

REPORT NO. 1076

MICROBIOLOGICAL WATERS

PROFICIENCY TESTING PROGRAM

ROUND 61

APRIL 2018

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 March 2018 by Proficiency Testing Australia (PTA). The Program Coordinator was Mrs K Weller and the Technical Adviser was Ms S Mott from Global Proficiency Ltd (New Zealand). This is the sixty-first 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 all 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. Where a laboratory has reported more than one result for a test/technique, their code number will appear with a corresponding letter for each result.

Participants included laboratories from Australia, Philippines and Singapore.

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

The normalised IQR is a measure of the spread of the results. It is calculated by multiplying the interquartile range (IQR) by a correction factor which converts the IQR to an estimate of the standard deviation. The IQR is the difference between the upper and lower quartiles (i.e. the values above and below which a quarter of the results lie, respectively).

For normally distributed data, the uncertainty of the median is approximated by:

$$\sqrt{\frac{\pi}{2}} \times \frac{\text{normIQR}}{\sqrt{n}} \quad n = \text{number of results}$$

Please see reference [1] for further details on these robust summary statistics.

(iii) Ordered z-score charts

On these charts each laboratory's robust z-score is shown, in order of magnitude, and is marked with its code number. From these charts, each laboratory can readily compare its performance relative to the other laboratories.

These charts contain solid lines at +3.0 and -3.0, so that outliers are clearly identifiable as those laboratories whose "bar" extends beyond these "cut-off" lines. The y-axis of these charts has been limited, so very large z-scores appear to extend beyond the chart boundary.

- (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 6. Also included in this table are the laboratories that reported false 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
<i>E. coli</i> orgs/100mL	MF	1	22	1.570	0.124
		2	22	N/A	N/A
	MPN	1	13	1.360	0.340
		2	14	N/A	N/A
	Colilert	1	29	1.490	0.230
		2	29	N/A	N/A
Faecal Coliforms orgs/100mL	MF	1	32	1.540	0.154
		2	32	1.340	0.078
	MPN	1	16	1.360	0.340
		2	15	1.520	0.293
Total Coliforms orgs/100mL	MF	1	26	1.695	0.107
		2	26	1.390	0.104
	MPN	1	12	1.500	0.278
		2	13	1.540	0.126
	Colilert	1	26	1.695	0.107
		2	29	1.670	0.185
Enterococci orgs/100mL	MF	1	13	1.540	0.059
		2	14	1.370	0.148
Plate Count orgs/mL	All	1	7	1.857	0.087
		2	7	1.991	0.078

All statistics (including No. of Results), except for Plate Count, 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.
3. Target CVs were used to calculate the z-scores for the *E. coli* MPN and Faecal Coliforms via MPN results for Sample 1 due to the wide spread of data observed for this sample. The CV selected was 25% for both tests which aligns with the values seen in a previous round using samples with similar levels of microorganisms.

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
<i>E. coli</i>	MF	3	-
	MPN	-	1
	Colilert	-	-
Faecal Coliforms	MF	2	-
	MPN	-	1
Total Coliforms	MF	-	-
	MPN	1, 3	1
	Colilert	-	-
Enterococci	MF	-	-
Plate Count	All	-	-

There were no incomplete results reported.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

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

Sample PTA 1 contained *E. coli* and *Citrobacter freundii* as the coliform organisms in the sample. Sample PTA 2 contained *Klebsiella pneumoniae* as the only coliform organism. The strain used is a member of the Thermotolerant (Faecal) coliforms and was included to provide an additional challenge to laboratories. *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'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 Total Coliforms, three laboratories reported results for the MF technique, three laboratories reported results for the MPN technique and three laboratories reported results for the Colilert technique. Two participants (Laboratories 1 and 3) recorded outliers using the MPN technique with results lower than expected. Laboratory 1 reported a result rated as an outlier for Sample PTA 1 and a false negative (identification outlier) result for Sample PTA 2; Laboratory 3 received an outlier for Sample PTA 2. It is recommended that results classified as outliers are investigated further.

A variety of methods were used by laboratories.

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 equation on page 3.

Total Coliforms via:	Sample PTA 1 Median \pm Uncertainty (Log ₁₀ cfu/MPN/100mL)	Sample PTA 2 Median \pm Uncertainty (Log ₁₀ cfu/MPN /100mL)
Membrane Filtration	1.695 \pm 0.026	1.390 \pm 0.026
Most Probable Number	1.500 \pm 0.101	1.540 \pm 0.044
Colilert	1.695 \pm 0.026	1.670 \pm 0.043

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

Measurement Uncertainty - Total Coliforms via Membrane Filtration (MF):

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

Of the reported MUs for the Total Coliforms methods, eight 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: MUs for all three methods; Membrane Filtration, MPN and Colilert - sample PTA 1, and in addition, PTA 2 for Colilert only.
- Laboratory 5: MUs for Membrane Filtration and Colilert – both samples.

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.

