



MICROBIOLOGICAL WATERS

PROFICIENCY TESTING PROGRAM

ROUND 60

NOVEMBER 2017

REPORT NO. 1051

ACKNOWLEDGMENTS

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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 2017 by Proficiency Testing Australia (PTA). The Program Coordinator was Mrs K Weller and the Technical Adviser was Mrs S Mott from Global Proficiency Ltd (New Zealand). This is the sixtieth round in a series of on-going water proficiency testing programs. This report was authorised by Mrs F Watton, PTA Quality Manager.

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 six separate laboratories received samples for the program with five 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. $100 \times \text{Normalised IQR} \div \text{Median}$;
- * 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. $z \leq -3.0$ or $z \geq 3.0$). 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
<i>E. coli</i> orgs/100mL	MF	1	17	4.670	0.141
		2	17	4.360	0.163
	MPN	1	18	4.755	0.130
		2	18	4.420	0.226
	Colilert	1	18	4.755	0.130
		2	18	4.420	0.226
Thermotolerant (Faecal) Coliforms orgs/100mL	MF	1	18	4.725	0.137
		2	18	4.360	0.209
	MPN	1	11	4.720	0.096
		2	11	4.340	0.219
Total Coliforms orgs/100mL	MF	1	11	4.810	0.452
		2	11	4.480	0.549
	MPN	1	15	5.240	0.241
		2	15	5.240	0.326
	Colilert	1	15	5.240	0.241
		2	15	5.240	0.326
Enterococci orgs/100mL	MF	1	13	4.680	0.200
		2	13	4.260	0.074
	Enterolert	1	8	4.320	0.293
		2	8	3.875	0.216
Plate Count orgs/mL	All	1	8	3.525	0.141
		2	8	3.330	0.139

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 log₁₀ values before they were analysed.
2. Table A does not include open ended, incomplete or approximate results.
3. Data for Total Coliforms and *E. coli* Most Probable Number (MPN) tests obtained using either Traditional (e.g. APHA 9221B and E) or Enzyme substrate (Colilert) techniques have been assessed together in this round. Global Proficiency Ltd undertakes this as a routine procedure for the Coliform tests only. Visual method comparisons can be seen in the method differentiation graphs presented for these tests (pages 7 and 9).

SUMMARY OF OUTLIER RESULTS

Outlier Results and False Results

There were no outliers or false results for this round.

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 *Enterobacter cloacae* (*E. cloacae*). *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°C.

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, with the exception of Plate Count results.

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

Total Coliforms:

For the three different Total Coliforms techniques, five laboratories reported results. No laboratories reported outliers for these tests.

All laboratories reported using AS or APHA methods.

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:

$$\sqrt{\frac{\pi}{2}} \times \frac{\text{normIQR}}{\sqrt{n}}$$

Total Coliforms via:	Sample PTA 1 Median ± Uncertainty (Log ₁₀ cfu/100mL)	Sample PTA 2 Median ± Uncertainty (Log ₁₀ cfu/100mL)
Membrane Filtration	4.810 ± 0.171	4.480 ± 0.207
Most Probable Number	5.240 ± 0.078	5.240 ± 0.106
Colilert	5.240 ± 0.078	5.240 ± 0.106

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

Measurement Uncertainty: Total Coliforms

Four 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, only one 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 7 submitted results for the Colilert technique. Although the result for sample PTA 2 was not rated as an outlier, the result and stated uncertainty was still below 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.

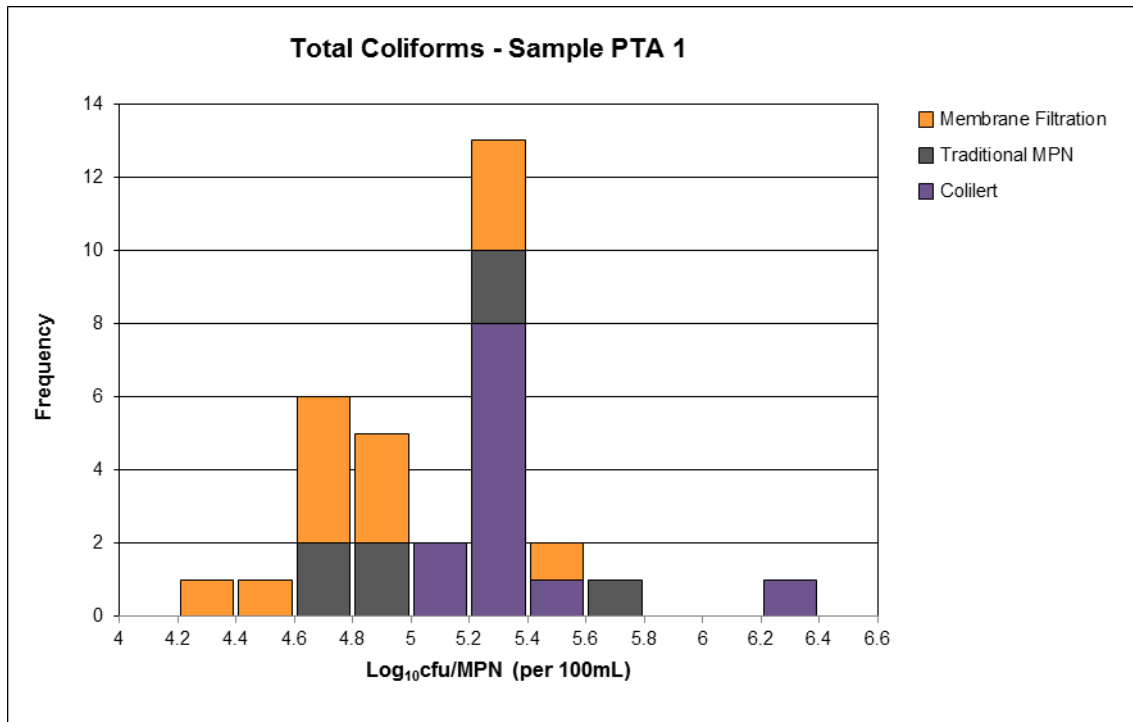


Figure TA-1. Total Coliform results for Sample PTA 1

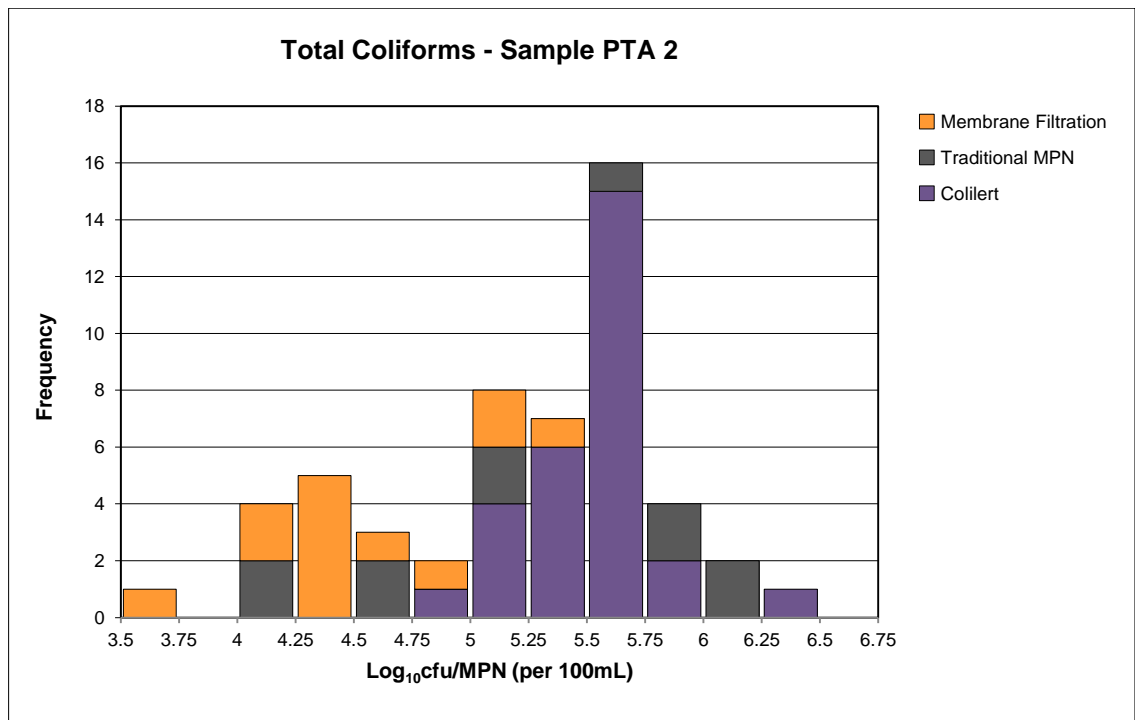


Figure TA-2. Total Coliform results for Sample PTA 2

E. coli:

For the three different *E.coli* techniques, five laboratories reported results. No outliers were reported for this test.

All laboratories reported using AS or APHA methods.

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.

<i>E. coli</i> via:	Sample PTA 1 Median \pm Uncertainty (Log ₁₀ cfu/100mL)	Sample PTA 2 Median \pm Uncertainty (Log ₁₀ cfu/100mL)
Membrane Filtration	4.670 \pm 0.043	4.360 \pm 0.050
Most Probable Number	4.755 \pm 0.038	4.420 \pm 0.067
Colilert	4.755 \pm 0.038	4.420 \pm 0.067

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

Measurement Uncertainty: *E. coli*

Four laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways; i.e. \pm log values, a range of cfu/100mL values and % relative expanded uncertainty.

- Laboratory 6 may need to re-examine their test results or their MU calculations for the Traditional MPN method as their results for PTA 2 and the stated uncertainty was 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.

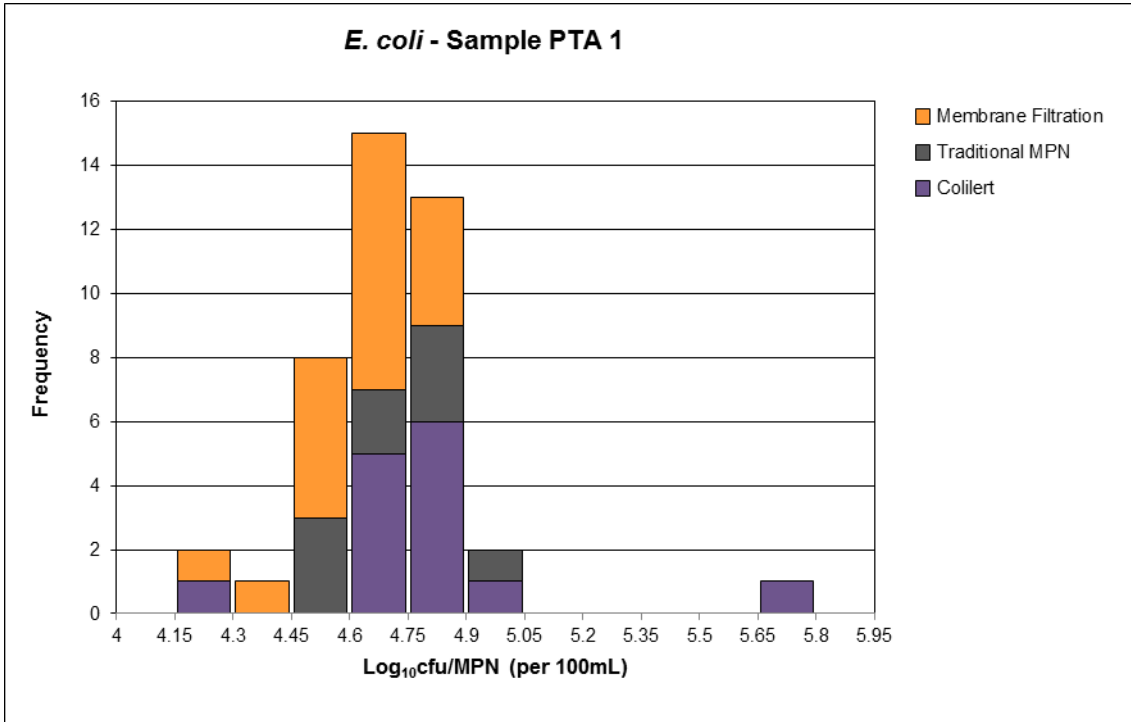


Figure TA-3. *E. coli* results for Sample PTA 1.

