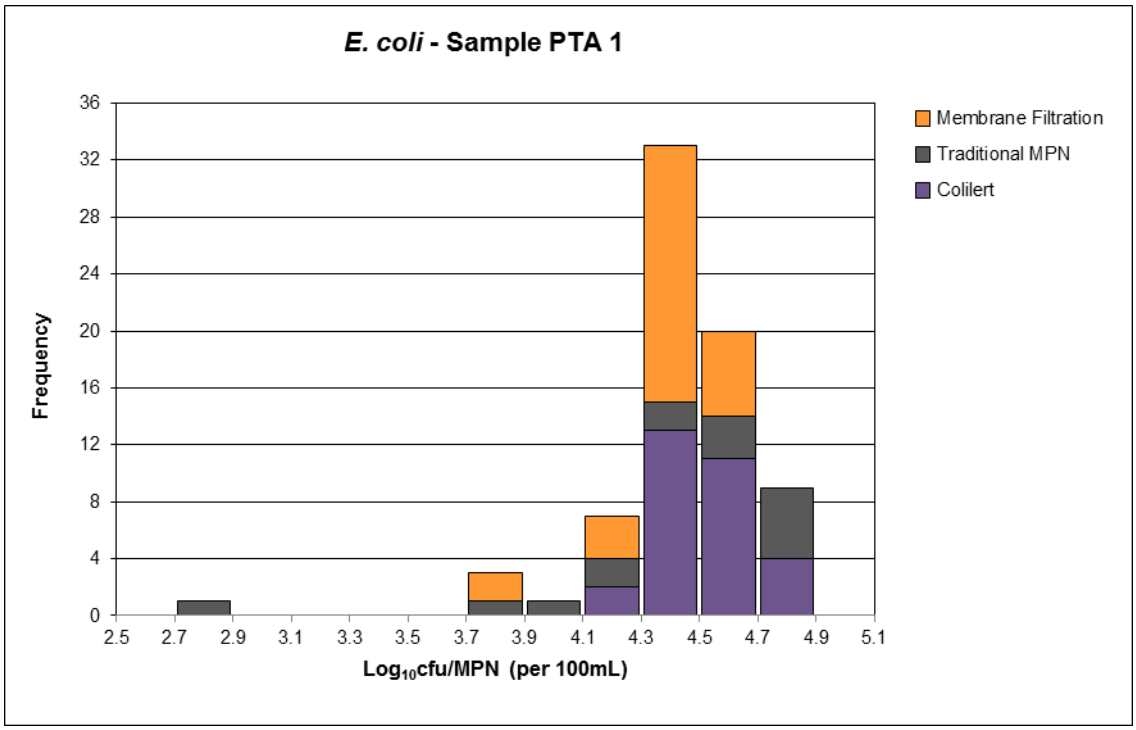


Figure TA-3. *E. coli* results for Sample PTA 1.

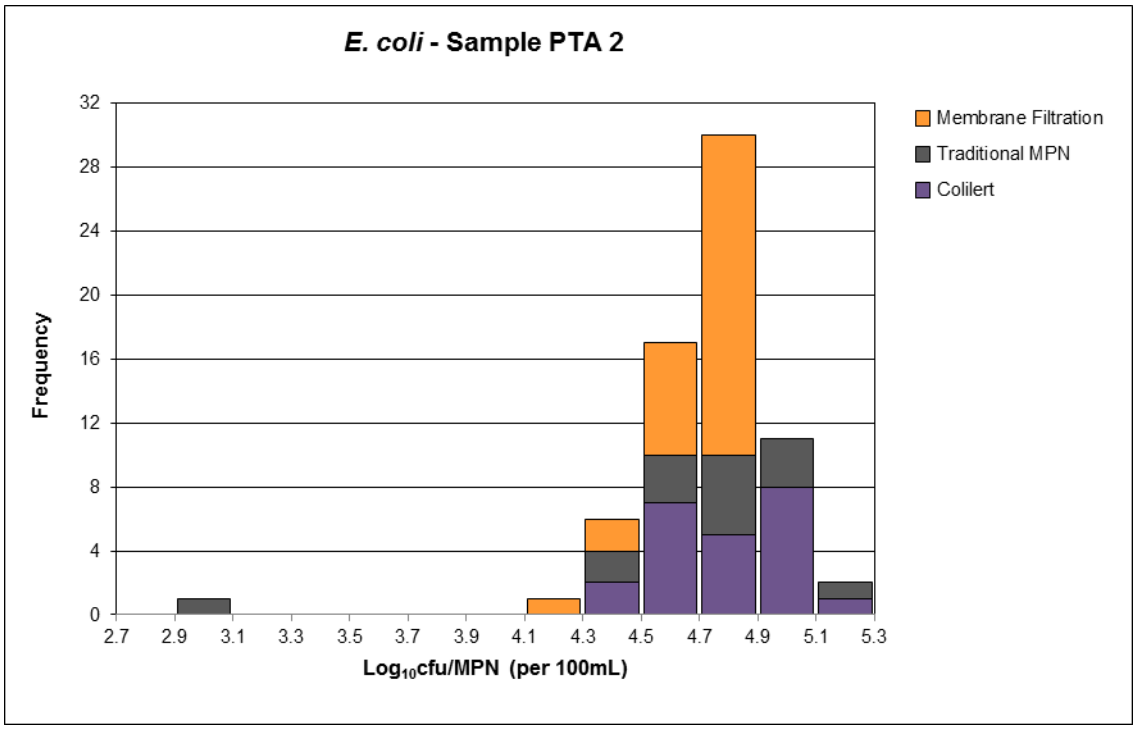


Figure TA-4. *E. coli* results for Sample PTA 2.

Thermotolerant (Faecal) Coliforms:
Four and five laboratories reported results for the two different Thermotolerant (Faecal) Coliforms techniques; MF and MPN respectively.

The majority of laboratories reported using AS or APHA methods. Two laboratories reported MPN results where one stated they had used Colilert with incubation at 44.5^{o}C, and the other stated they had used Colitag as an in-house method (presumably also incubated at 44.5^{o}C). No outliers were reported for this test.

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} cfu/100mL)	**Sample PTA 2** Median ± Uncertainty (Log_{10} cfu/100mL)
Membrane Filtration	4.440 ± 0.022	4.720 ± 0.023
Most Probable Number	4.540 ± 0.098	4.790 ± 0.114

Statistics from Global Proficiency Ltd's results using the same samples were used for all methods.

Measurement Uncertainty: Faecal Coliforms via Membrane Filtration (MF):
Four laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways; i.e. ± log values, a range of cfu/100mL values and % relative expanded uncertainty.

- Laboratory 5 may need to re-examine their MU calculations for the Membrane Filtration method as their result and stated uncertainty for both samples were outside the expected range of the median and its associated uncertainty.

Graphs showing the differentiation of methods used for Faecal Coliform testing are included below. These graphs show the distribution of results from the two methods used in this round.

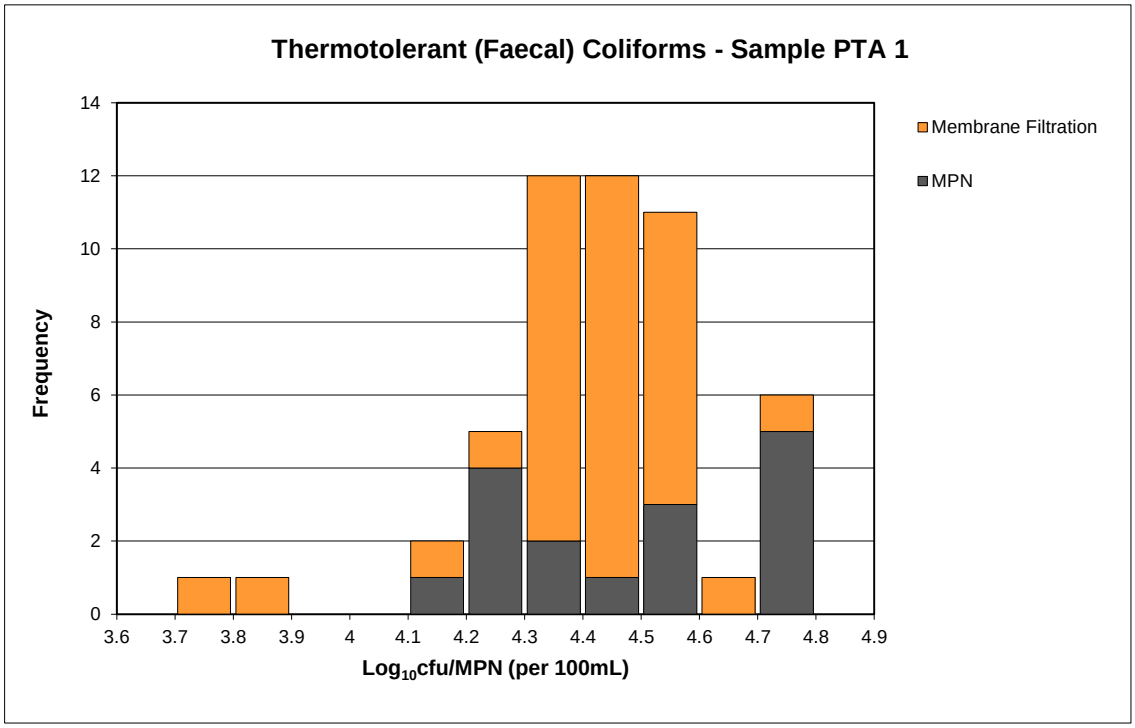


Figure TA-5. Thermotolerant (Faecal) Coliforms results for Sample PTA 1.