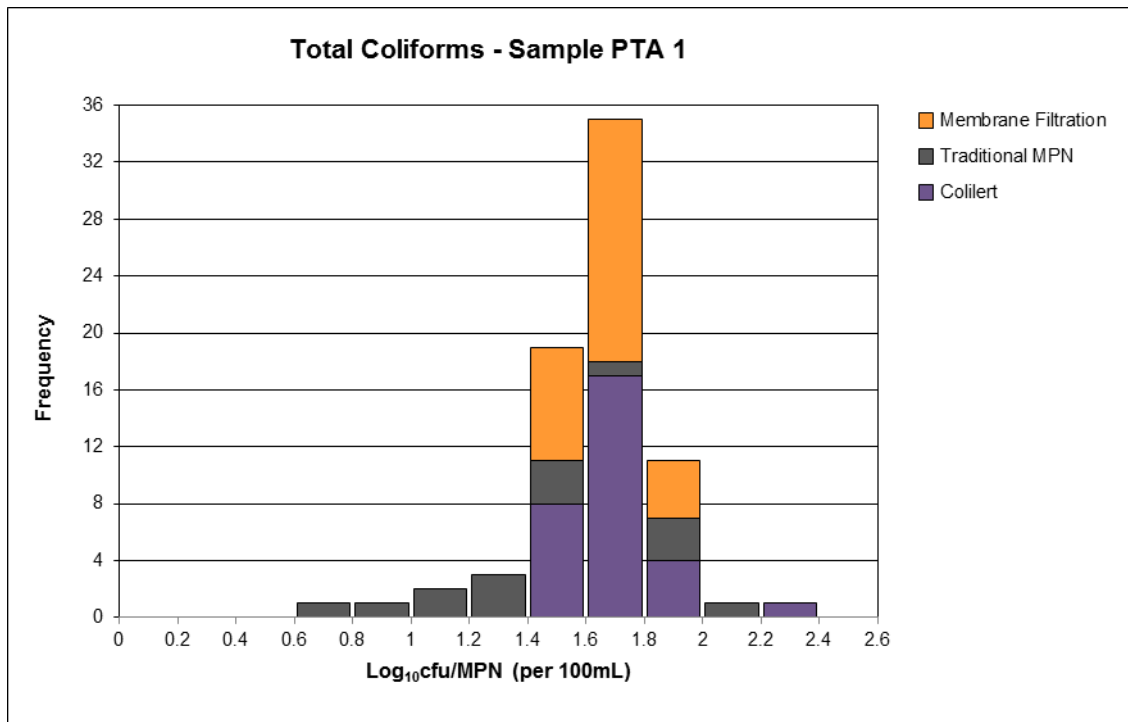


Figure TA-1. Total Coliform results for Sample PTA 1

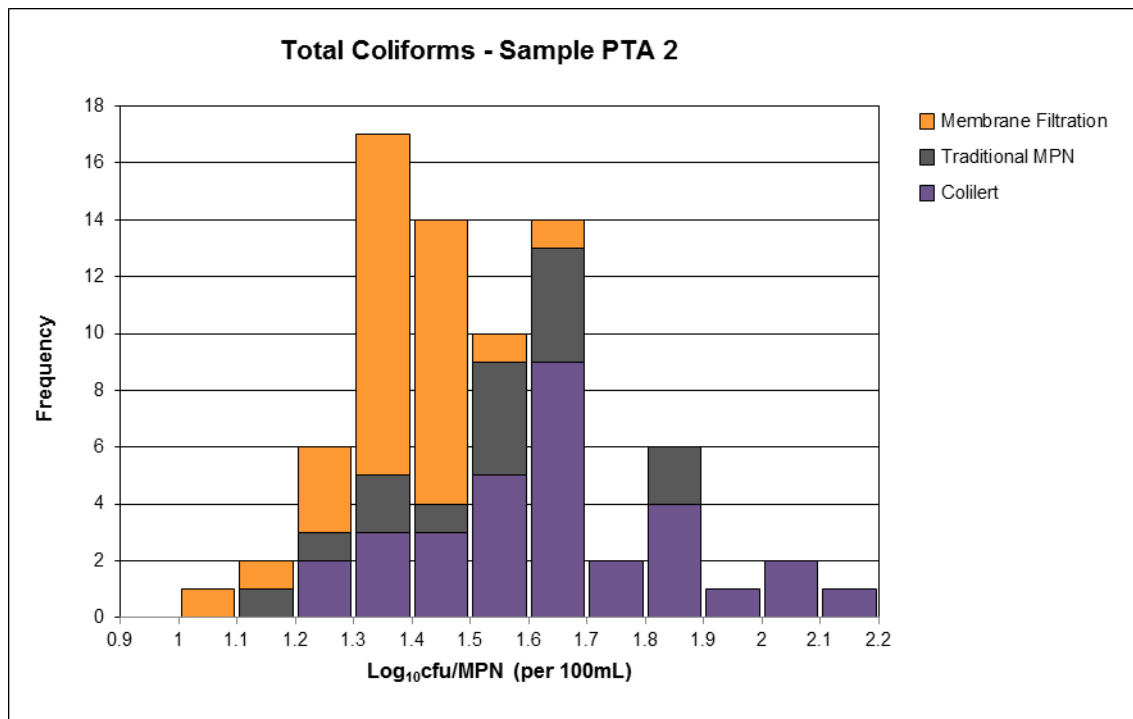


Figure TA-2. Total Coliform results for Sample PTA 2

E. coli:

Between three and four laboratories reported results for the three different *E. coli* techniques. One participant (Laboratory 3) recorded an outlier for the MF technique for Sample PTA 1 with results lower than expected. One participant (Laboratory 1) recorded an identification outlier (false positive) for Sample PTA 2 using the MPN technique.

All results classified as outliers should be investigated further.

A variety of methods were used by participating laboratories.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 3 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)
Membrane Filtration	1.570 \pm 0.033
Most Probable Number	1.360 \pm 0.118
Colilert	1.490 \pm 0.053

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

Measurement Uncertainty - *E. coli* 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. \pm log values, \pm cfu values and a range of cfu/100mL.

Laboratories 2 and 3 may need to re-examine their test results or their MU calculations for the MF method as their results for PTA 1 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.