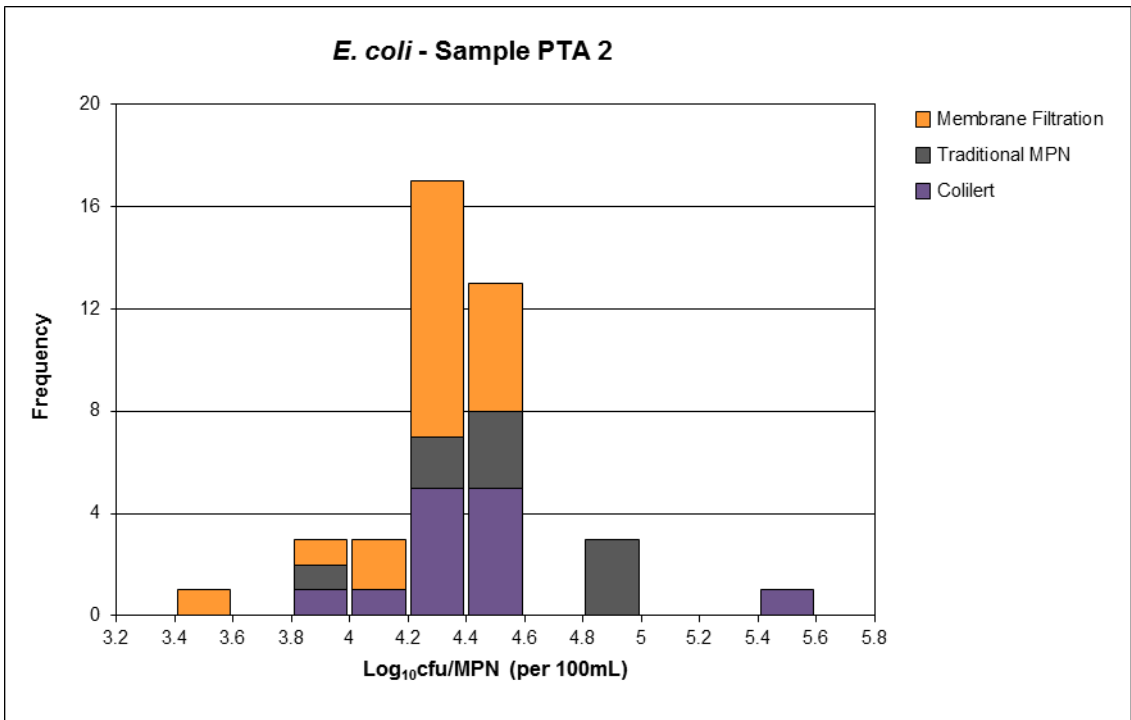


Figure TA-4. *E. coli* results for Sample PTA 2.

Thermotolerant (Faecal) Coliforms:

While this test is still referred to as the “Faecal Coliforms” test in many instances, the correct terminology is “Thermotolerant Coliforms” (those that are able to ferment lactose to produce gas at 44.5°C), as there are documented cases of detection of these organisms in the absence of faecal contamination (APHA *Standard methods for the examination of water and wastewater*, 22nd Edition (2012) – Section 9221 E). This is the reason that testing for *E. coli* specifically is recommended to identify faecal contamination.

A total of four laboratories reported results for the two different Thermotolerant (Faecal) Coliforms techniques; MF and MPN respectively. No outliers were reported for this test.

The majority of laboratories reported using AS or APHA methods.

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	4.725 ± 0.041	4.360 ± 0.062
Most Probable Number	4.720 ± 0.036	4.340 ± 0.083

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 6 may need to re-examine their test results or their MU calculations for the Traditional MPN method as their results for PTA 2 and the stated uncertainty was 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.

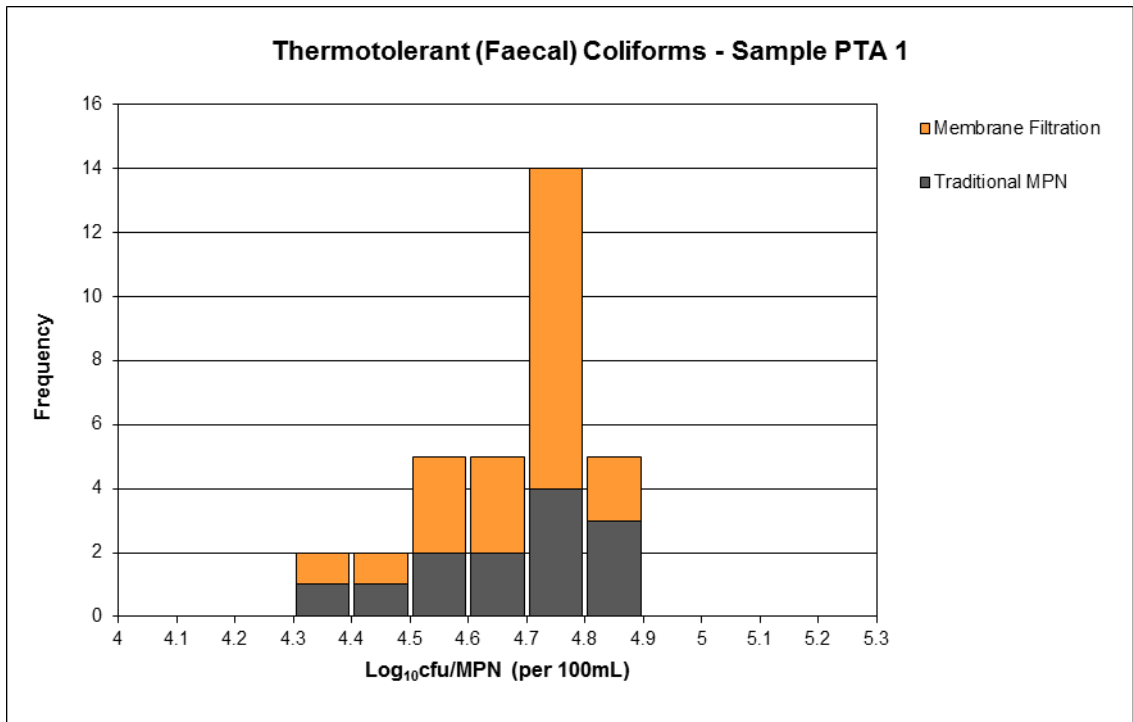


Figure TA-5. Thermotolerant (Faecal) Coliforms results for Sample PTA 1

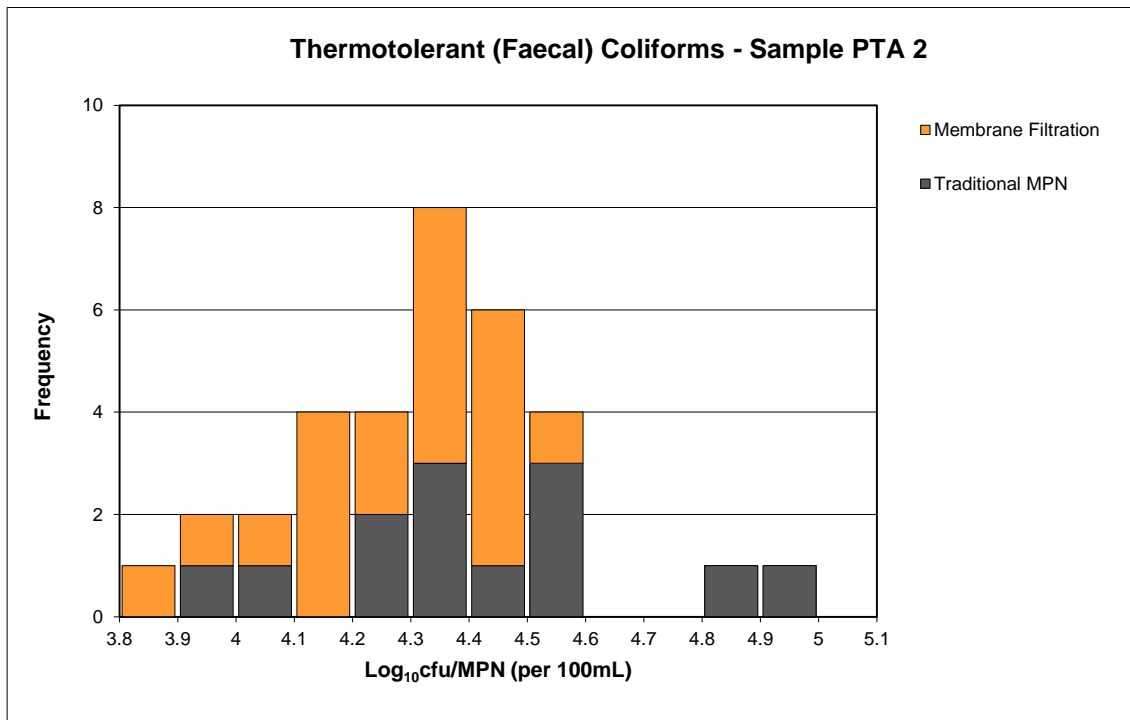


Figure TA-6. Thermotolerant (Faecal) Coliforms results for Sample PTA 2

Enterococci:

A total of three laboratories reported results for the two different Enterococci techniques; MF and Enterolert. No outliers were reported for this test.

AS methods and an ISO method were quoted as being used in this round.

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 \pm Uncertainty (Log ₁₀ cfu/100mL)	Sample PTA 2 Median \pm Uncertainty (Log ₁₀ cfu/100mL)
Membrane Filtration	4.680 \pm 0.070	4.260 \pm 0.026
Enterolert	4.320 \pm 0.130	3.875 \pm 0.096

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

Measurement Uncertainty: Enterococci via Membrane Filtration (MF):

Two laboratories reported MU estimations associated with their test results in this round. MU was reported in two different ways; i.e. \pm 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.