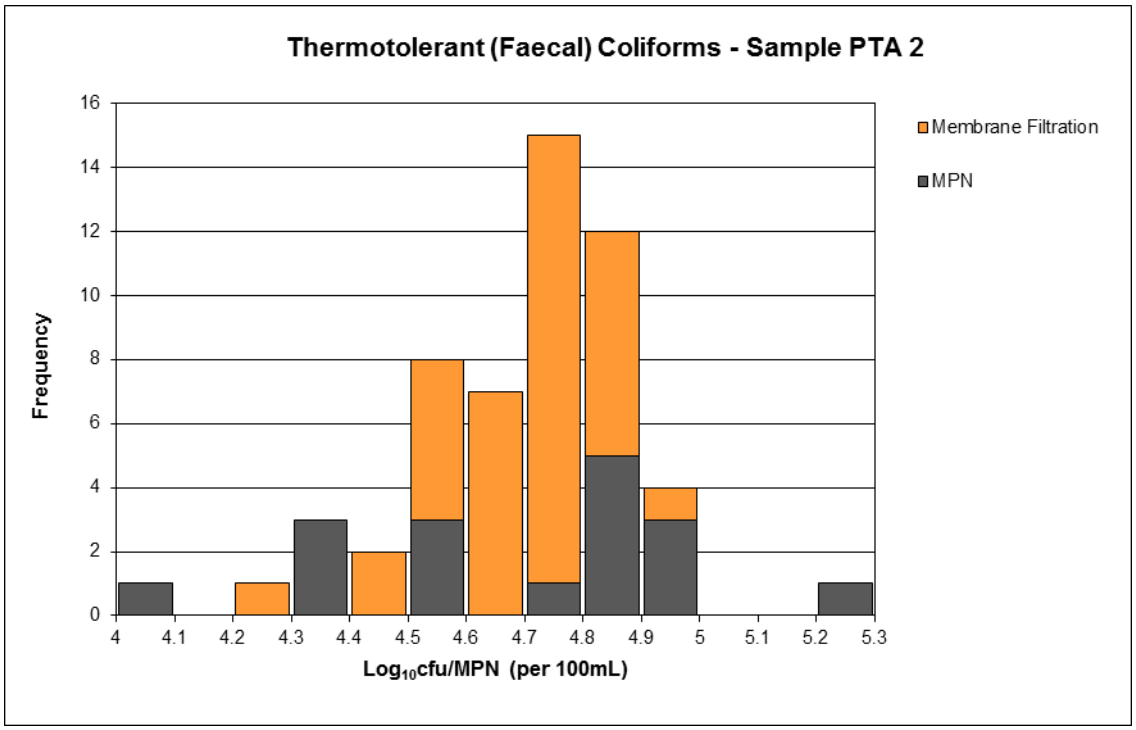


Figure TA-6. Thermotolerant (Faecal) Coliforms results for Sample PTA 2.

Enterococci:
Five and two laboratories reported results for the two different Enterococci techniques; MF and Enterolert respectively. No outliers were reported for this test

The majority of laboratories reported using AS or APHA methods. For Enterococci results reported in the Enterolert fields, it is assumed that the Enterolert Quanti-Tray (multi-well trays) format has been used.

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} cfu/100mL)	**Sample PTA 2** Median ± Uncertainty (Log_{10} cfu/100mL)
Membrane Filtration	4.490 ± 0.038	4.820 ± 0.040
Enterolert	4.300 ± 0.018	4.610 ± 0.032

Statistics from Global Proficiency Ltd's results using the same samples were used for this method.

Measurement Uncertainty: Enterococci via Membrane Filtration (MF):
Four laboratories reported MU estimations associated with their test results in this round. MU was reported in two different ways; i.e. ± log values and a range of cfu/100mL values.

Graphs showing the 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.

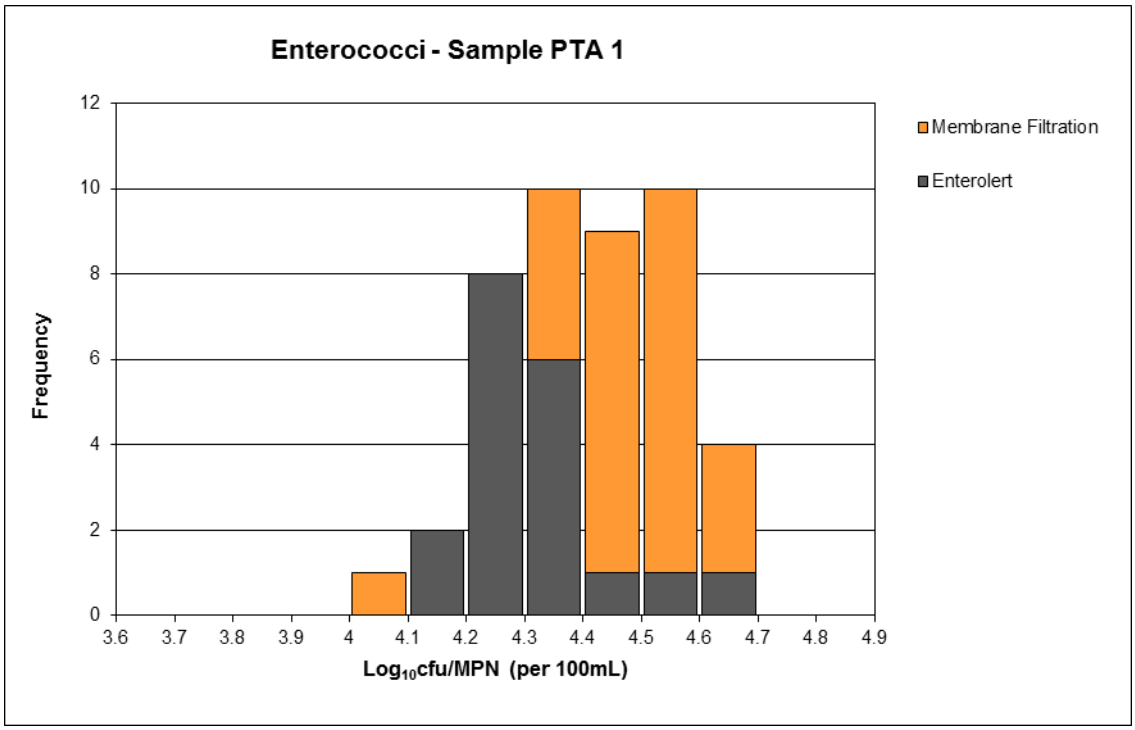


Figure TA-7. Enterococci results for Sample PTA 1

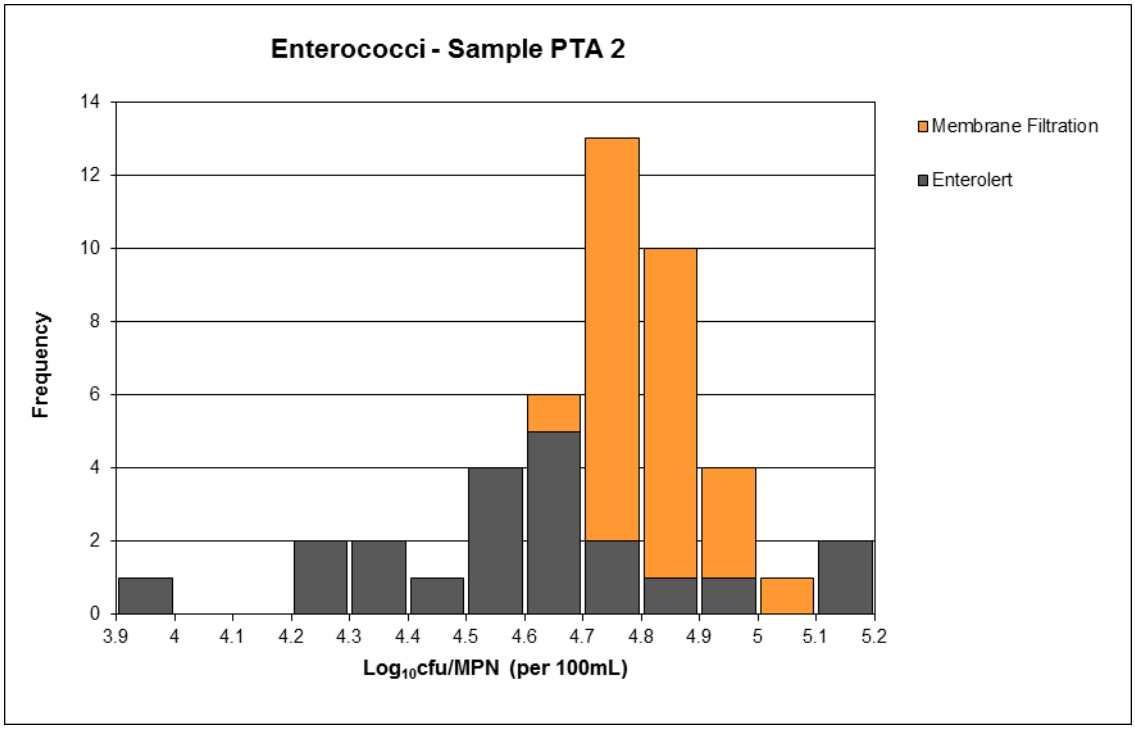


Figure TA-8. Enterococci results for Sample PTA 2

Plate Count:
Six sets of results were submitted for the Plate Count test. Two sets of results were obtained using APHA methods, one set of results was obtained using the Australian/New Zealand Standard method (AS/NZS 4276.3.1), one using another country standard/ISO method and two laboratories did not record the method used.

One participant (laboratory 3) reported an outlier for Sample PTA 2 with a result lower than expected. This laboratory reported using an incubation temperature of 30^{o}C however the mesophilic organisms used in these samples would be expected to grow just as well at this incubation temperature as at 35^{o}C, so we would not expect this to have influenced the results obtained.

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.

	Sample PTA 1 Median ± Uncertainty (Log_{10} cfu/mL)	**Sample PTA 2** Median ± Uncertainty (Log_{10} cfu/mL)
Plate Count:	3.330 ± 0.044	3.565 ± 0.040

Statistics from Global Proficiency Ltd's results using the same samples were used for this method.

Measurement Uncertainty: Plate Count:
Five laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways; i.e. ± log values, a range of cfu/100mL values and % relative expanded uncertainty.

General Comments

A total of 86 results were submitted for analysis in this round. Of these results 7 (8%) were outlier results. This is higher than the 0% of results which were outlier results in the previous potable water round (Round 60).

Outlier results 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* (2016). (This document can be found on the PTA website, www.pta.asn.au)

[2] ISO 13528:2015: *Statistical methods for use in proficiency testing by interlaboratory comparison*

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

[4] AS 4276.2-1995 (R2013): *Water microbiology - Culture media, diluents and reagents*

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

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

[7] AS/NZS 4276.6-2007: *Water microbiology – Coliforms, Escherichia coli and thermotolerant coliforms - Determination of most probable number (MPN)*

[8] AS/NZS 4276.7-2007: *Water microbiology - Escherichia coli and thermotolerant coliforms - Membrane filtration method*

[9] AS/NZS 4276.9-2007: *Water microbiology - Enterococci - Membrane filtration method* (ISO 7899-2:2000, MOD)

[10] AS 4276.21-2005: *Water microbiology - Examination for coliforms and Escherichia coli - Determination of most probable number (MPN) using enzyme hydrolysable substrates*

[11] APHA 9230C – *Fecal Enterococcus/Streptococcus Groups – Membrane Filtration techniques. American Public Health Association: Standard methods for the examination of water and wastewater, 23rd Edition (2017)*

[12] APHA 9230D – *Fecal Enterococcus/Streptococcus Groups – Fluorogenic Substrate Enterococcus test. American Public Health Association: Standard methods for the examination of water and wastewater, 23rd Edition (2017)*

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
2	30000	NA	61000	NA	4.48	4.79	0.20	0.07
4	25000	13900 - 45000	55000	29200-104000	4.40	4.74	-0.39	-0.24
5	16000	0.20	27000	0.20	4.20	4.43	-1.82	-2.37
6	33000	±0.14 log10	61000	±0.14 log10	4.52	4.79	0.51	0.07

Summary Statistics

Sample - PTA 1

No. of Results	26
Median	4.450
Norm IQR	0.135
Robust CV*	3.0%
Minimum	3.78
Maximum	4.66
Range	0.88
Uncertainty (Median)	0.033