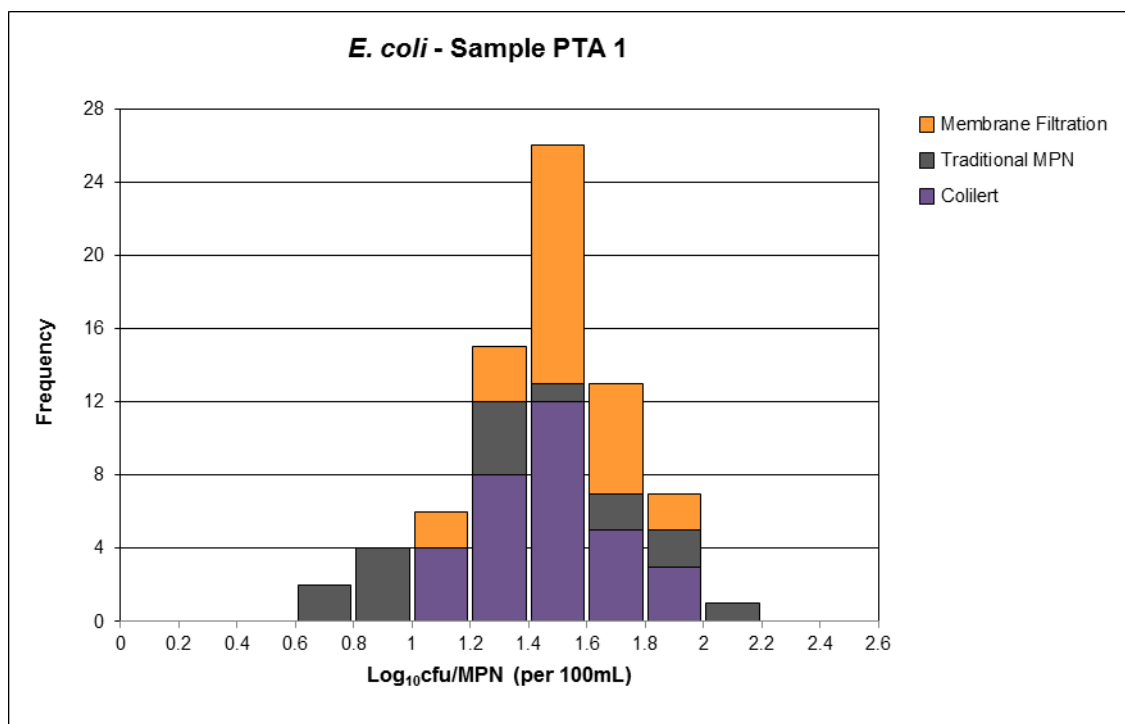


Figure TA-3. *E. coli* results for Sample PTA 1.

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.

For Faecal Coliforms, four laboratories reported results for the MF technique and four laboratories reported results for the MPN technique. One participant (Laboratory 2) recorded an outlier for the MF technique (Sample PTA 2) with a result lower than expected. One participant (Laboratory 1) recorded an identification outlier (false negative) for Sample PTA 2 using the MPN technique.

All results classified as outliers should be investigated further.

A variety of methods were used by participating laboratories.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 3 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	1.540 ± 0.034	1.340 ± 0.017
Most Probable Number	1.360 ± 0.107	1.520 ± 0.095

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, ± cfu values and a range of cfu/100mL values.

Laboratory 3 may need to re-examine their test results or their MU calculations for the MF method as their results for both samples and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

Laboratory 5 may need to re-examine their test results or their MU calculations for the MF method as their results for sample 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.