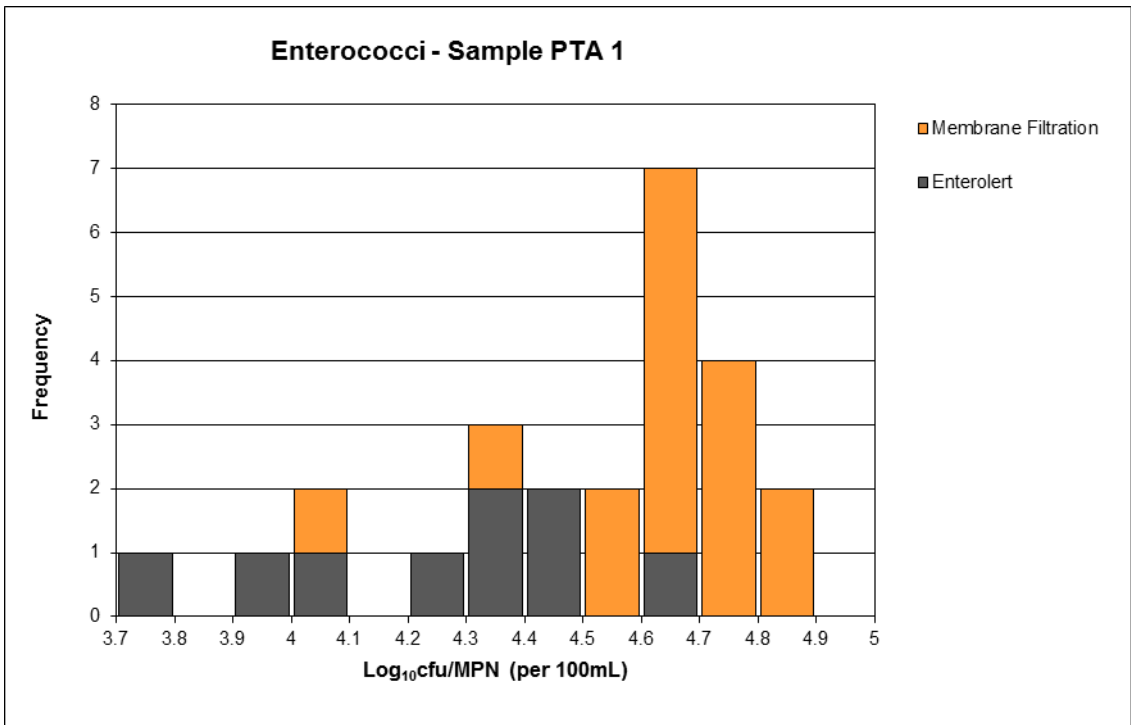


Figure TA-7. Enterococci results for Sample PTA 1

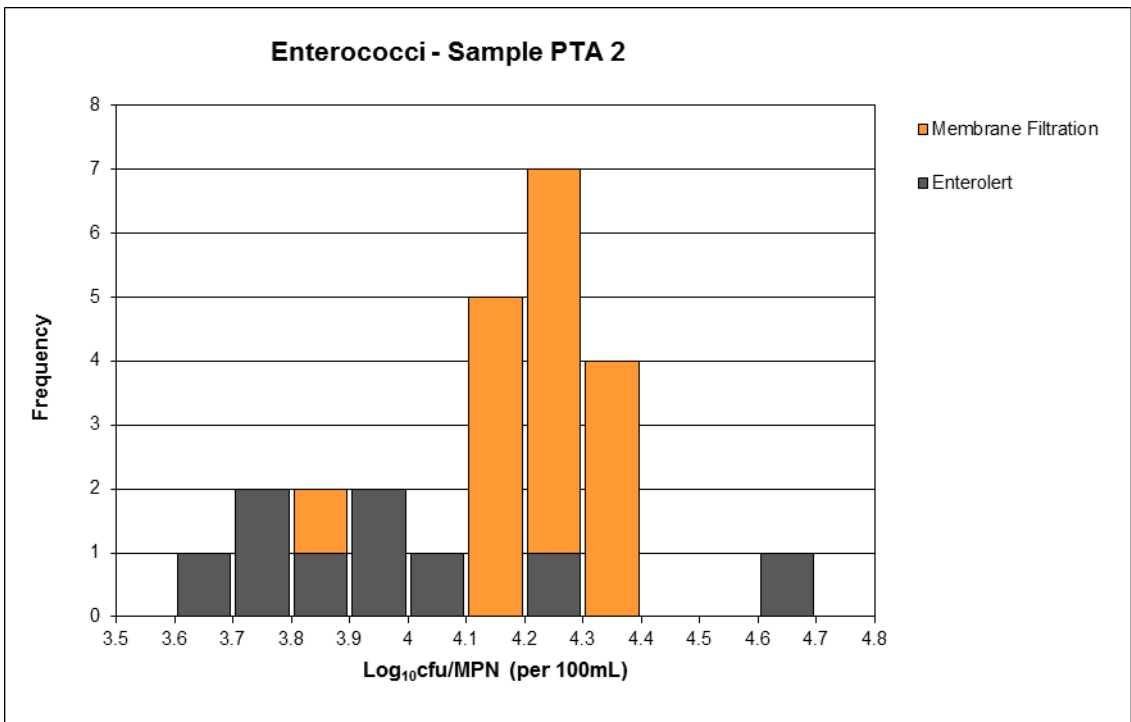


Figure TA-8. Enterococci results for Sample PTA 2

Plate Count:

Three sets of results were submitted for the Plate Count test. Two sets of results were obtained using the Australian/New Zealand Standard method (AS/NZS 4276.3.1) and one using another country standard/ISO method. 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.

	Sample PTA 1	Sample PTA 2
	Median \pm Uncertainty (Log ₁₀ cfu/mL)	Median \pm Uncertainty (Log ₁₀ cfu/mL)
Plate Count:	3.525 \pm 0.062	3.330 \pm 0.061

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

Measurement Uncertainty: Plate Count:

Three laboratories reported MU estimations associated with their test results in this round. MU was reported in three different ways; i.e. \pm log values, a range of cfu/100mL values and % relative expanded uncertainty.

General Comments

A total of 48 results were submitted for analysis in this round. Of these results none were outlier results. This is lower than the 5% of results which were outlier results in Round 58.

Outlying 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, 22nd Edition (2012)*
- [12] 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
7	56000	40000 - 72000	17000	13000 - 22000	4.75	4.23	0.56	-0.79
8	44000	0.20	19000	0.20	4.64	4.28	-0.19	-0.50

Summary Statistics

Sample - PTA 1

No. of Results	17
Median	4.670
Norm IQR	0.141
Robust CV	3.0%
Minimum	4.28
Maximum	4.88
Range	0.60
Uncertainty (Median)	0.043

Sample - PTA 2

No. of Results	17
Median	4.360
Norm IQR	0.163
Robust CV	3.7%
Minimum	3.48
Maximum	4.54
Range	1.06
Uncertainty (Median)	0.050

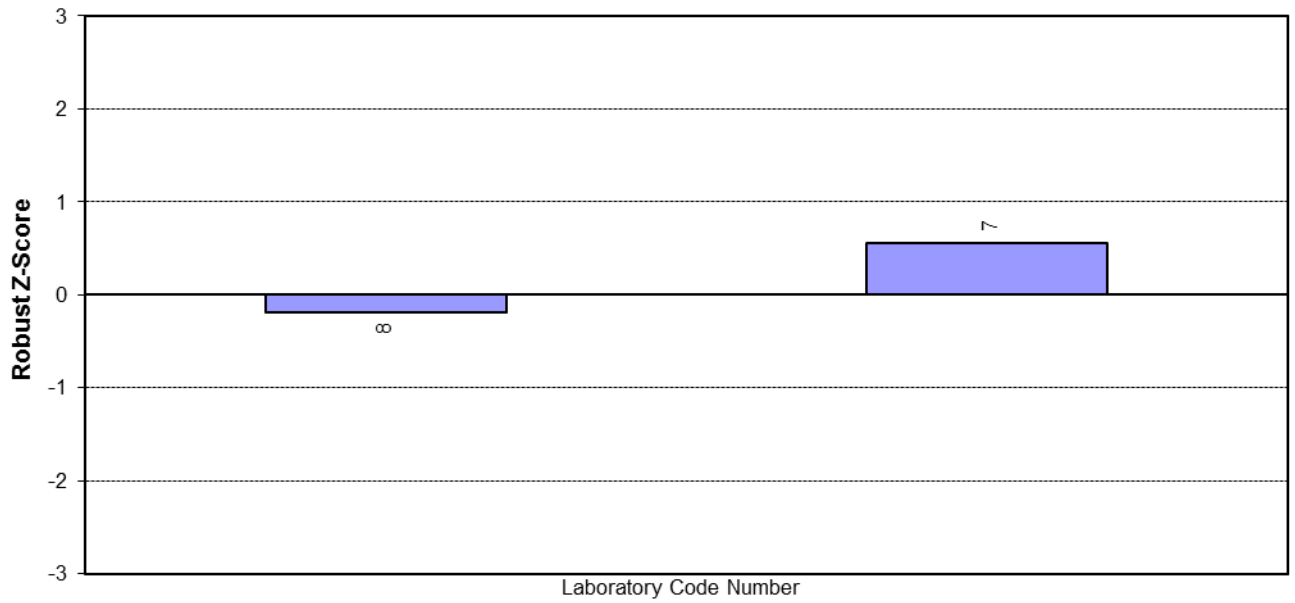
Note:

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).