Sample - PTA 2

No. of Results	26
Median	4.775
Norm IQR	0.145
Robust CV*	3.0%
Minimum	4.28
Maximum	4.90
Range	0.62
Uncertainty (Median)	0.036

Note:

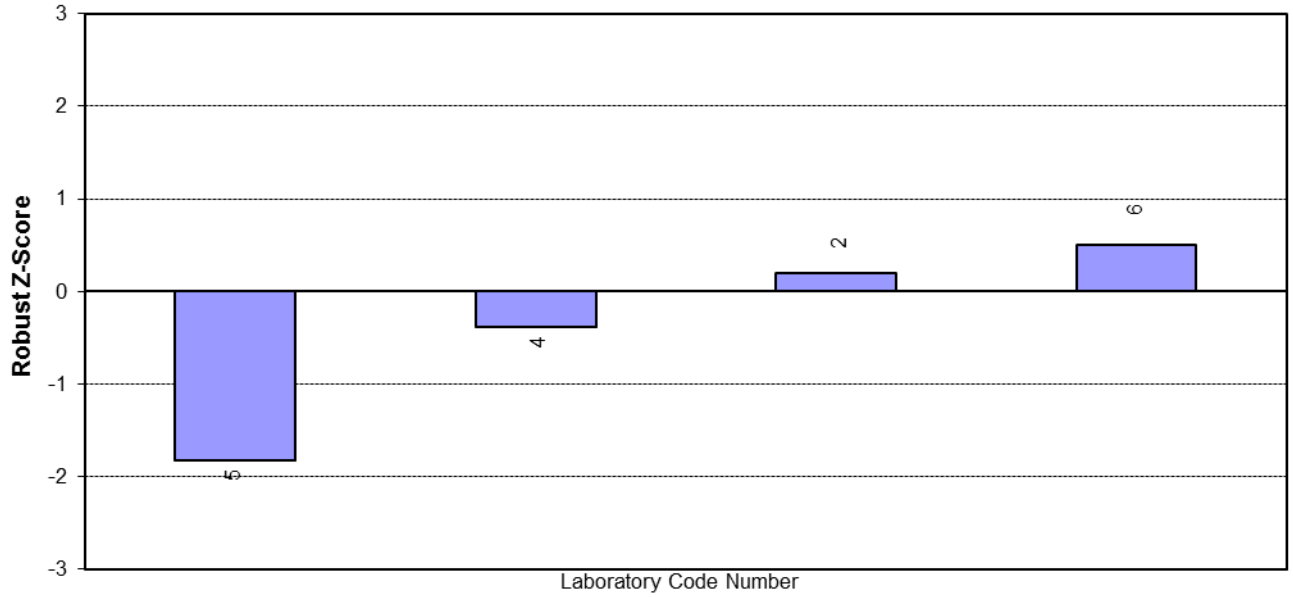
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).

2. *The robust CV's achieved for both samples were low (robust CV = 1.8% for Sample PTA 1 and 2.1% for Sample PTA 2) so in this case a target robust CV of 3.0% 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].

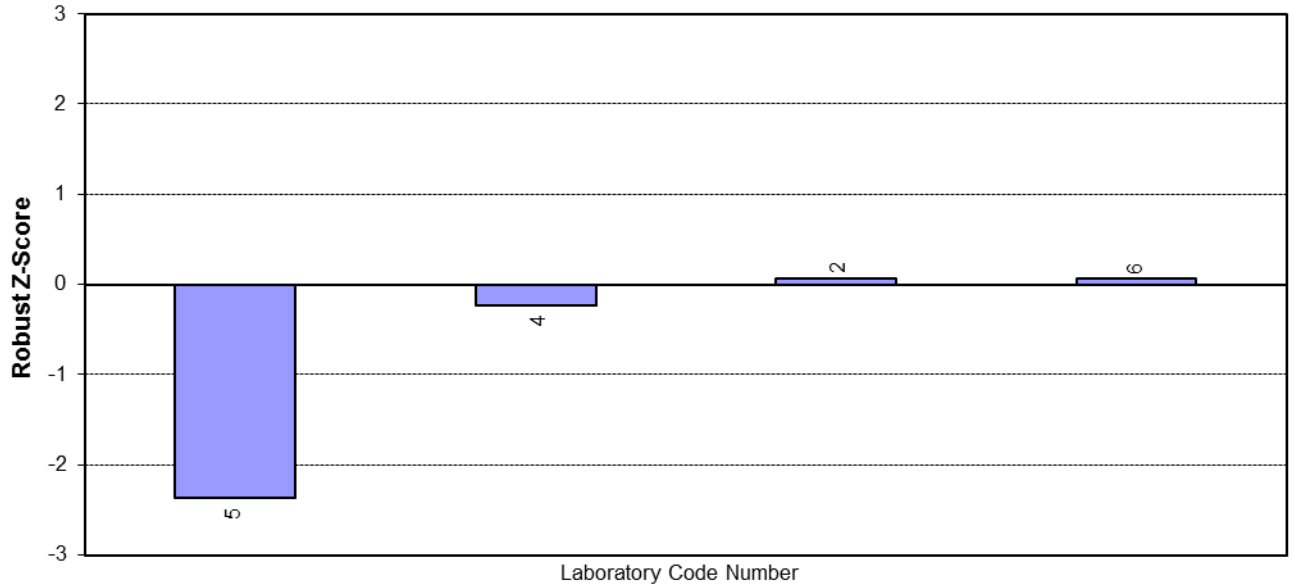
A1.2

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

Sample - PTA 1



Sample - PTA 2



A2.1

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
1	17000		24000	2% (REU)	4.23	4.38	-0.91	-1.51
3	540		920		2.73	2.96	-5.32 **§**	-6.06 **§**
5	54000	0.44	79000	0.44	4.73	4.90	0.57	0.15
6	7900	±0.59 log10	79000	±0.59 log10	3.90	4.90	-1.89	0.15

Note:

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

Summary Statistics

Sample - PTA 1

No. of Results	11
Median	4.540
Norm IQR	0.340
Robust CV	7.5%
Minimum	4.04
Maximum	4.73
Range	0.69
Uncertainty (Median)	0.128

Sample - PTA 2

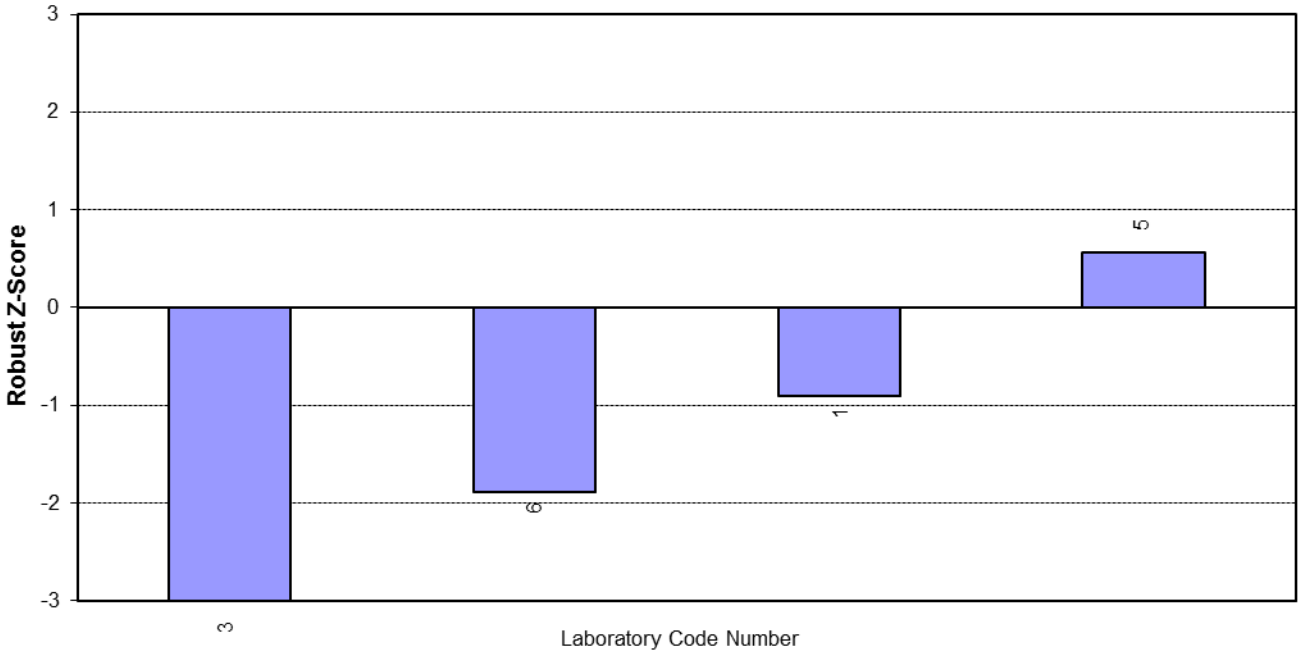
No. of Results	11
Median	4.850
Norm IQR	0.311
Robust CV	6.4%
Minimum	4.38
Maximum	5.23
Range	0.85
Uncertainty (Median)	0.118

Notes:

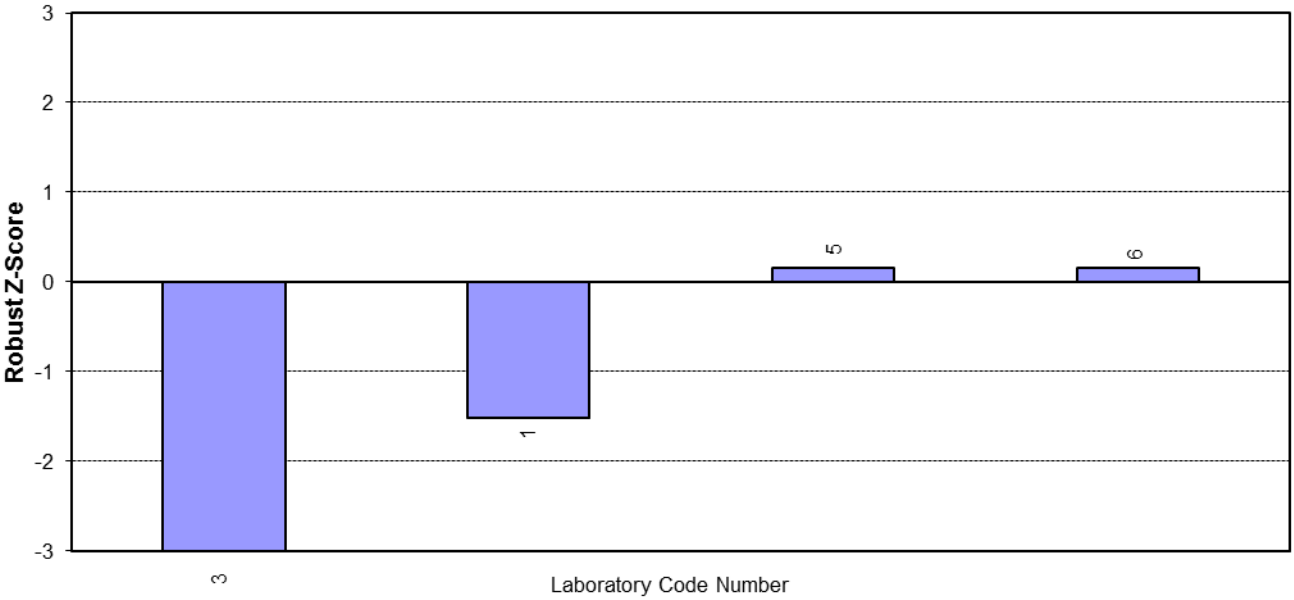
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).