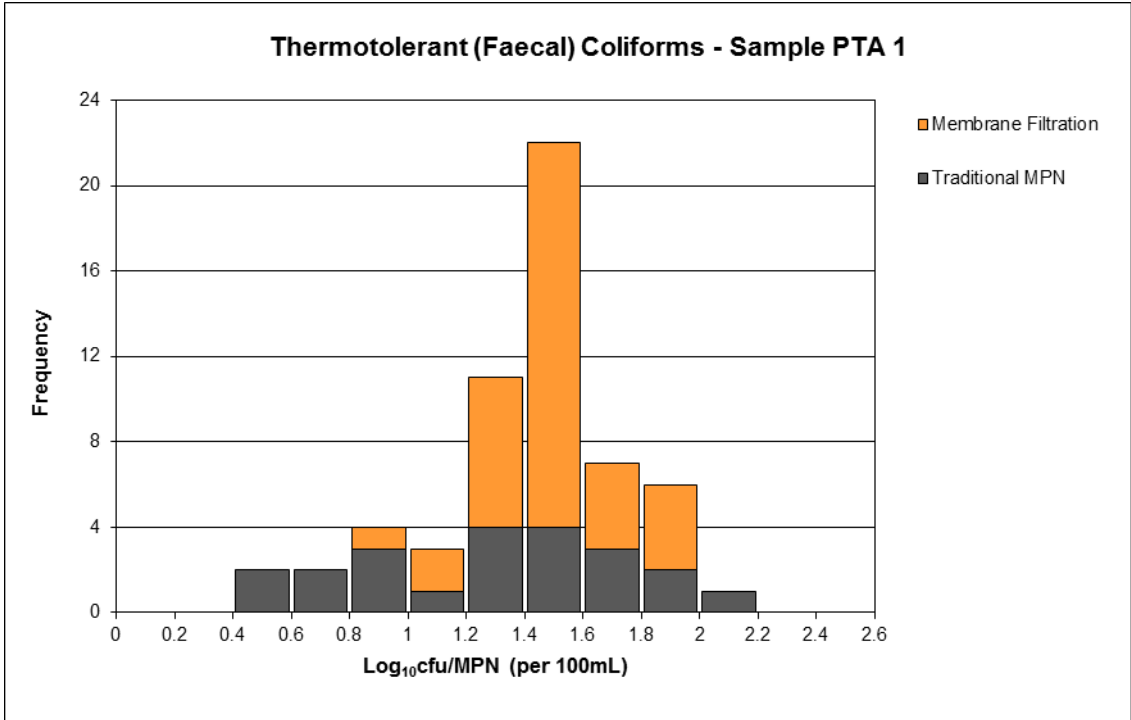


Figure TA-4 Faecal Coliform results for Sample PTA 1

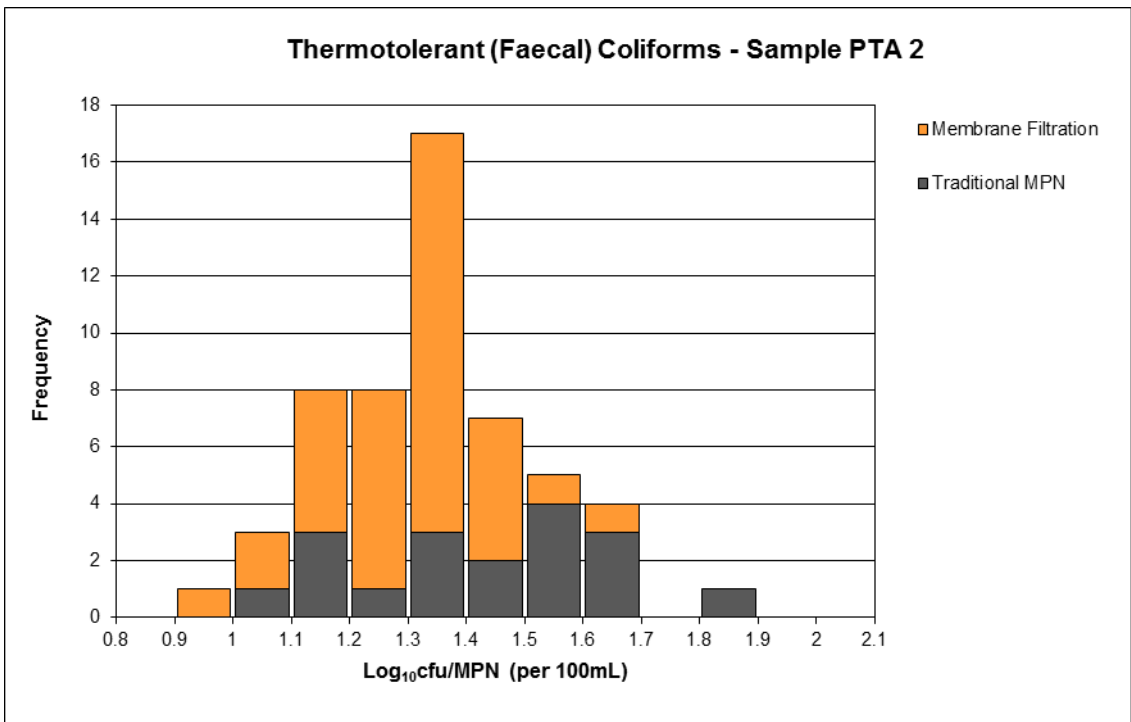


Figure TA-5. Faecal Coliform results for Sample PTA 2

Enterococci:

Four laboratories reported results for Enterococci using the Enterococci MF technique (with one laboratory reporting two results). No laboratories recorded outliers for this test.

The majority of laboratories reported using Australian Standard methods.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 3 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	1.540 \pm 0.021	1.370 \pm 0.050

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 three different ways; i.e. \pm log values, \pm cfu values and a range of cfu/100mL values.

Plate Count:

Seven sets of results were submitted for the Plate Count test (with Laboratory 4 submitting 2 sets of results). Six laboratories reported using a Pour Plate technique, while one laboratory reported using Petrifilm. No outliers were recorded for this test.

Confidence in the medians can be expressed as the uncertainty of the median (as defined on page 3 of this report), which was calculated for each test and/or method within a test.

	Sample PTA 1 Median \pm Uncertainty (Log ₁₀ cfu/mL)	Sample PTA 2 Median \pm Uncertainty (Log ₁₀ cfu/mL)
Plate Count:	1.857 \pm 0.041	1.991 \pm 0.037

Measurement Uncertainty - Plate Count:

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, \pm cfu values and a range of cfu/100mL values.

General Comments

A total of 64 results were submitted for analysis in this round. Of these results seven (11%) were outlier results. This is lower than the 19% of results which were outlier results in Round 59.

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 1 Robust z-score
2	20	±4	0	-	1.30	-2.17
3	14	±0.14 log ₁₀	<1	±0.14 log ₁₀	1.15	-3.41 §
5	28	23-34	<1	N/A	1.45	-0.99
6	27	0.20	<1	0.20	1.43	-1.12

Note:

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

Summary Statistics

Sample - PTA 1

No. of Results	22
Median	1.570
Norm IQR	0.124
Robust CV	7.9%
Minimum	1.08
Maximum	1.84
Range	0.76
Uncertainty (Median)	0.033

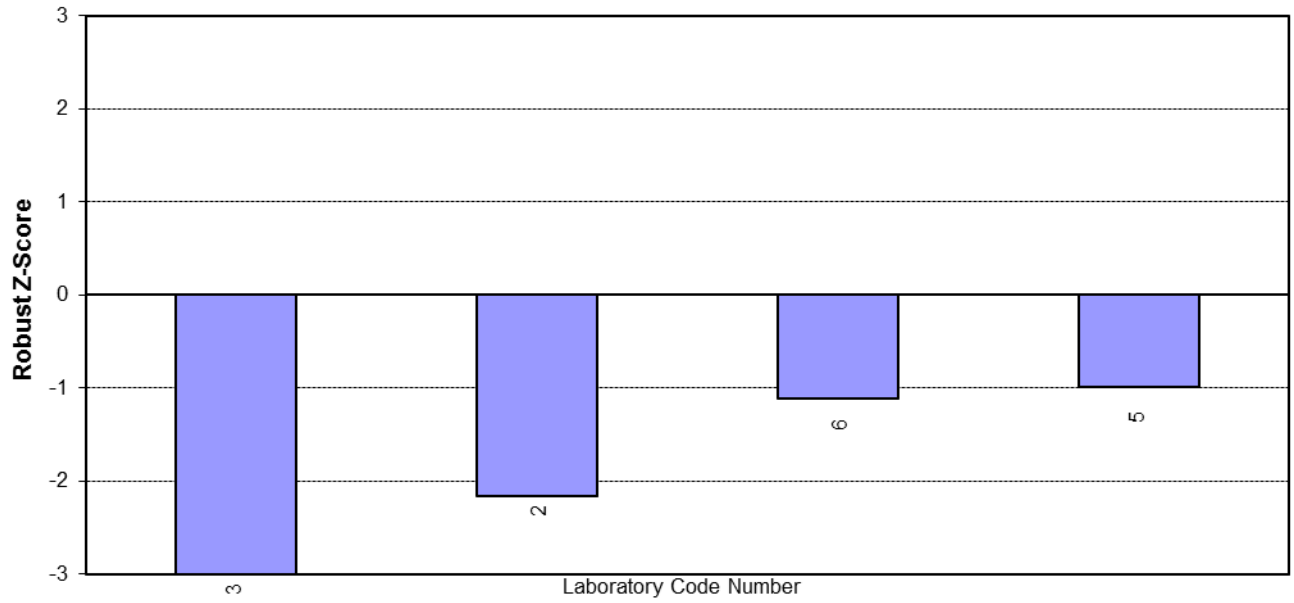
Note:

- 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



A2.1

***E. coli* (orgs/100mL) – MPN Technique**

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log ₁₀ Result	PTA 1 Robust z-score
1	4.3	Not available	3.8 ‡	Not available	0.63	-2.14
2	17	±4	0 (<2)	-	1.23	-0.38
3	8	±0.59 log ₁₀	<2	±0.59 log ₁₀	0.90	-1.34

Note:

- ‡ denotes a false positive.

Summary Statistics

Sample - PTA 1

No. of Results	13
Median	1.360
Norm IQR	0.340
Robust CV	25.0%
Minimum	0.70
Maximum	2.04
Range	1.34
Uncertainty (Median)	0.118

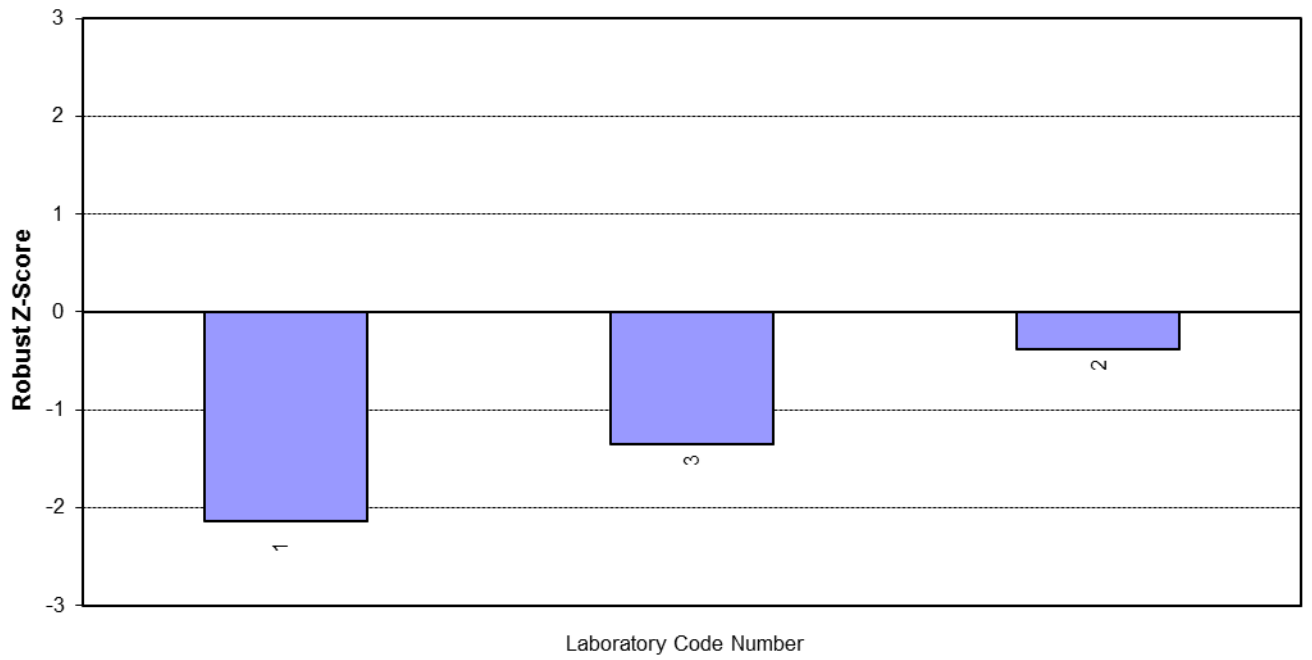
Notes:

- 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).
- Target CVs were used to calculate the z-scores for the *E. coli* MPN results for Sample 1 due to the wide spread of data observed for this sample. The CV selected was 25% which aligns with the values seen in a previous round using samples with similar levels of microorganisms.

A2.2

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

Sample - PTA 1



A3.1

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

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log ₁₀ Result	PTA 1 Robust z-score
2	25	±3	0	-	1.40	-0.40
3	32	21-47	<1	0 - 3.7	1.51	0.07
5	23	19-29	<1	N/A	1.36	-0.56

Summary Statistics

Sample - PTA 2

No. of Results	29
Median	1.490
Norm IQR	0.230
Robust CV	15.4%
Minimum	1.08
Maximum	2.00
Range	0.92
Uncertainty (Median)	0.053