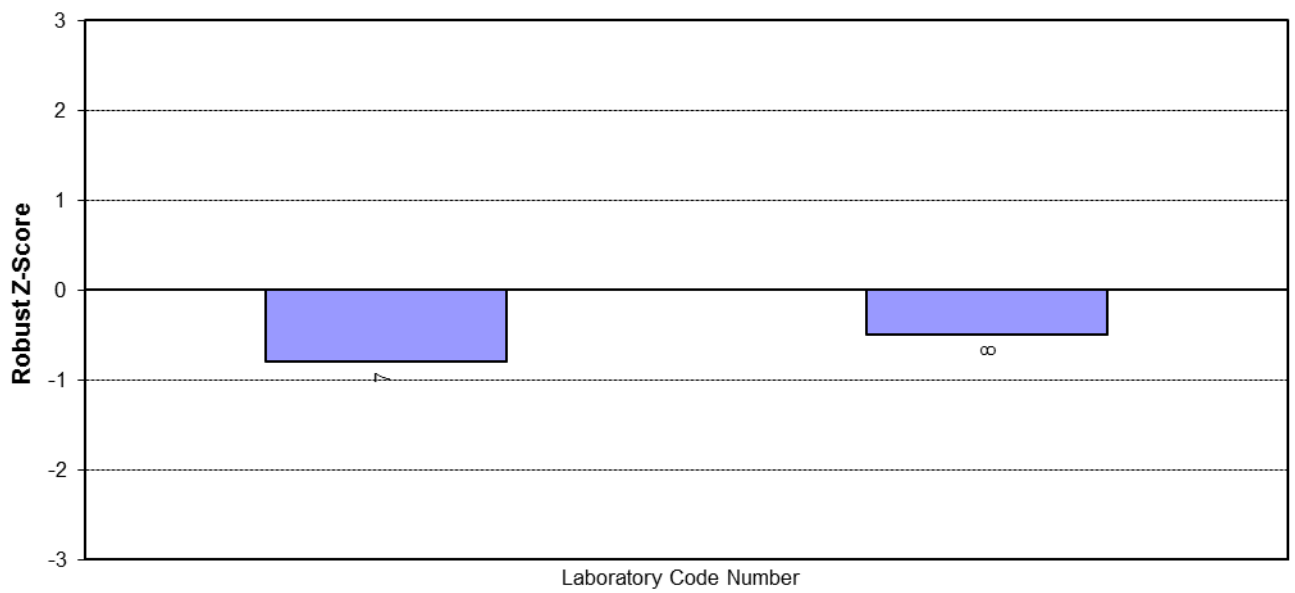
A1.2

***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
4	110000	-	79000	-	5.04	4.90	2.21	2.11
6	70000	-	70000	2% (REU)	4.85	4.85	0.69	1.88

Summary Statistics

Sample - PTA 1

No. of Results	18
Median	4.755
Norm IQR	0.130
Robust CV	2.7%
Minimum	4.52
Maximum	5.76
Range	1.24
Uncertainty (Median)	0.038

Sample - PTA 2

No. of Results	18
Median	4.420
Norm IQR	0.226
Robust CV	5.1%
Minimum	3.87
Maximum	5.49
Range	1.62
Uncertainty (Median)	0.067

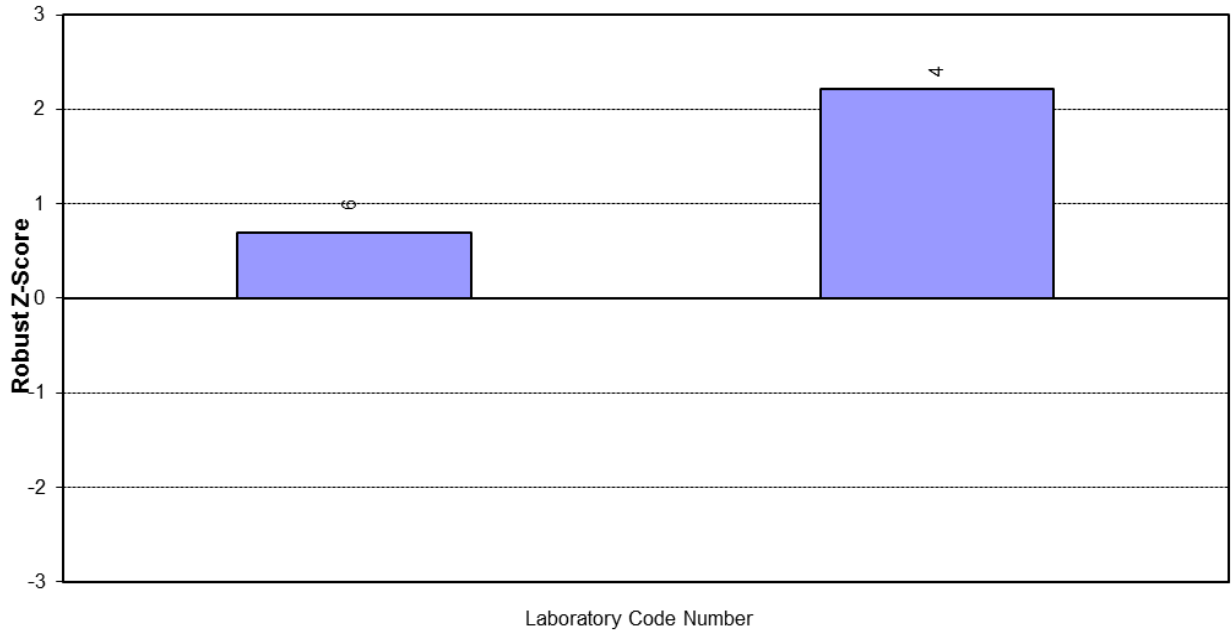
Notes:

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).
2. The data presented consists of Traditional MPN and Colilert techniques assessed together.
3. Visual presentation of method comparisons is included on page 9.

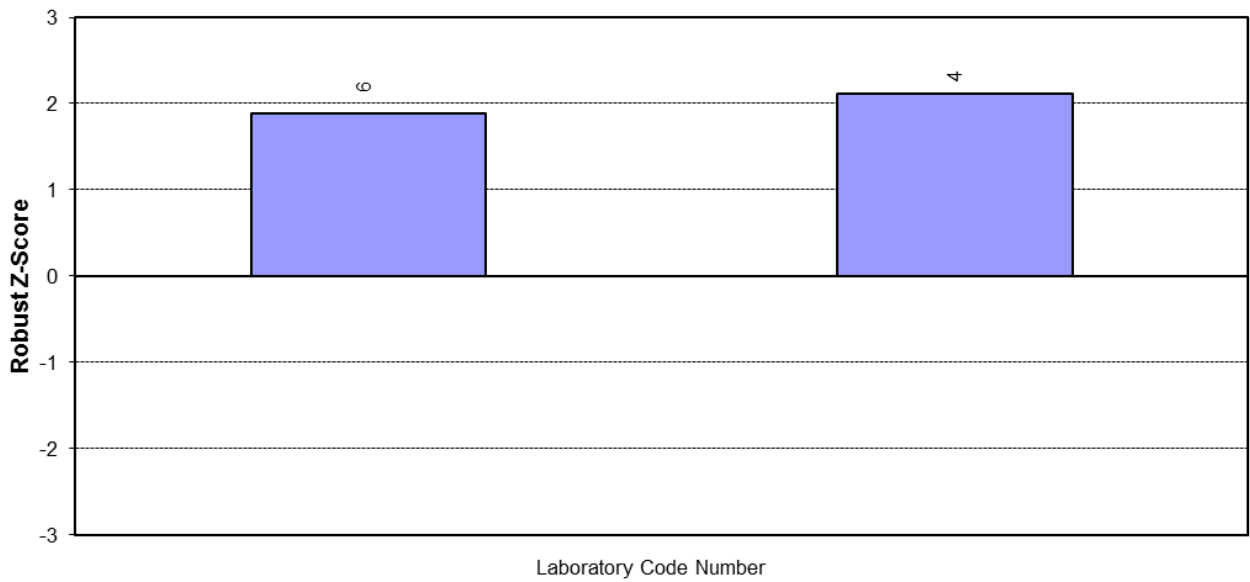
A2.2

***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
3	Result reported as Log 10 value	±0.24	Result reported as Log 10 value	±0.24	4.76	4.32	0.04	-0.44
7	51000	39000 - 67000	24000	19000 - 30000	4.71	4.38	-0.37	-0.18

Summary Statistics

Sample - PTA 1

No. of Results	18
Median	4.755
Norm IQR	0.130
Robust CV	2.7%
Minimum	4.52
Maximum	5.76
Range	1.24
Uncertainty (Median)	0.038

Sample - PTA 2

No. of Results	18
Median	4.420
Norm IQR	0.226
Robust CV	5.1%
Minimum	3.87
Maximum	5.49
Range	1.62
Uncertainty (Median)	0.067

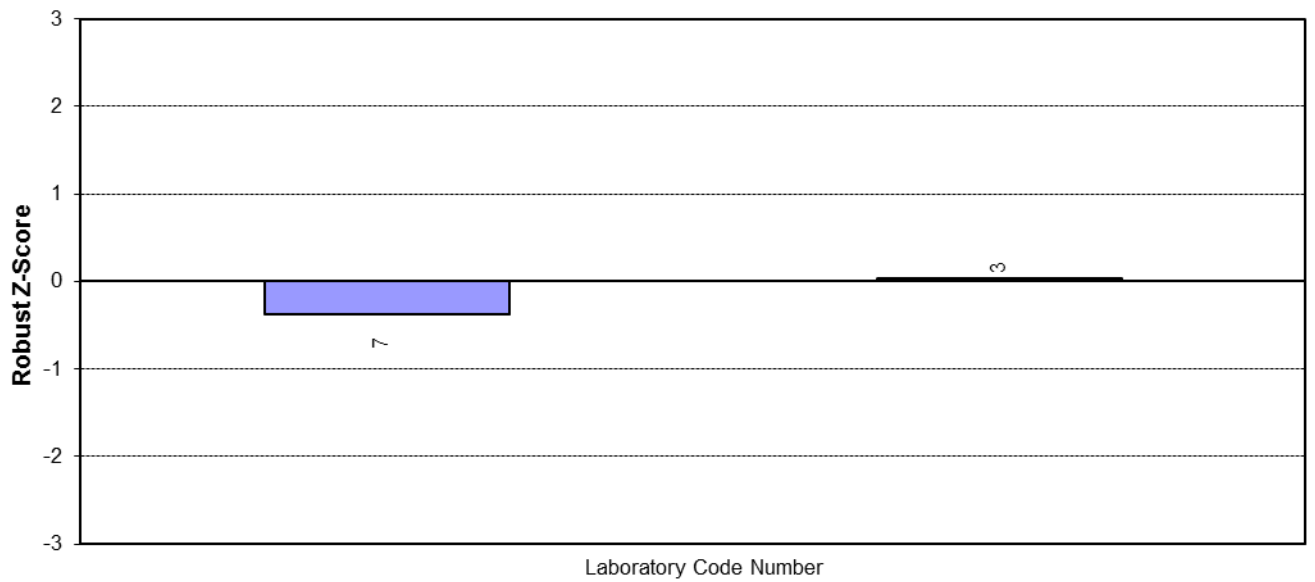
Note:

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 data presented consists of Traditional MPN and Colilert techniques assessed together.
3. Visual presentation of method comparisons is included on page 9.