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

Sample - PTA 1



Sample - PTA 2



A3.1

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
2	31000	18000-54000	200000	120000-350000	4.49	5.30	-0.16	2.93
4	26000	12900 - 52500	44000	21000 - 92000	4.41	4.64	-0.80	-0.84
6	37000	23000 - 56000	77000	55000 - 110000	4.57	4.89	0.49	0.55

Summary Statistics

Sample - PTA 1

No. of Results	27
Median	4.510
Norm IQR	0.119
Robust CV	2.6%
Minimum	4.24
Maximum	4.83
Range	0.59
Uncertainty (Median)	0.029

Sample - PTA 2

No. of Results	27
Median	4.790
Norm IQR	0.174
Robust CV	3.6%
Minimum	4.35
Maximum	4.80
Range	0.45
Uncertainty (Median)	0.042

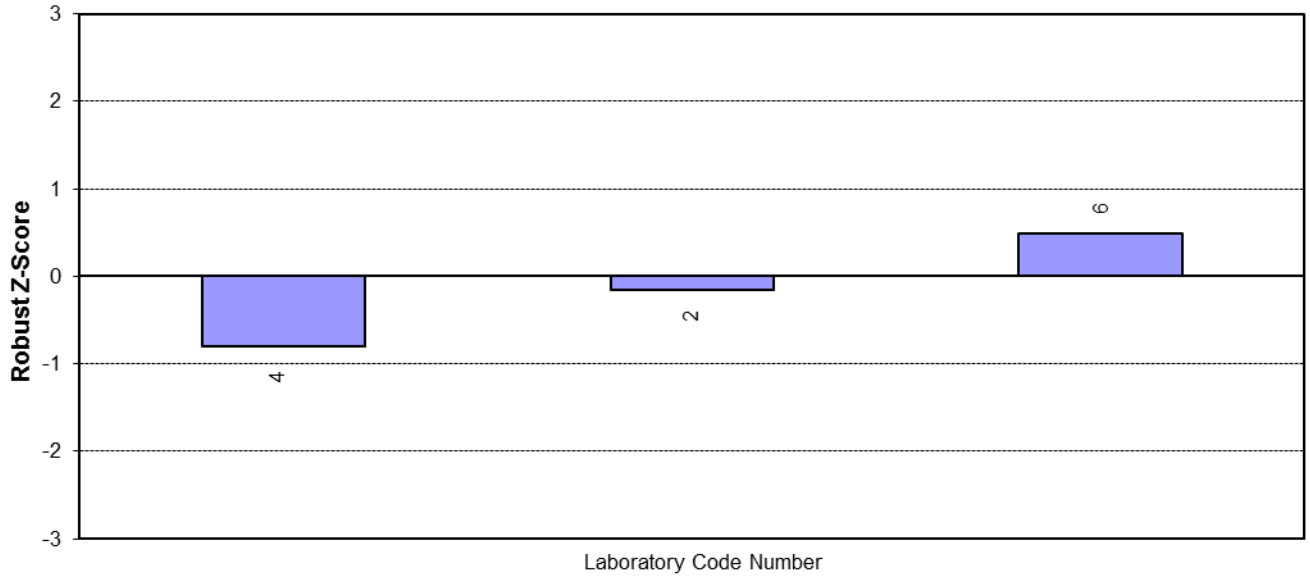
Note:

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

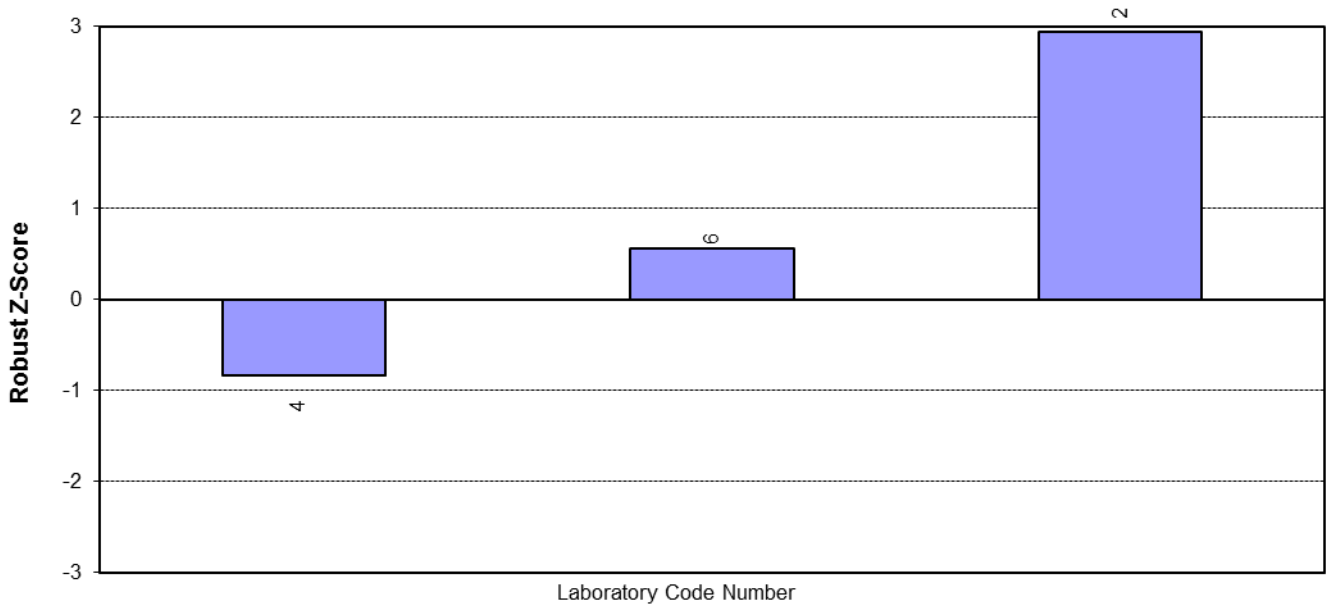
A3.2

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

Sample - PTA 1



Sample - PTA 2



SECTIONS A4 to A5

Thermotolerant (Faecal) Coliforms

Thermotolerant (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
2	31000	NA	65000	NA	4.49	4.81	0.51	0.90
4	25000	13700 - 45700	55000	28700 - 105000	4.40	4.74	-0.42	0.20
5	16000	0.13	27000	0.13	4.20	4.43	-2.36	-2.78
6	33000	±0.14 log10	61000	±0.14 log10	4.52	4.79	0.78	0.63

Summary Statistics
Sample - PTA 1

No. of Results	32
Median	4.440
Norm IQR	0.100
Robust CV	2.3%
Minimum	3.78
Maximum	4.78
Range	1.00
Uncertainty (Median)	0.022

Sample - PTA 2

No. of Results	33
Median	4.720
Norm IQR	0.104
Robust CV	2.2%
Minimum	4.28
Maximum	4.92
Range	0.64
Uncertainty (Median)	0.023

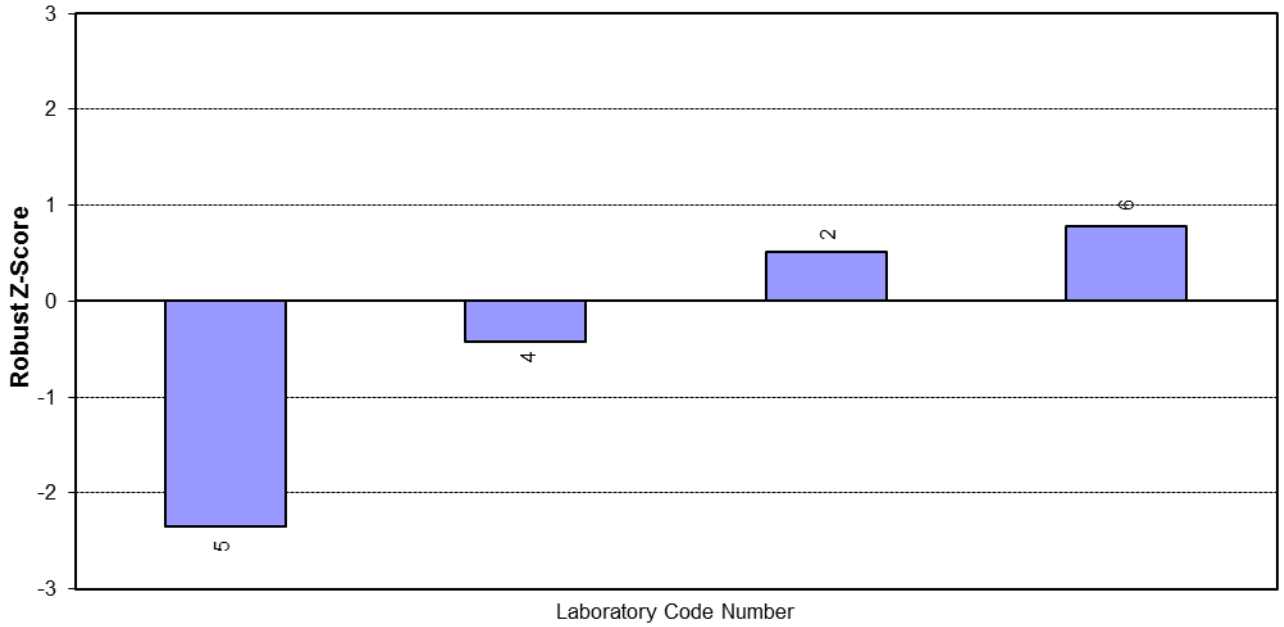
Note:

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

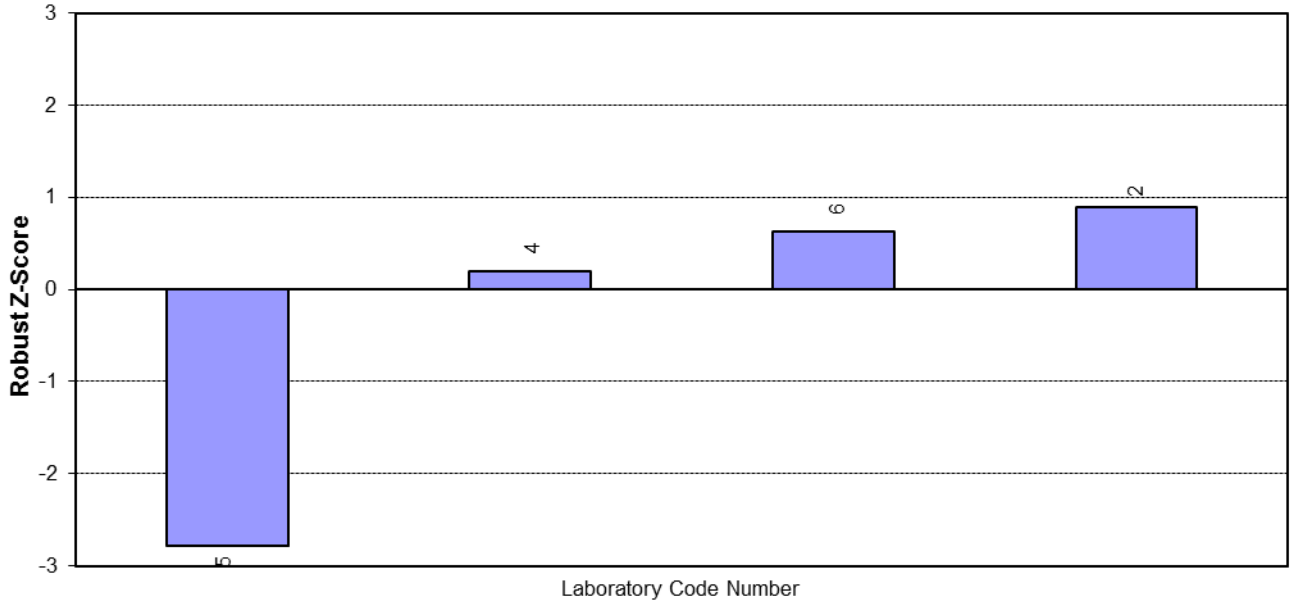
A4.2

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

Sample - PTA 1



Sample - PTA 2



Thermotolerant (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
1	17000		24000	2% (REU)	4.23	4.38	-1.19	-1.30
2	17000	NA	10000	NA	4.23	4.00	-1.19	-2.51
4	29000	11900 - 70400	65000	25000 - 169000	4.46	4.81	-0.30	0.07
5	54000	0.32	79000	0.32	4.73	4.90	0.74	0.34
6	13000	±0.59 log10	79000	±0.59 log10	4.11	4.90	-1.64	0.34

Summary Statistics
Sample - PTA 1

No. of Results	11
Median	4.540
Norm IQR	0.259
Robust CV	5.7%
Minimum	4.23
Maximum	4.73
Range	0.50
Uncertainty (Median)	0.098

Sample - PTA 2

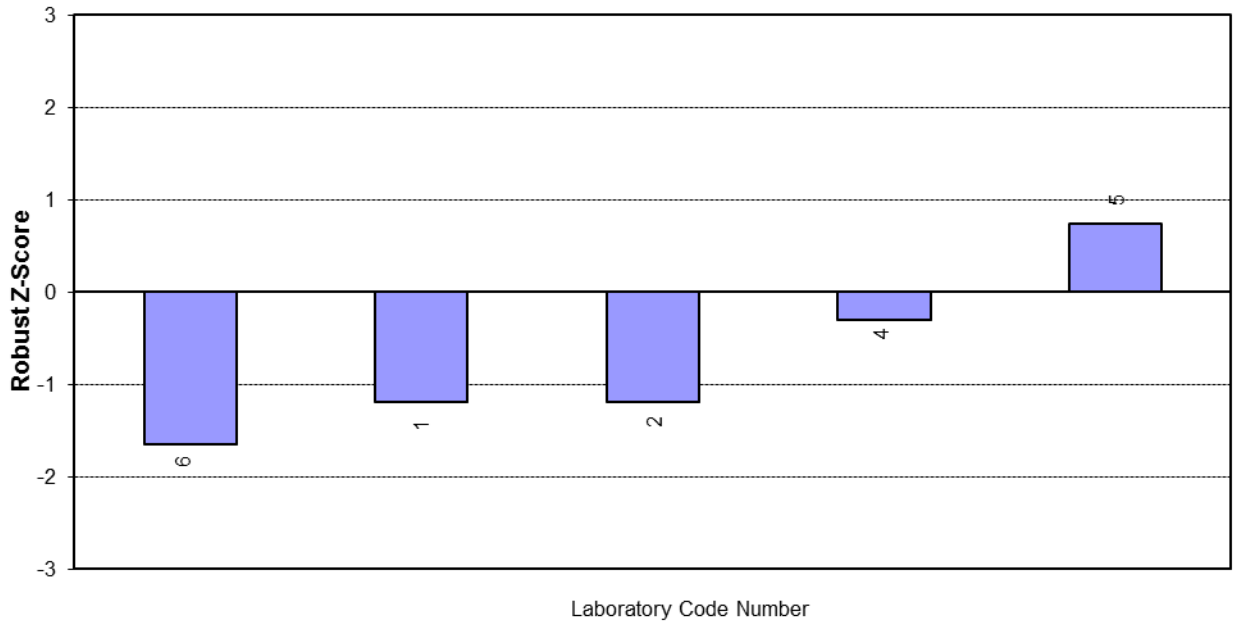
No. of Results	12
Median	4.790
Norm IQR	0.315
Robust CV	6.6%
Minimum	4.34
Maximum	5.23
Range	0.89
Uncertainty (Median)	0.114

Note:

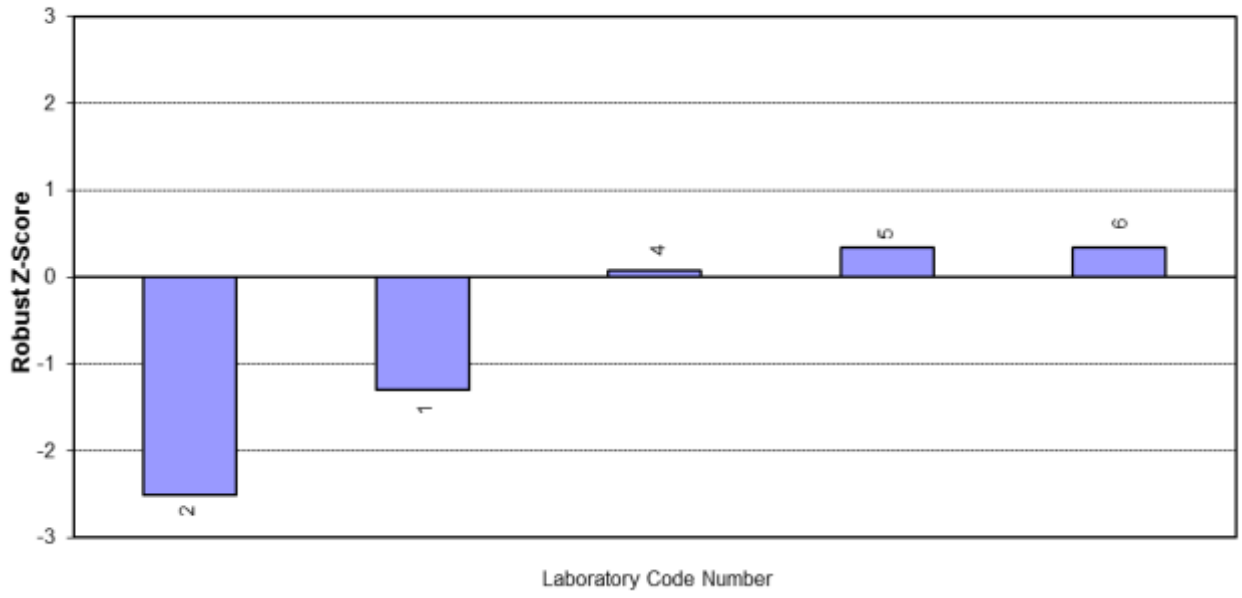
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).

Thermotolerant (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	35000	NA	61000	NA	4.54	4.79	-0.36	0.04
4	25000	13600 - 46100	55000	28400 - 106000	4.78	4.49	-1.41	-0.27
5	24000	0.15	30000	0.15	4.65	4.30	-1.53	-2.09

Summary Statistics
Sample - PTA 1

No. of Results	16
Median	4.595
Norm IQR	0.140
Robust CV*	3.0%
Minimum	4.39
Maximum	4.82
Range	0.43
Uncertainty (Median)	0.044

Sample - PTA 2

No. of Results	17
Median	4.780
Norm IQR	0.145
Robust CV*	3.0%
Minimum	4.61
Maximum	4.96
Range	0.35
Uncertainty (Median)	0.044

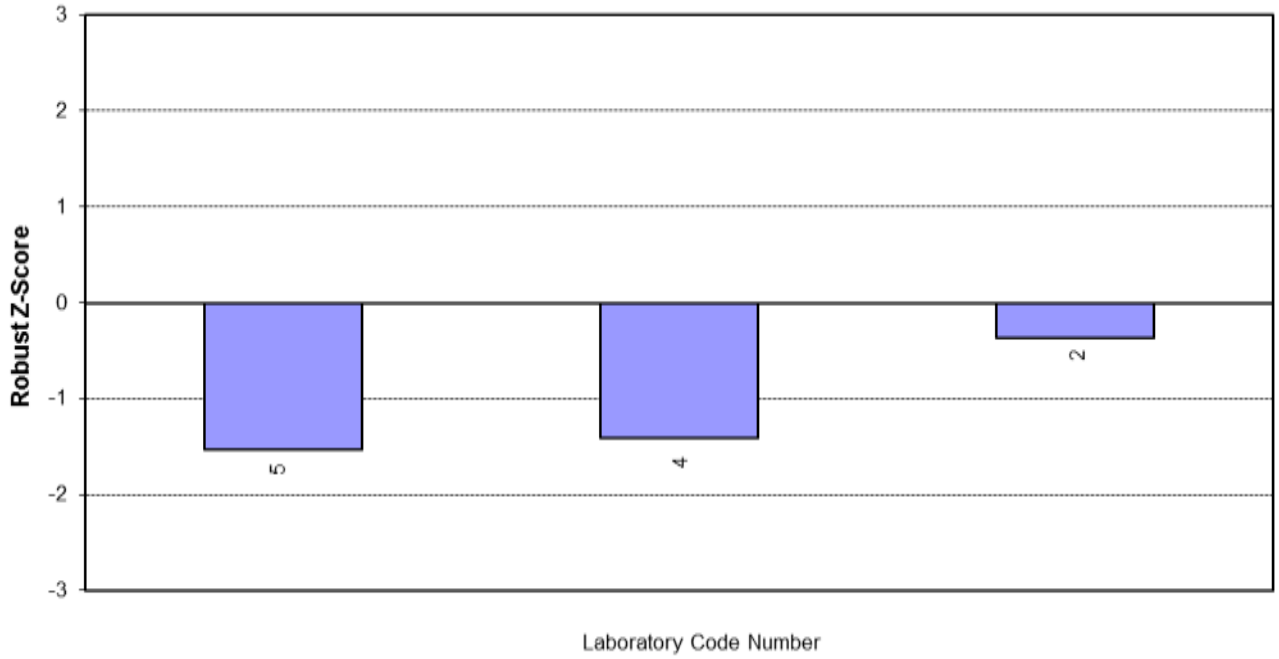
Note:

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

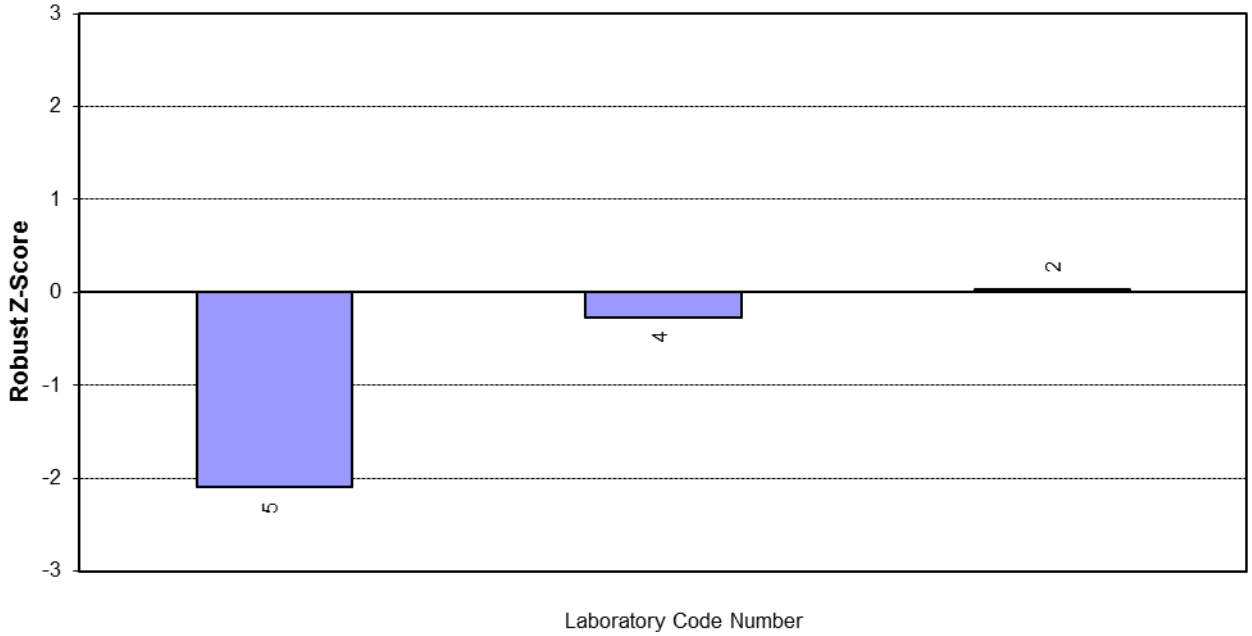
2. *The robust CV's achieved for both samples were low (robust CV = 1.4% for both samples) so in this case a target robust CV of 3.0% was considered more appropriate for both samples and was used to determine z-scores. For more information on calculating z-scores using target CVs refer to reference [1].

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
1	17000		24000	2.4% (REU)	4.23	4.38	-2.87	-2.59
3	920		920		2.96	2.96	-10.44 **§**	-9.63 **§**
5	92000	0.32	79000	0.32	4.96	4.90	1.52	-0.01
6	13000	±0.72 log10	79000	±0.72 log10	4.11	4.90	-3.56 **§**	-0.01

Note:

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

Summary Statistics

Sample - PTA 1

No. of Results	8
Median	4.710
Norm IQR	0.167
Robust CV*	3.6%
Minimum	4.52
Maximum	4.96
Range	0.44
Uncertainty (Median)	0.074

Sample - PTA 2

No. of Results	9
Median	4.900
Norm IQR	0.201
Robust CV	4.1%
Minimum	4.38
Maximum	5.23
Range	0.85
Uncertainty (Median)	0.084

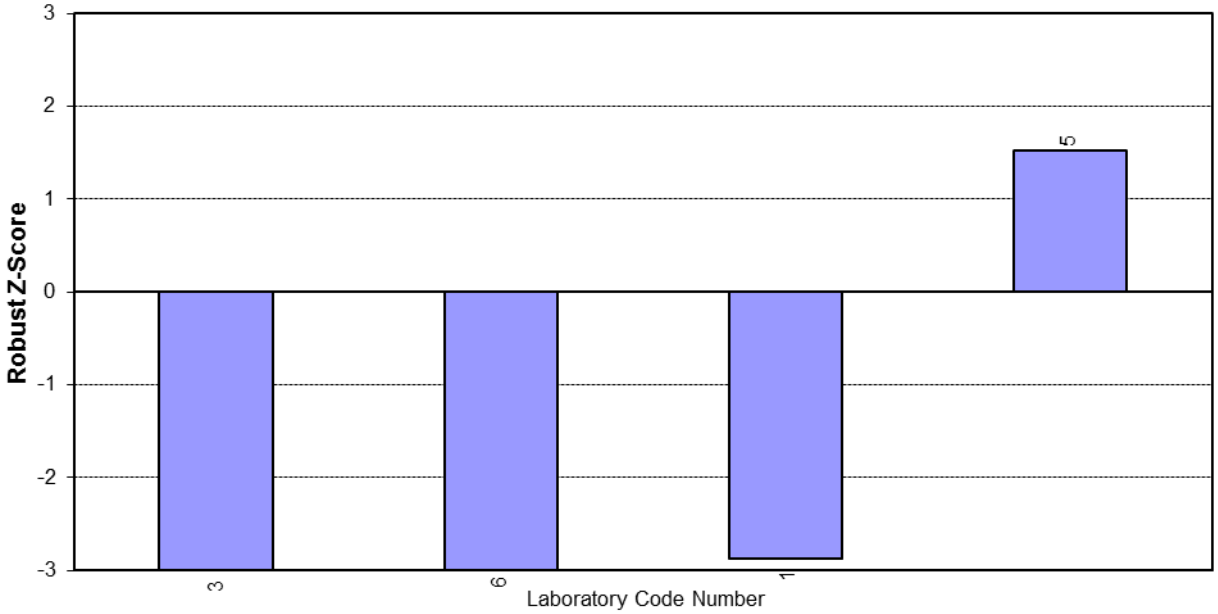
Notes:

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

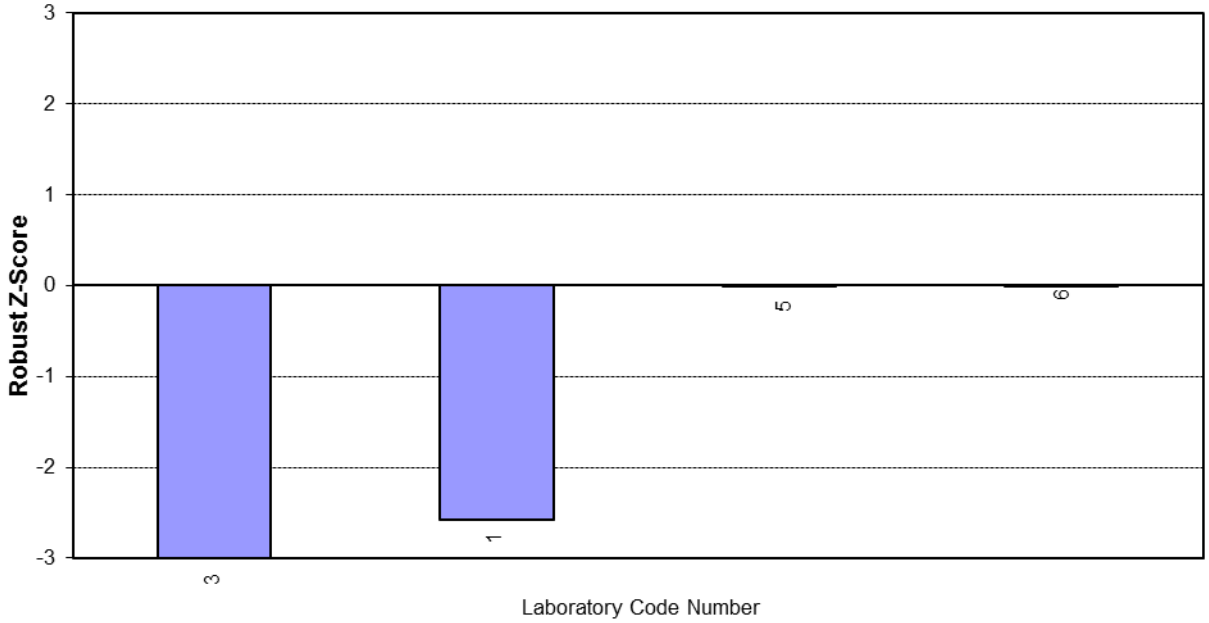
A7.2

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
2	31000	19000-50000	200000	120000-320000	4.49	5.30	-1.16	3.13 **§**
4	26000	12900 - 48500	48000	24800 - 93000	4.41	4.68	-1.71	-0.67
6	58000	38000 - 85000	77000	55000 - 110000	4.76	4.89	0.83	0.59

Note:

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

Summary Statistics
Sample - PTA 1

No. of Results	24
Median	4.650
Norm IQR	0.137
Robust CV	2.9%
Minimum	4.38
Maximum	4.99
Range	0.61
Uncertainty (Median)	0.035

Sample - PTA 2

No. of Results	24
Median	4.790
Norm IQR	0.163
Robust CV	3.4%
Minimum	4.45
Maximum	5.04
Range	0.59
Uncertainty (Median)	0.042

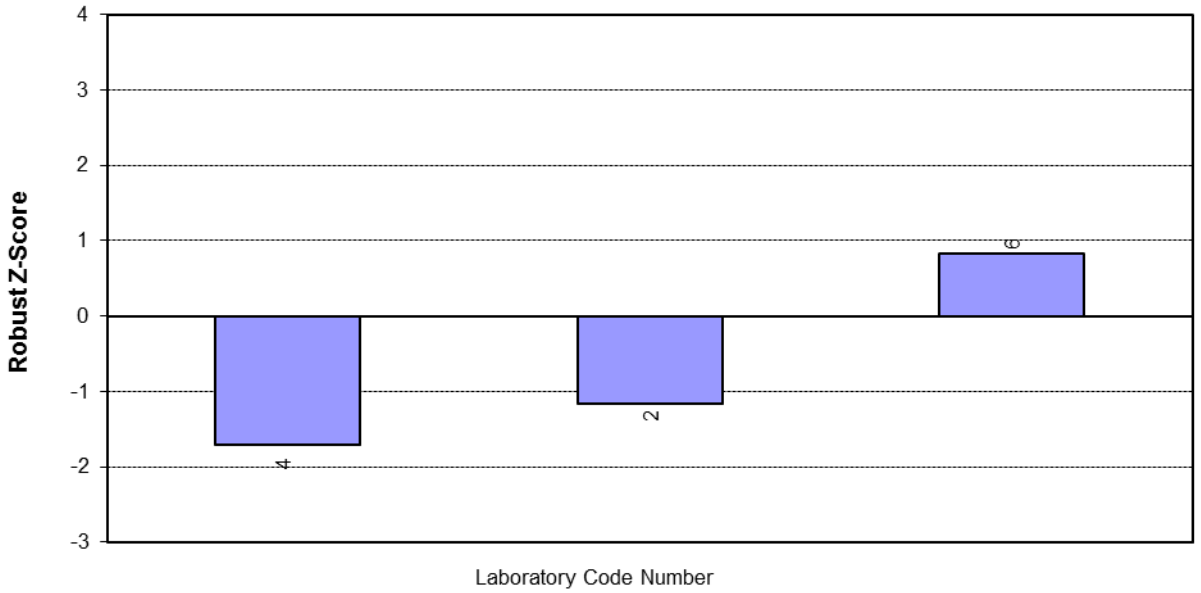
Note:

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

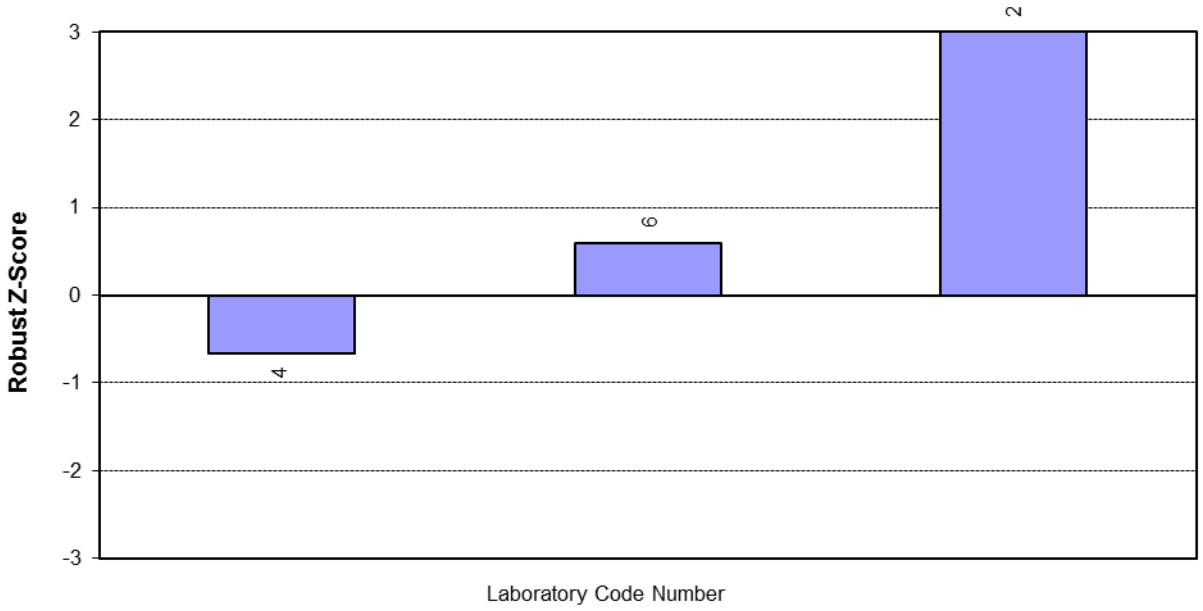
A8.2

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

Sample - PTA 1



Sample - PTA 2



SECTION A9 - 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	39600		115000		4.60	5.06	0.80	1.66
2	36000	22000 - 60000	61000	37000 - 100000	4.56	4.79	0.49	-0.24
4	33000	17300 - 63000	86000	42400 - 174000	4.52	4.93	0.21	0.79
5	30000	0.1	52000	0.1	4.48	4.72	-0.10	-0.72
6	39000	±0.28 log10	60000	±0.28 log10	4.59	4.78	0.75	-0.29

Summary Statistics
Sample - PTA 1

No. of Results	20
Median	4.490
Norm IQR	0.135
Robust CV*	3.0%
Minimum	4.00
Maximum	4.67
Range	0.67
Uncertainty (Median)	0.038

Sample - PTA 2

No. of Results	21
Median	4.820
Norm IQR	0.145
Robust CV*	3.0%
Minimum	4.67
Maximum	4.95
Range	0.28
Uncertainty (Median)	0.040

Note:

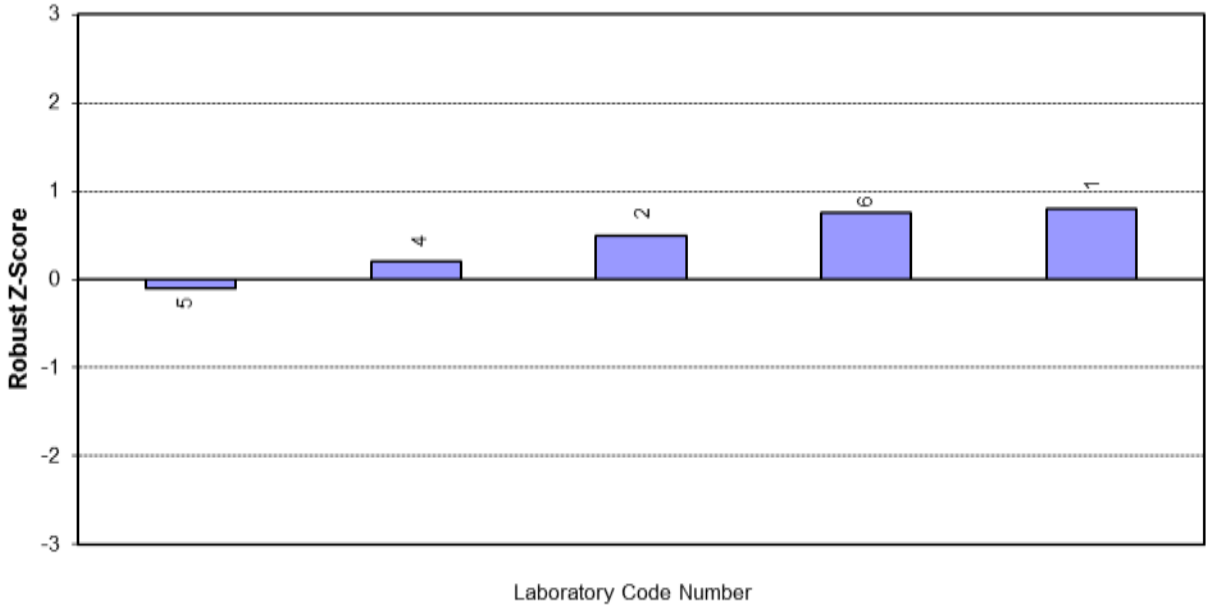
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).

2. *The robust CV's achieved for both samples were low (robust CV = 2.5% for Sample PTA 1 and 1.5% for Sample PTA 2) so in this case a target robust CV of 3.0% 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].

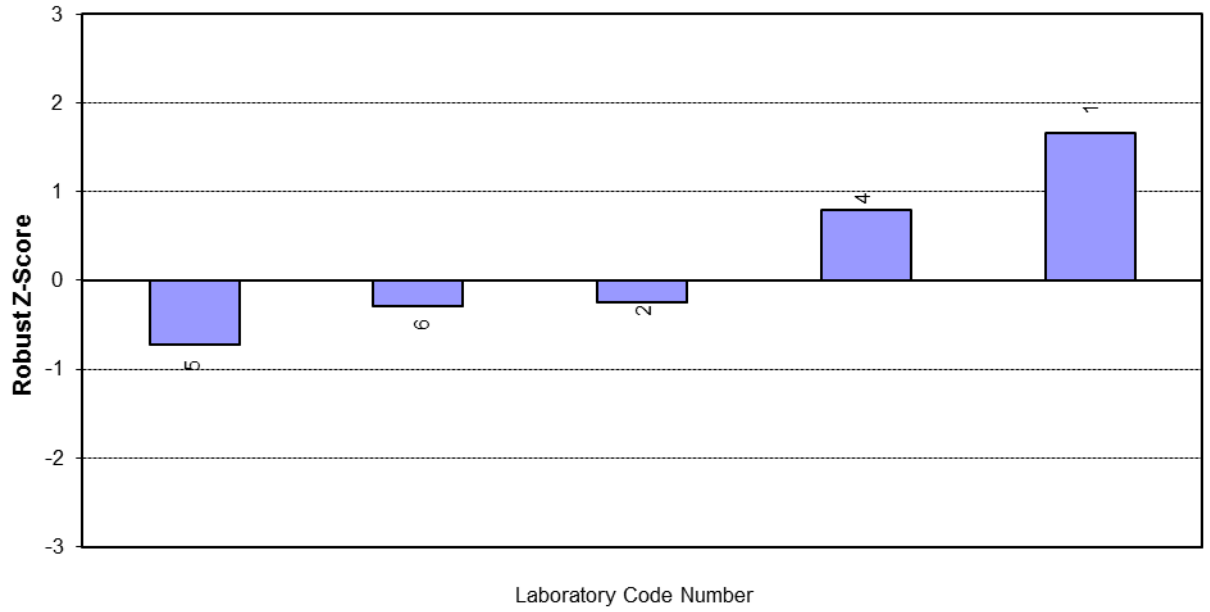
A9.2

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

Sample - PTA 1



Sample - PTA 2



A10.1

Enterococci (orgs/100mL) – Enterolert 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
4	22000	13800 - 35000	49000	29600 - 81000	4.34	4.69	-0.56	-0.37
6	24000	±0.39 log10	70000	±0.39 log10	4.38	4.85	-0.49	-0.23

Summary Statistics
Sample - PTA 1

No. of Results	17
Median	4.300
Norm IQR	0.059
Robust CV	1.4%
Minimum	4.11
Maximum	4.64
Range	0.53
Uncertainty (Median)	0.018

Summary Statistics
Sample - PTA 2

No. of Results	17
Median	4.610
Norm IQR	0.104
Robust CV	2.3%
Minimum	3.97
Maximum	5.11
Range	1.14
Uncertainty (Median)	0.032