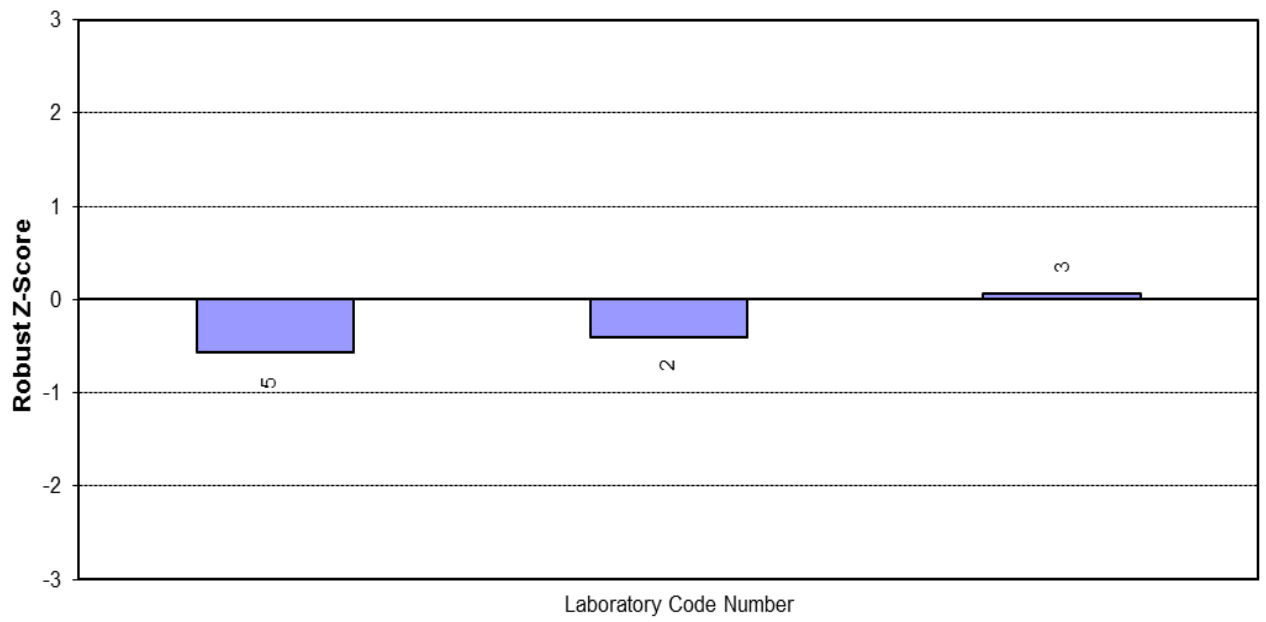
Note:

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) – MPN Technique
Ordered Robust Z-Score Charts**

Sample - PTA 1



SECTIONS A4 to A5

Faecal Coliforms

A4.1

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	20	±4	12	±2	1.30	1.08	-1.55	-3.35 §
3	14	±0.14 log ₁₀	14	±0.14 log ₁₀	1.15	1.15	-2.56	-2.49
5	28	23-34	16	14-19	1.45	1.20	-0.60	-1.75
6	27	0.13	20	0.13	1.43	1.30	-0.71	-0.50

Note:

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

Summary Statistics

Sample - PTA 1

No. of Results	32
Median	1.540
Norm IQR	0.154
Robust CV	10.0%
Minimum	0.95
Maximum	1.89
Range	0.94
Uncertainty (Median)	0.034

Sample - PTA 2

No. of Results	32
Median	1.340
Norm IQR	0.078
Robust CV	5.8%
Minimum	1.00
Maximum	1.65
Range	0.65
Uncertainty (Median)	0.017

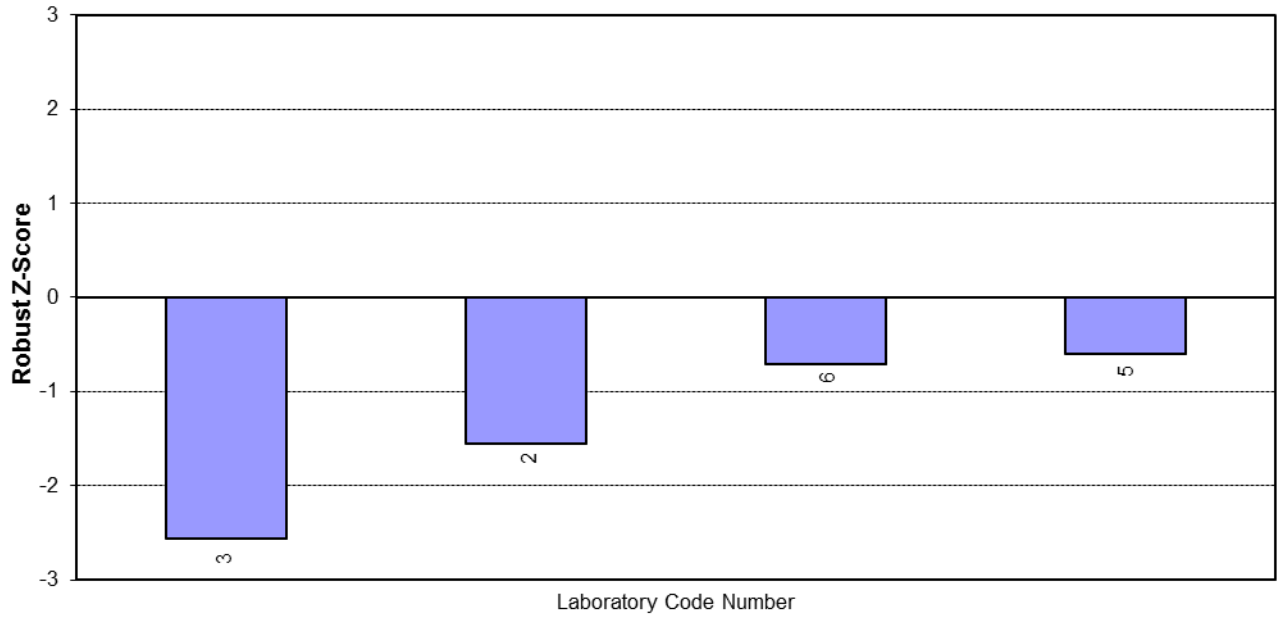
Note:

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).