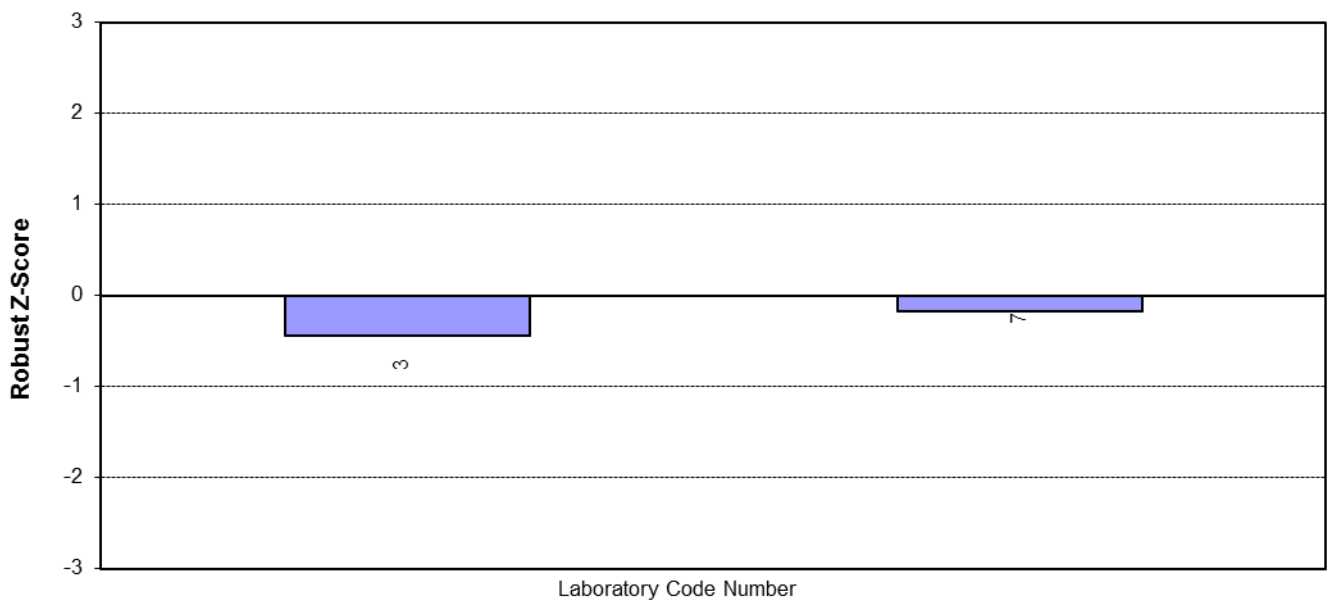
A3.2

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

Sample - PTA 1



Sample - PTA 2



SECTIONS A4 to A5

Thermotolerant (Faecal) Coliforms

A4.1

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
7	56000	40000 - 72000	17000	13000 - 22000	4.75	4.23	0.17	-0.62
8	44000	0.13	19000	0.13	4.64	4.28	-0.59	-0.39

Summary Statistics Sample - PTA 1

No. of Results	18
Median	4.725
Norm IQR	0.137
Robust CV	2.9%
Minimum	4.38
Maximum	4.88
Range	0.50
Uncertainty (Median)	0.041

Sample - PTA 2

No. of Results	18
Median	4.360
Norm IQR	0.209
Robust CV	4.8%
Minimum	3.86
Maximum	4.54
Range	0.68
Uncertainty (Median)	0.062

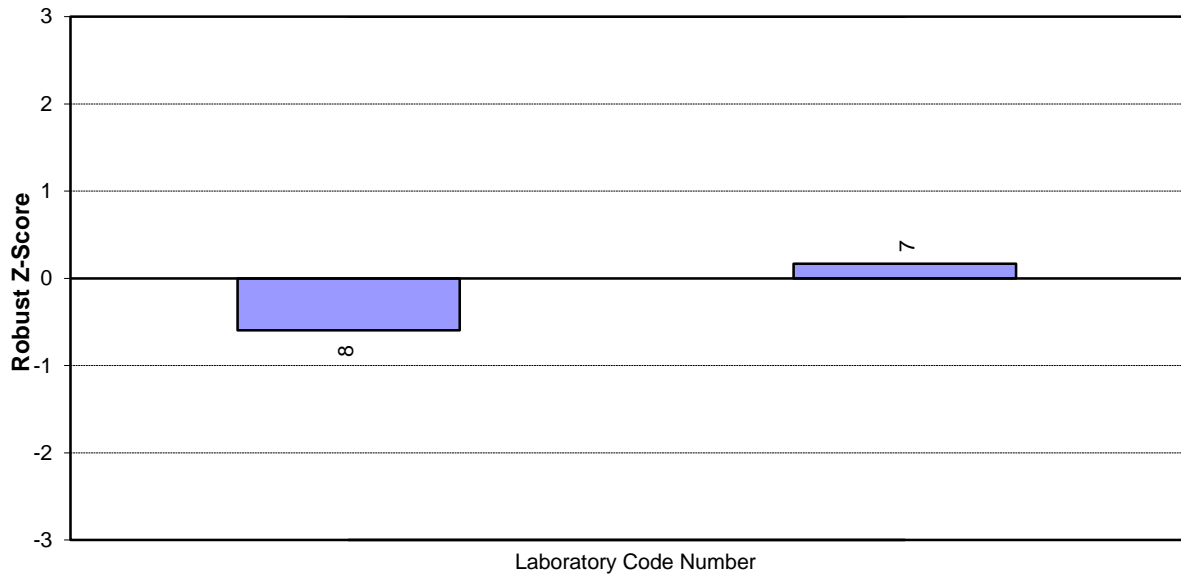
Note:

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).

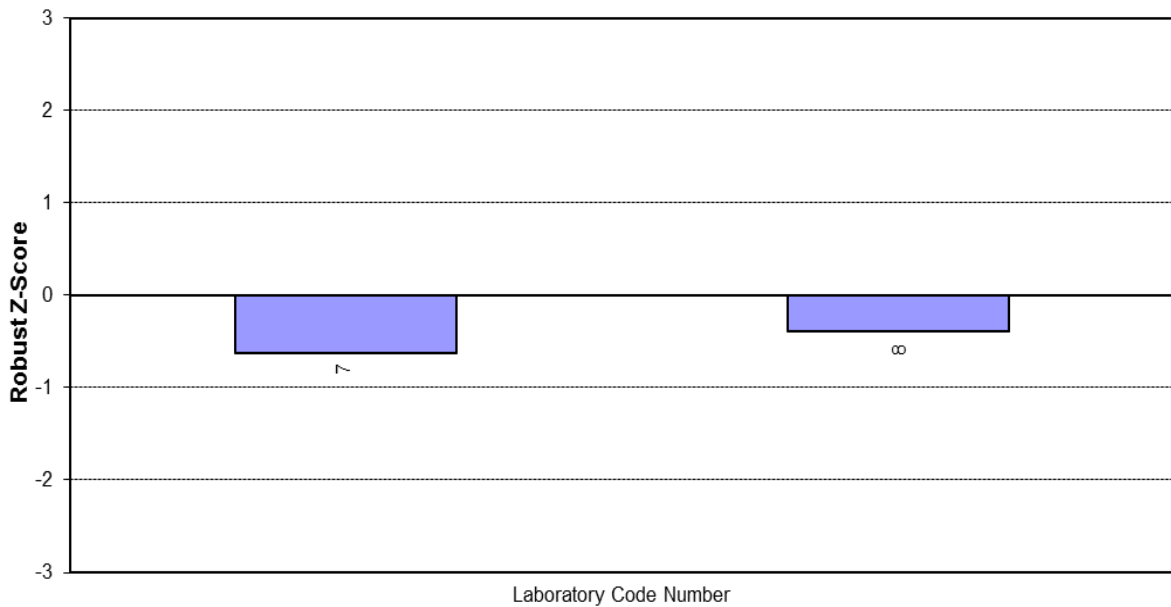
A4.2

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

Sample - PTA 1



Sample - PTA 2



A5.1

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
3	Result reported as Log 10 value	±0.11	Result reported as Log 10 value	±0.11	4.69	4.26	-0.31	-0.37
6	79000	-	70000	2% (REU)	4.90	4.85	1.84	2.31
7	52000	37000 - 73000	22000	17000 - 29000	4.72	4.34	-0.04	0.01

Summary Statistics Sample - PTA 1

No. of Results	11
Median	4.720
Norm IQR	0.096
Robust CV	2.0%
Minimum	4.49
Maximum	4.90
Range	0.41
Uncertainty (Median)	0.036

Sample - PTA 2

No. of Results	11
Median	4.340
Norm IQR	0.219
Robust CV	5.0%
Minimum	3.99
Maximum	4.96
Range	0.97
Uncertainty (Median)	0.083

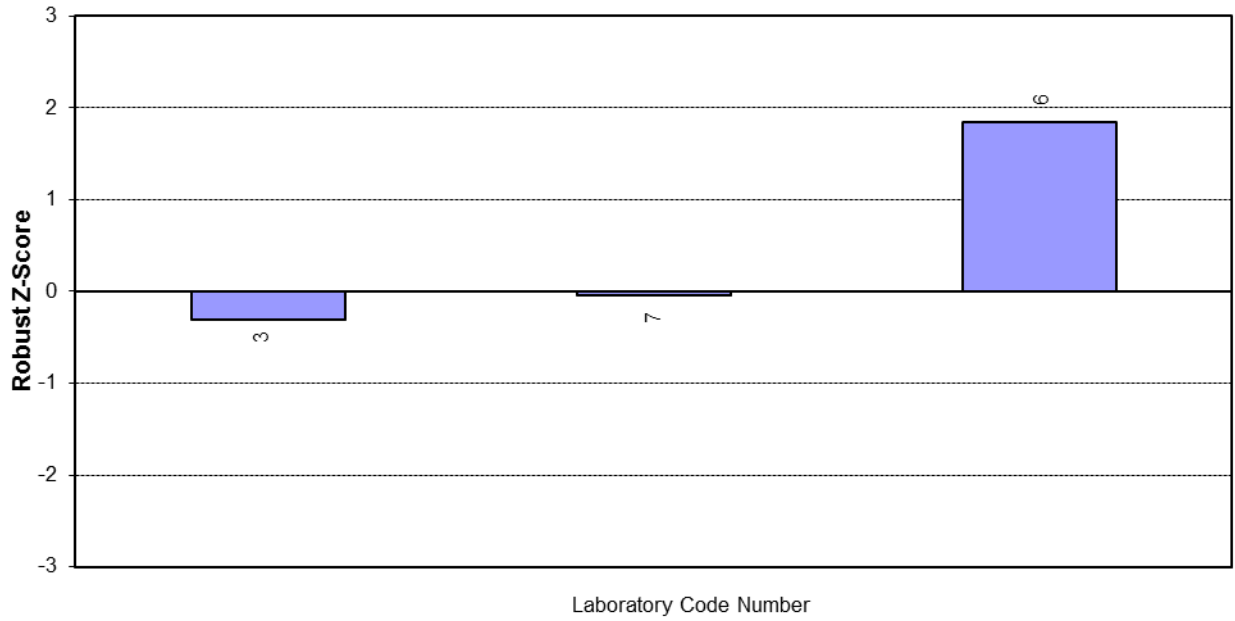
Note:

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).