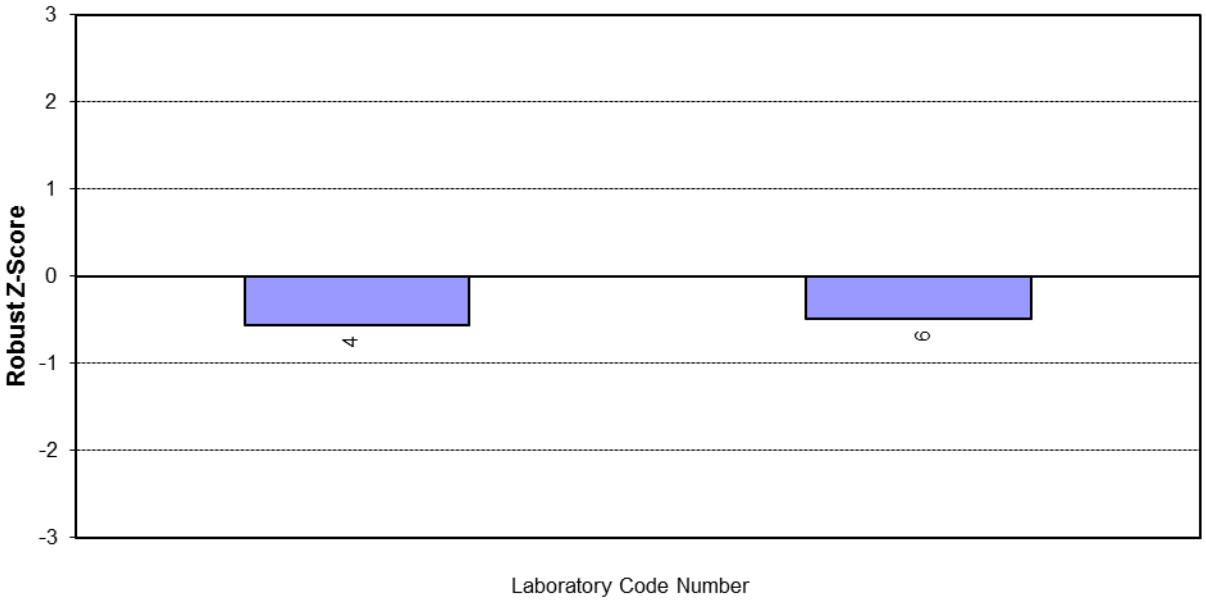
Notes:

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

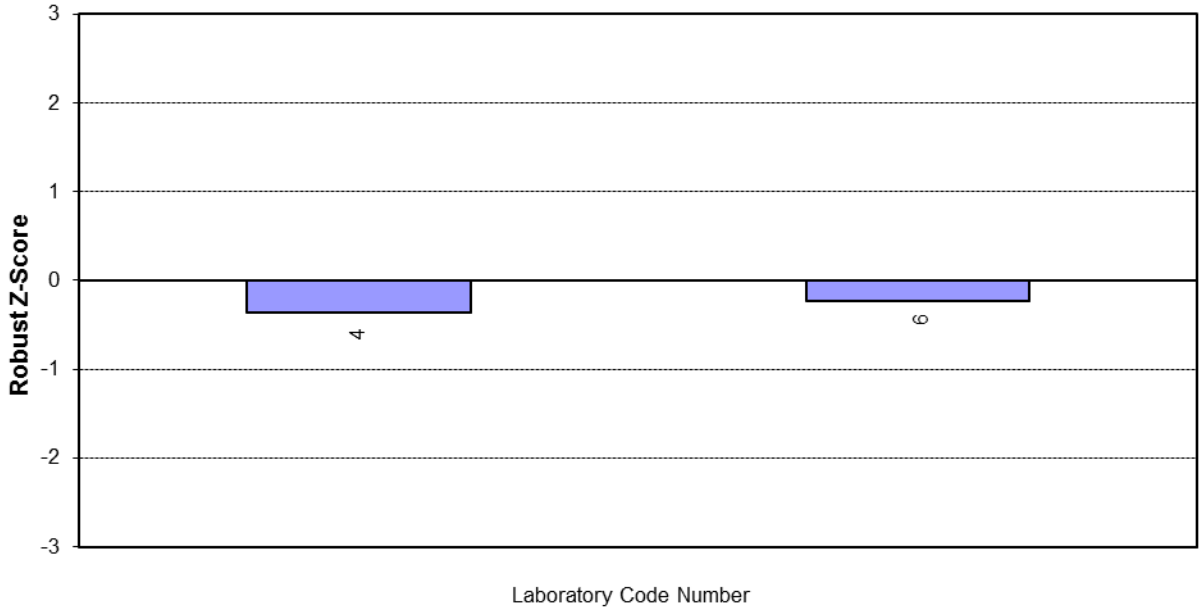
A10.2

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

Sample - PTA 1



Sample - PTA 2



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	1700		2800	12.9% (REU)	3.23	3.45	-0.90	-1.18
2	1700	1300-2100	3100	2500-3900	3.23	3.49	-0.90	-0.73
3	1200		1200		3.08	3.08	-2.26	-4.85 **§**
4	2400	1350 - 4270	2400	1350 - 4270	3.38	3.38	0.45	-1.84
5	1900	0.07	2800	0.07	3.28	3.45	-0.46	-1.18
6	1800	±0.18 log10	3400	±0.18 log10	3.26	3.53	-0.67	-0.33

Note:

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

Summary Statistics
Sample - PTA 1

No. of Results	10
Median	3.330
Norm IQR	0.111
Robust CV	3.3%
Minimum	3.11
Maximum	3.51
Range	0.40
Uncertainty (Median)	0.044

Sample - PTA 2

No. of Results	10
Median	3.565
Norm IQR	0.100
Robust CV	2.8%
Minimum	3.32
Maximum	3.78
Range	0.46
Uncertainty (Median)	0.040

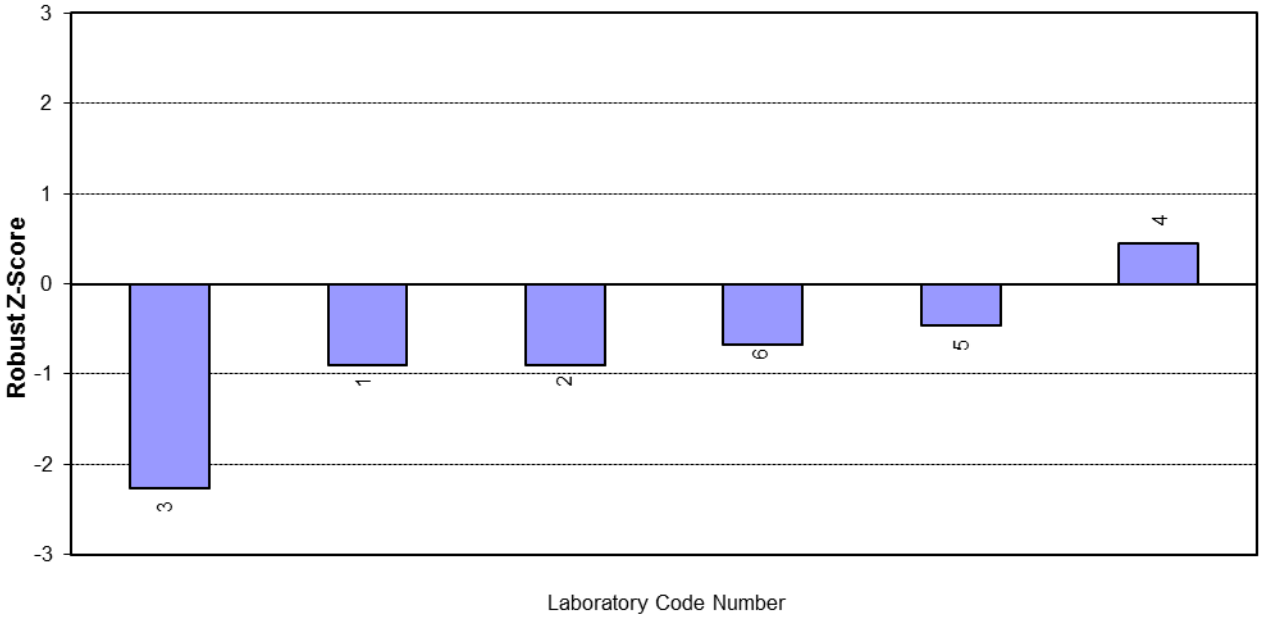
Note:

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

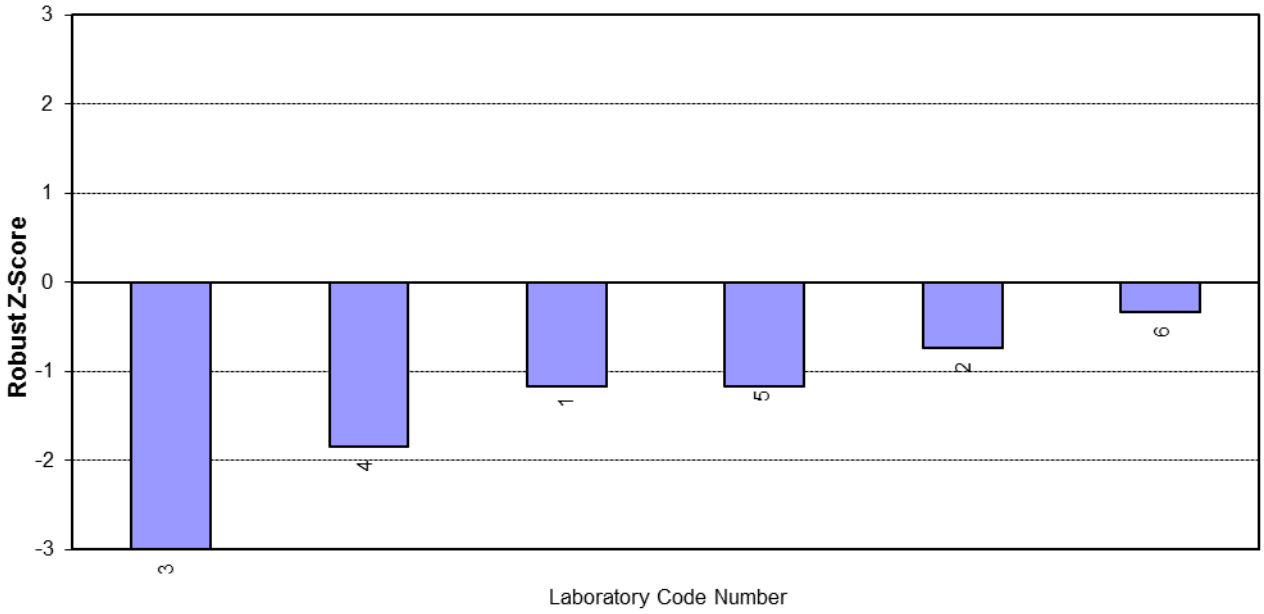
A11.2

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 12 June 2018. When reconstituted and added to the specified volume of sterile water, each sample was representative of an effluent water sample.

HOMOGENEITY AND STABILITY TESTING

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

During sample preparation for this program, five randomly selected samples from Sample 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.

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
71000	4.8513	64000	4.8062	67000	4.8261	65000	4.8129
70000	4.8451	77000	4.8865	69000	4.8388	63000	4.7993
74000	4.8692	73000	4.8633	71000	4.8513	76000	4.8808
70000	4.8451	62000	4.7924				
87000	4.9395	71000	4.8513				

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

C1

PROFICIENCY TESTING AUSTRALIA

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

INSTRUCTIONS TO PARTICIPANTS

ROUND 62 – JUNE 2018

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*, thermotolerant (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 if confidence limits from MPN tables 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 Tuesday 26 June 2018 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

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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

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RESULTS SHEET

Laboratory Code: 6

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