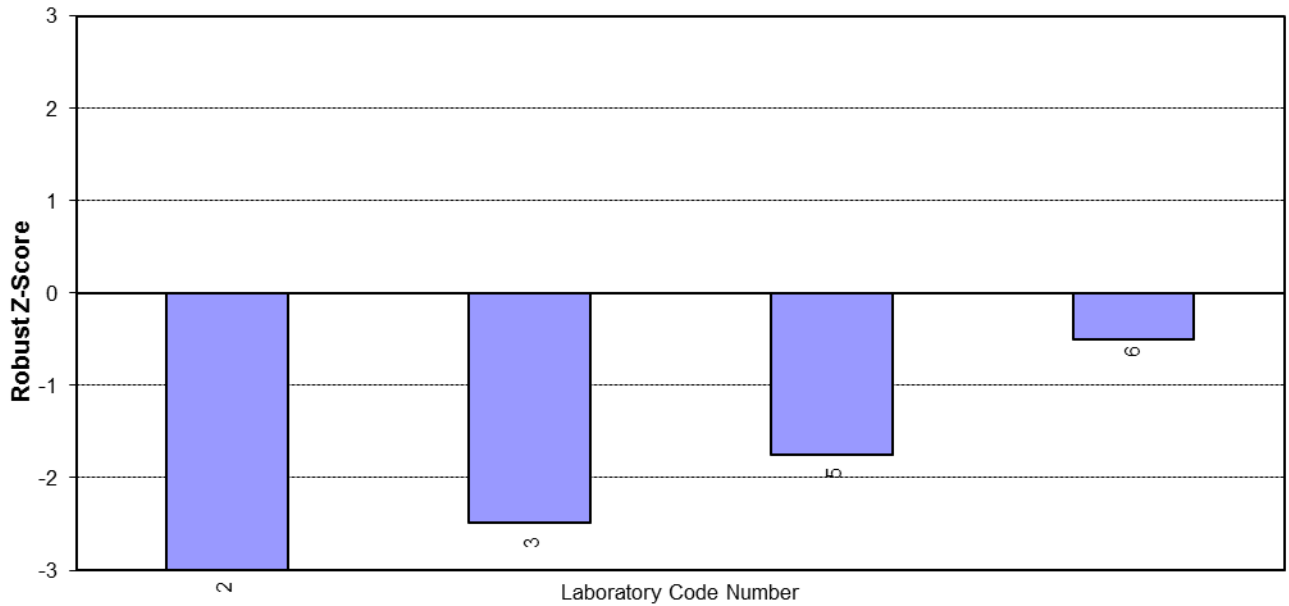
A4.2

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
1	4.3	Not available	<0.3 †	Not available	0.63	N/A	-2.14	N/A
2	17	±4	25	±6	1.23	1.40	-0.38	-0.42
3	8	±0.59 log ₁₀	13	±0.59 log ₁₀	0.90	1.11	-1.34	-1.39
5	31	23-42	21	16-27	1.49	1.32	0.39	-0.68

Note:

- † denotes a false negative.

Summary Statistics

Sample - PTA 1

No. of Results	16
Median	1.360
Norm IQR	0.340
Robust CV	25.0%
Minimum	0.48
Maximum	2.04
Range	1.56
Uncertainty (Median)	0.107

Sample - PTA 2

No. of Results	15
Median	1.520
Norm IQR	0.293
Robust CV	19.3%
Minimum	1.04
Maximum	1.90
Range	0.86
Uncertainty (Median)	0.095

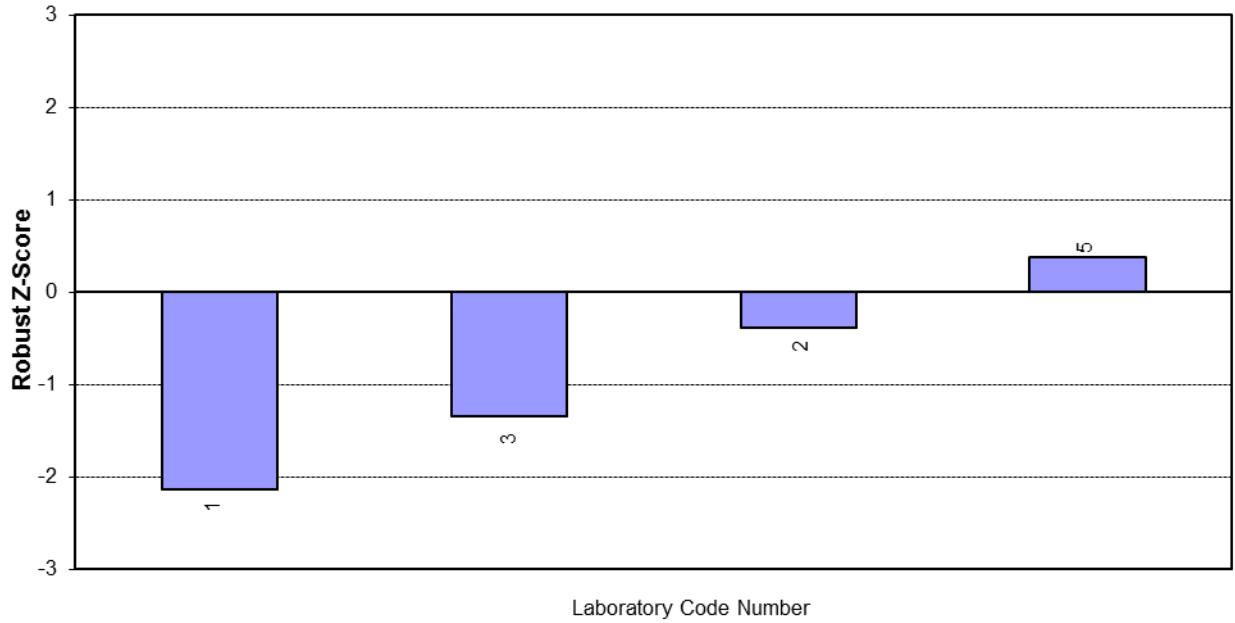
Notes:

- 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).
- Target CVs were used to calculate the z-scores for the Faecal Coliform MPN results for Sample 1 due to the wide spread of data observed for this sample. The CV selected was 25% which aligns with the values seen in a previous round using samples with similar levels of microorganisms.