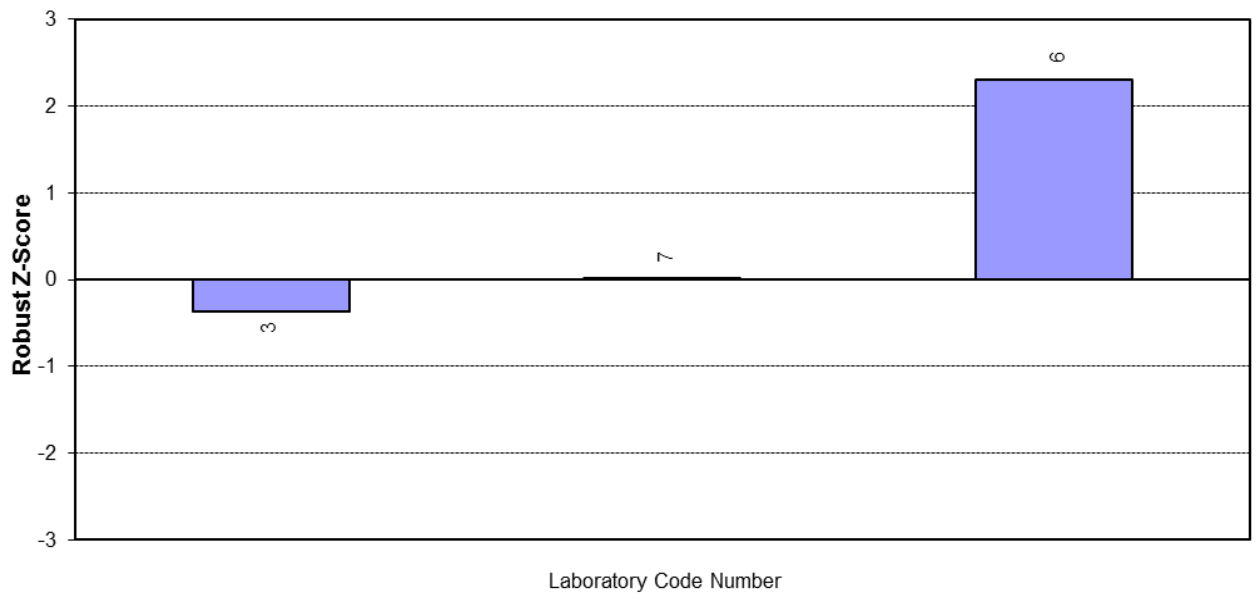
A5.2

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

Sample - PTA 1



Sample - PTA 2



SECTIONS A6 to A8

Total Coliforms

A6.1

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
7	60000	43000 - 77000	31000	24000 - 40000	4.78	4.49	-0.07	0.02
8	45000	0.15	20000	0.15	4.65	4.30	-0.35	-0.33

Summary Statistics

Sample - PTA 1

No. of Results	11
Median	4.810
Norm IQR	0.452
Robust CV	9.4%
Minimum	4.28
Maximum	5.57
Range	1.29
Uncertainty (Median)	0.171

Sample - PTA 2

No. of Results	11
Median	4.480
Norm IQR	0.549
Robust CV	12.2%
Minimum	3.51
Maximum	5.38
Range	1.87
Uncertainty (Median)	0.207

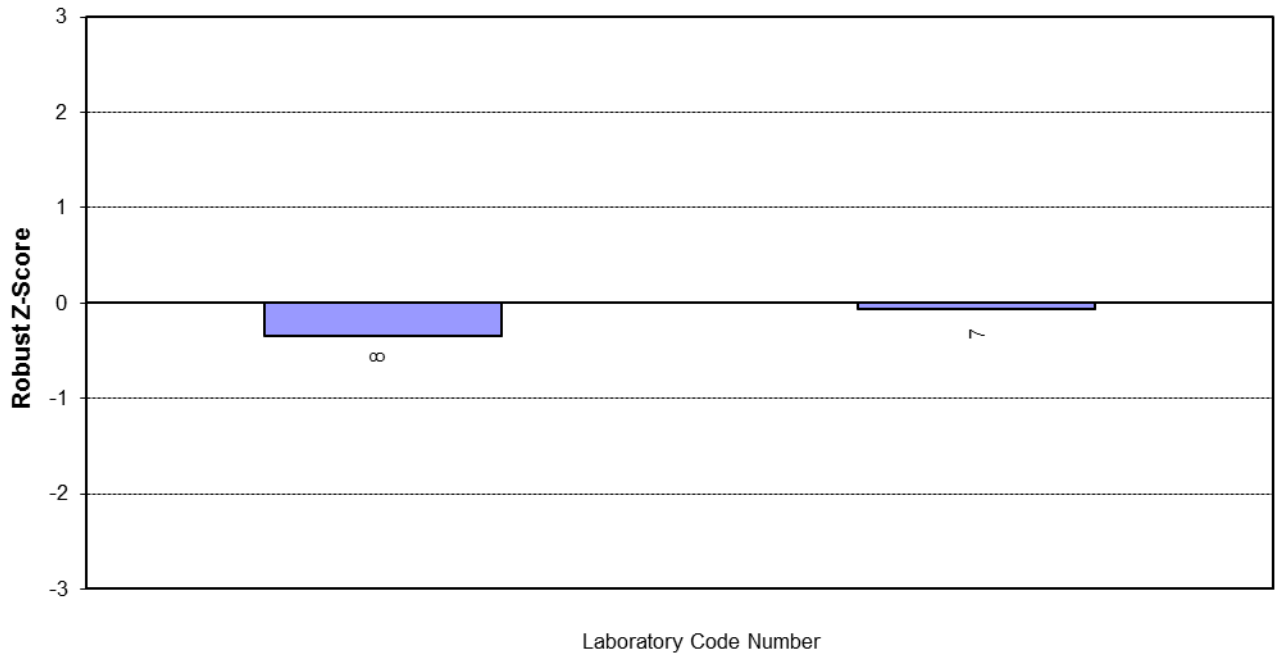
Note:

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).

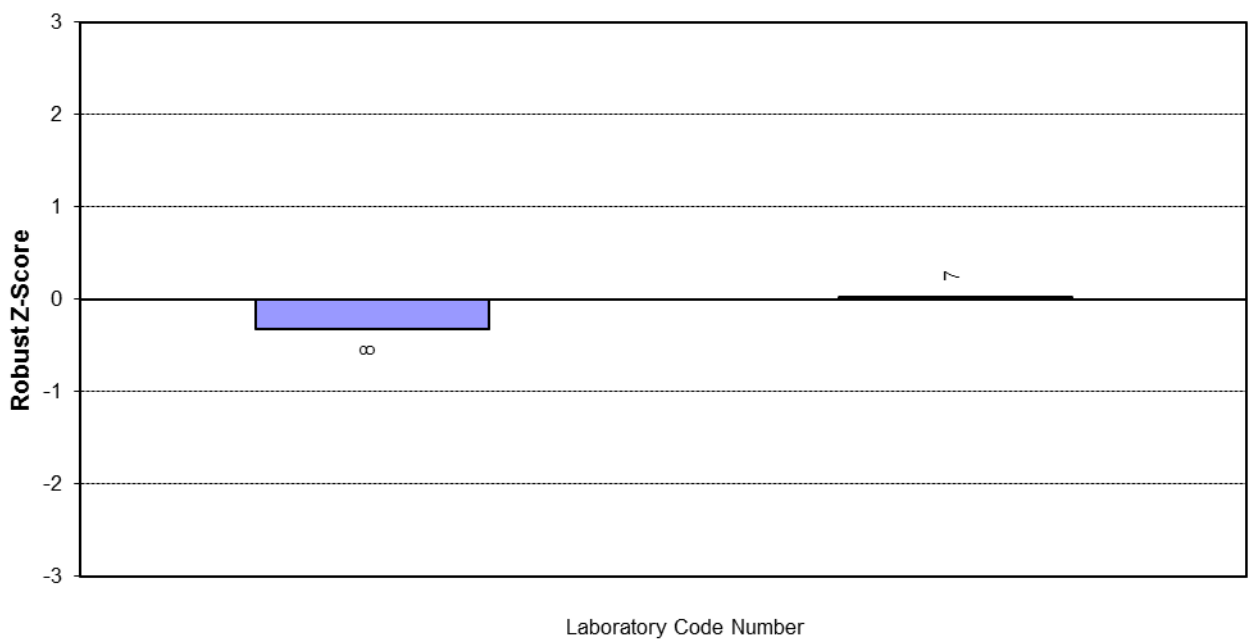
A6.2

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

Sample - PTA 1



Sample - PTA 2



A7.1

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
4	540000		350000		5.73	5.54	2.04	0.93
6	170000		140000	2.4% (REU)	5.23	5.15	-0.04	-0.29

Summary Statistics

Sample - PTA 1

No. of Results	15
Median	5.240
Norm IQR	0.241
Robust CV*	4.6%
Minimum	4.69
Maximum	5.38
Range	0.69
Uncertainty (Median)	0.078

Sample - PTA 2

No. of Results	15
Median	5.240
Norm IQR	0.326
Robust CV	6.2%
Minimum	4.04
Maximum	5.38
Range	1.34
Uncertainty (Median)	0.106

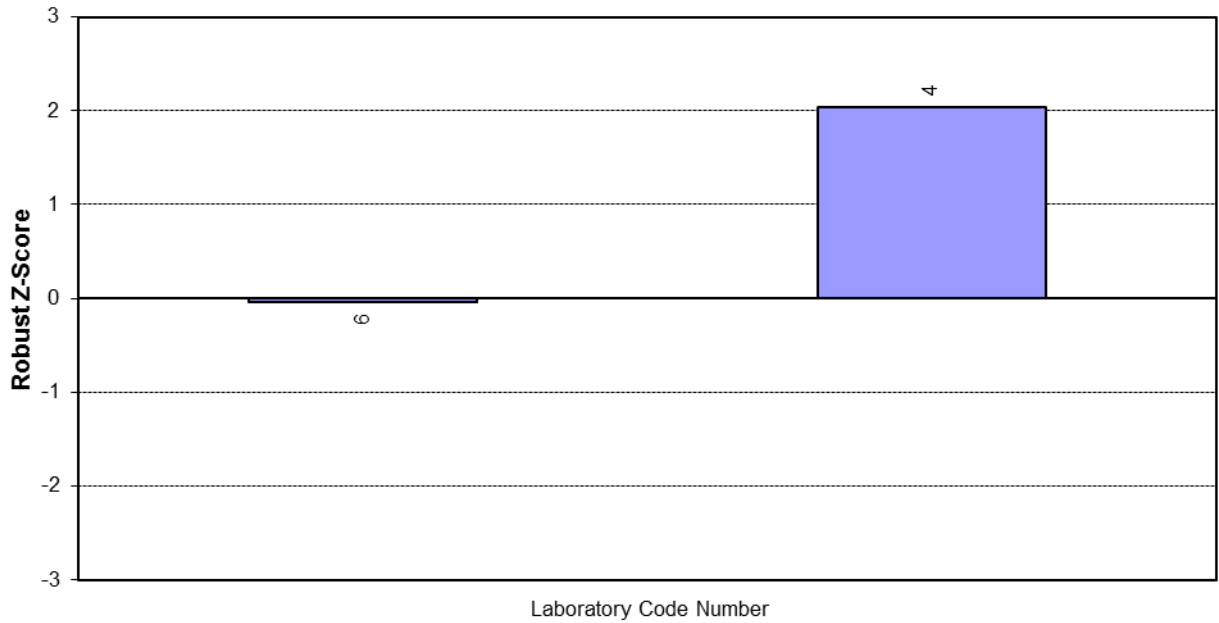
Notes:

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).
2. The data presented consists of Traditional MPN and Colilert techniques assessed together.
3. Visual presentation of method comparisons is included on page 7.

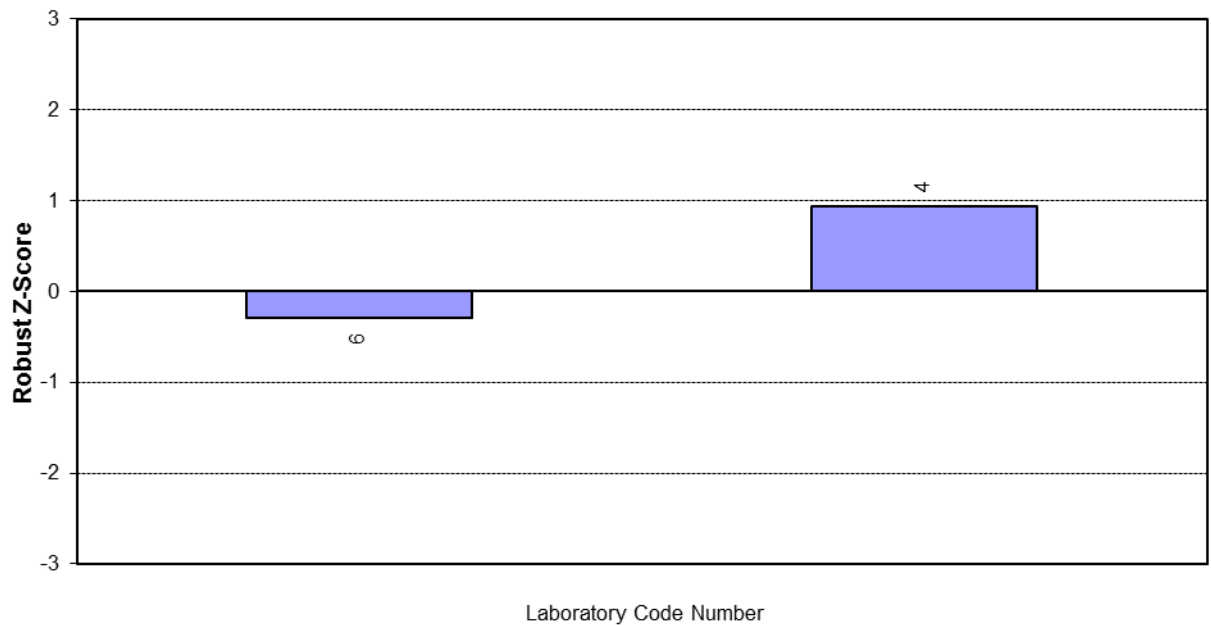
A7.2

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

Sample - PTA 1



Sample - PTA 2



A8.1

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
3	Result reported as Log 10 value	±0.21	Result reported as Log 10 value	±0.21	5.53	5.20	1.20	-0.12
7	160000	118000 - 220000	58000	45000 - 74000	5.20	4.76	-0.15	-1.46

Summary Statistics

Sample - PTA 1

No. of Results	15
Median	5.240
Norm IQR	0.241
Robust CV	4.6%
Minimum	4.69
Maximum	6.38
Range	1.69
Uncertainty (Median)	0.078

Sample - PTA 2

No. of Results	15
Median	5.240
Norm IQR	0.326
Robust CV	6.2%
Minimum	4.04
Maximum	6.38
Range	2.34
Uncertainty (Median)	0.106

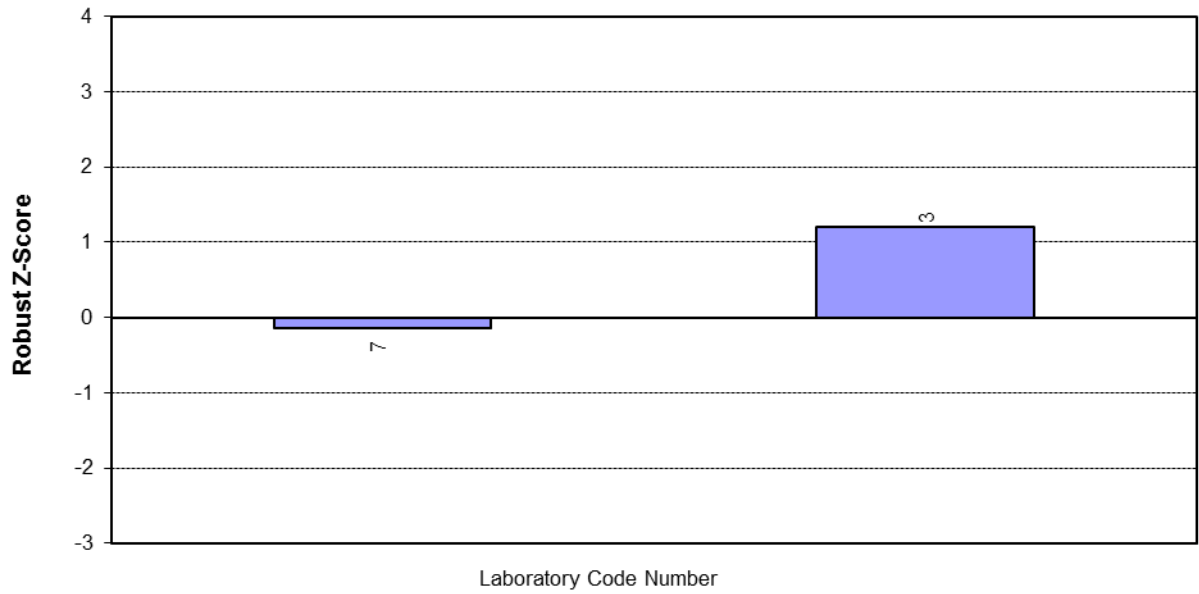
Notes:

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).
2. The data presented consists of Traditional MPN and Colilert techniques assessed together.
3. Visual presentation of method comparisons is included on page 7.

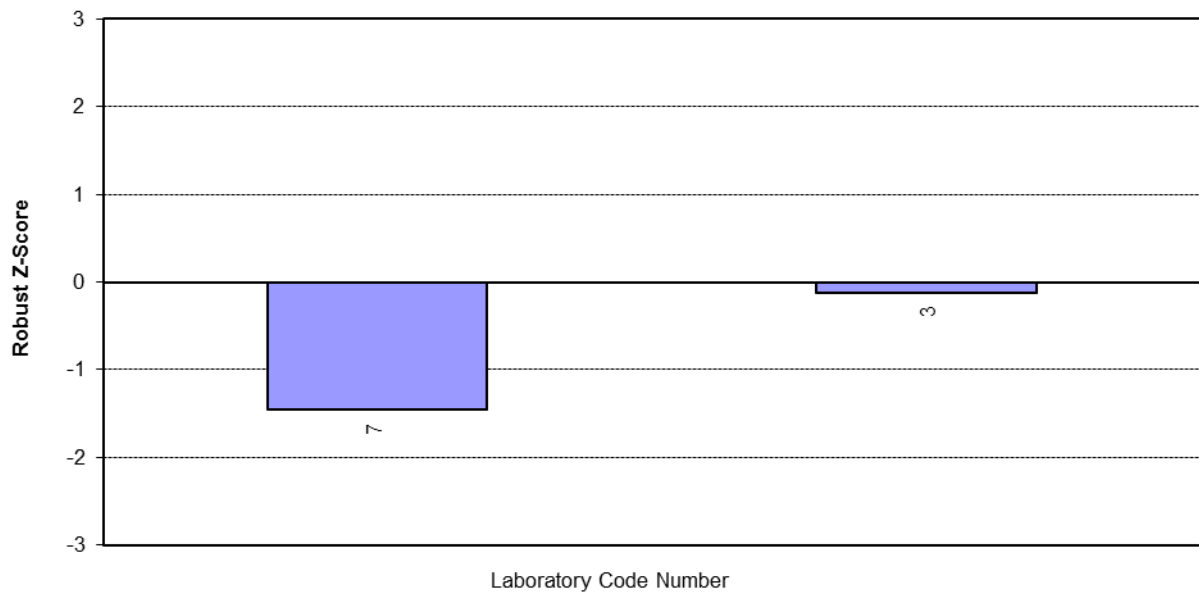
A8.2

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
6	53000		15400		4.72	4.19	0.22	-0.98
7	45000	36000 - 57000	22000	18000 - 27000	4.65	4.34	-0.13	1.11
8	45000	0.10	22000	0.10	4.65	4.34	-0.13	1.11

**Summary Statistics
Sample - PTA 1**

No. of Results	13
Median	4.680
Norm IQR	0.200
Robust CV	4.3%
Minimum	4.08
Maximum	4.82
Range	0.74
Uncertainty (Median)	0.070

**Summary Statistics
Sample - PTA 2**

No. of Results	13
Median	4.260
Norm IQR	0.074
Robust CV	1.7%
Minimum	3.84
Maximum	4.34
Range	0.50
Uncertainty (Median)	0.026

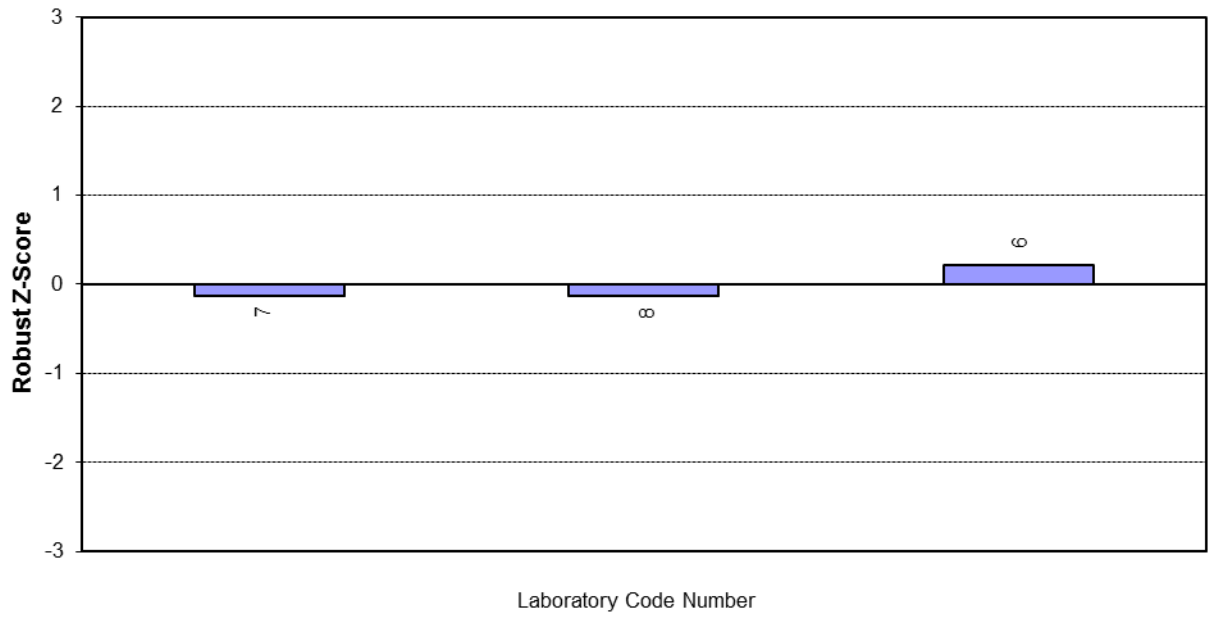
Note:

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).

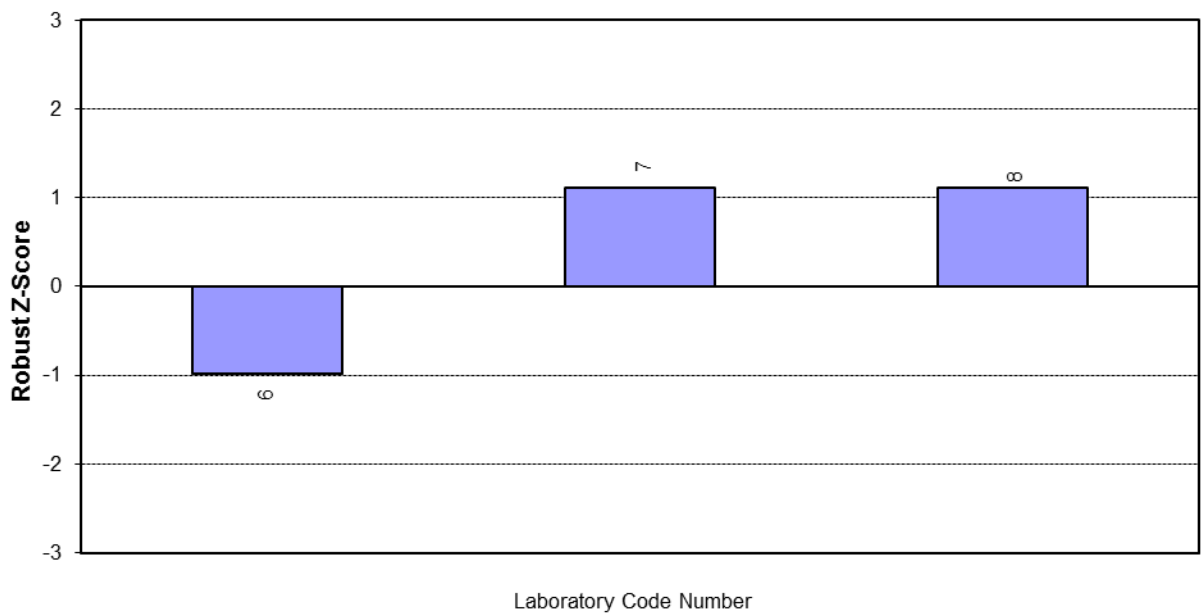
A9.2

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
7	30000	23000 - 39000	11000	8500 - 13000	4.48	4.04	0.54	0.77

Summary Statistics Sample - PTA 1

No. of Results	8
Median	4.320
Norm IQR	0.293
Robust CV	6.8%
Minimum	3.72
Maximum	4.61
Range	0.89
Uncertainty (Median)	0.130

Summary Statistics Sample - PTA 2

No. of Results	8
Median	3.875
Norm IQR	0.216
Robust CV	5.6%
Minimum	3.61
Maximum	4.69
Range	1.08
Uncertainty (Median)	0.096

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).
2. As there was only one robust z-score result reported, no graphs are shown for this technique.

SECTION A11

**Plate Count
All Techniques**

A11.1

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
6	3400		2100	12.9% REU	3.53	3.32	0.05	0.00
7	5800	4300 - 7800	2300	1800 - 2900	3.76	3.36	1.69	0.05
8	4200	0.07	2000	0.07	3.62	3.30	0.70	-0.47

Summary Statistics

Sample - PTA 1

No. of Results	8
Median	3.525
Norm IQR	0.141
Robust CV	4.0%
Minimum	3.28
Maximum	3.68
Range	0.40
Uncertainty (Median)	0.062

Sample - PTA 2

No. of Results	8
Median	3.330
Norm IQR	0.139
Robust CV	4.2%
Minimum	3.18
Maximum	3.85
Range	0.67
Uncertainty (Median)	0.061

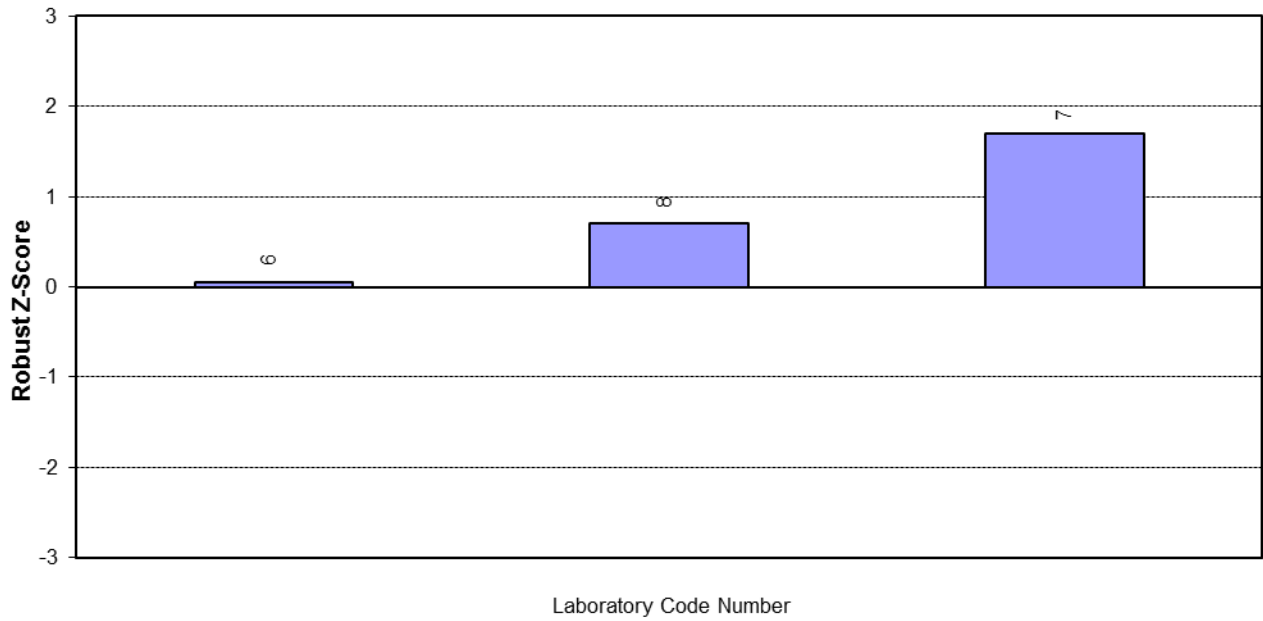
Note:

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).

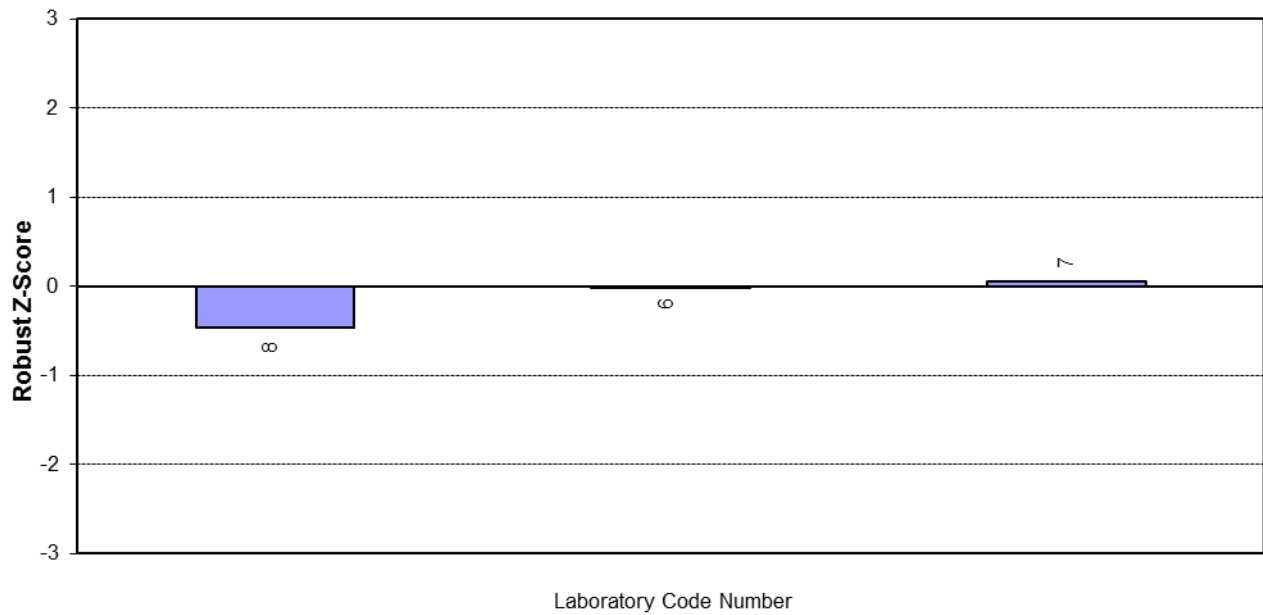
A11.2

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 5 September 2017. 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 44.5°C 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
27000	4.4314	32000	4.5051	22000	4.3424	28000	4.4472
23000	4.3617	30000	4.4771	24000	4.3802	24000	4.3802
33000	4.5185	30000	4.4771	20000	4.3010	23000	4.3617
23000	4.3617	28000	4.4472				
23000	4.3617	25000	4.3979				

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 60 – SEPTEMBER 2017

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 <math><4^{\circ}\text{C}</math> 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 $k \approx 2$). For microbiological testing, you may submit MU information as either a range of results if reporting in standard form (e.g. 6.2×10^1 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 19 September 2017 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 60 – SEPTEMBER 2017

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.

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

ROUND 60 SEPTEMBER 2017

RESULTS SHEET

Laboratory Code:

Test	Technique	PTA 1	MU	PTA 2	MU	Method Source/ Year/Technique
<i>E. coli</i> (cfu/100mL or MPN/100mL)	MF					<input type="checkbox"/> AS/NZS 4276.7-2007 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> Other:
	Colilert					<input type="checkbox"/> AS 4276.21-2005 <input type="checkbox"/> Other:
Thermotolerant (Faecal) Coliforms (cfu/100mL or MPN/100mL)	MF					<input type="checkbox"/> AS/NZS 4276.7-2007 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> Other:
Total Coliforms (cfu/100mL or MPN/100mL)	MF					<input type="checkbox"/> AS/NZS 4276.5-2007 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> Other:
	Colilert					<input type="checkbox"/> AS 4276.21-2005 <input type="checkbox"/> Other:
Enterococci (cfu/100mL or MPN/100mL)	MF					<input type="checkbox"/> AS/NZS 4276.9-2007 <input type="checkbox"/> Other:
	Enterolert					<input type="checkbox"/> APHA 9230D <input type="checkbox"/> Other:
Plate Count 37°C (or 35°C) (cfu/mL)	Pour Plate					<input type="checkbox"/> AS/NZS 4276.3.1-2007 <input type="checkbox"/> Other:
	Other					

Date Sample Received:

Temperature of samples on arrival:

Date Sample Processed:

Comments

.....

Signature: _____ Date: _____

-- End of Report --