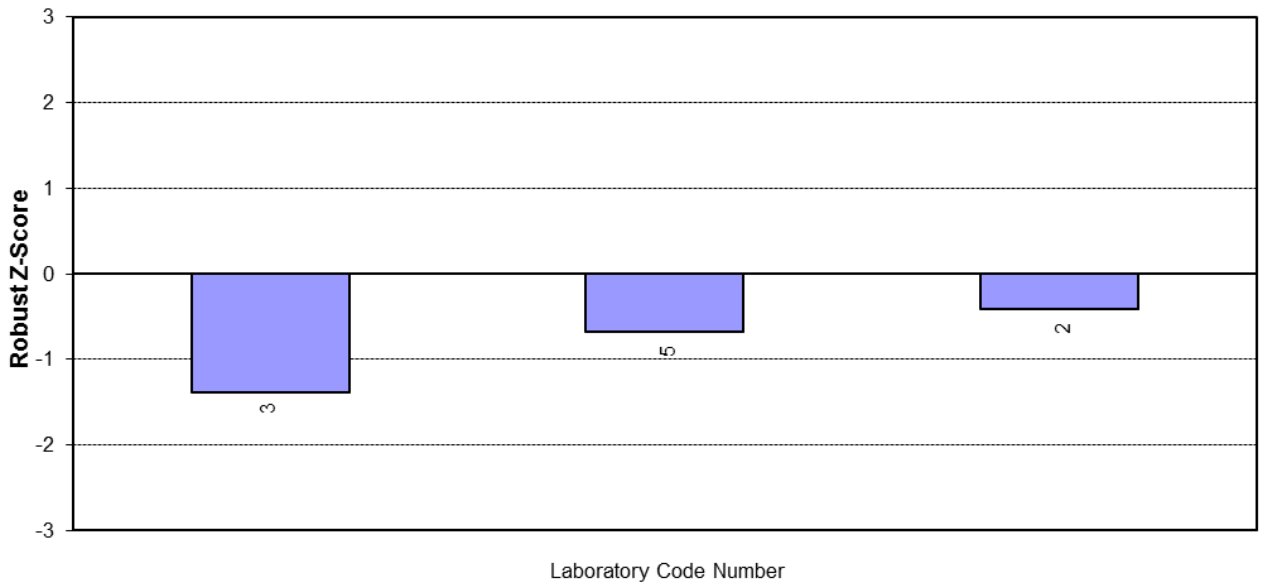
A5.2

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
2	36	±6	23	±4	1.56	1.36	-1.29	-0.27
4	44		12		1.64	1.08	-0.48	-2.99
5	29	24-36	17	14-20	1.46	1.23	-2.16	-1.54

Summary Statistics

Sample - PTA 1

No. of Results	26
Median	1.695
Norm IQR	0.107
Robust CV	6.3%
Minimum	1.48
Maximum	1.98
Range	0.50
Uncertainty (Median)	0.026

Sample - PTA 2

No. of Results	26
Median	1.390
Norm IQR	0.104
Robust CV	7.5%
Minimum	1.11
Maximum	1.67
Range	0.56
Uncertainty (Median)	0.026

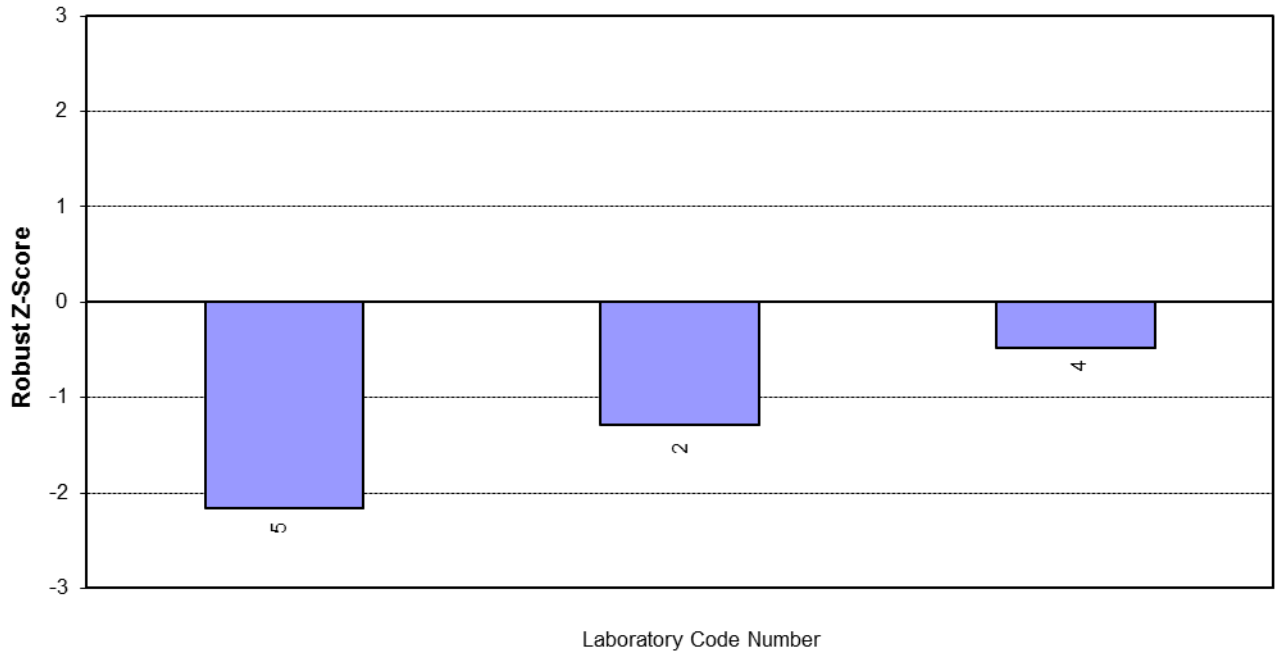
Note:

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).

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
1	4.3	Not available	<0.3 †	Not available	0.63	N/A	-3.12 §	N/A
2	80	±20	25	±6	1.90	1.40	1.45	-1.13
3	13	0.72 log ₁₀	13	±0.72 log ₁₀	1.11	1.11	-1.39	-3.38 §

Notes:

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. † denotes a false negative.

Summary Statistics

Sample - PTA 1

No. of Results	12
Median	1.500
Norm IQR	0.278
Robust CV*	18.5%
Minimum	0.90
Maximum	2.04
Range	1.14
Uncertainty (Median)	0.101

Sample - PTA 2

No. of Results	13
Median	1.540
Norm IQR	0.126
Robust CV	8.2%
Minimum	1.23
Maximum	1.90
Range	0.67
Uncertainty (Median)	0.044

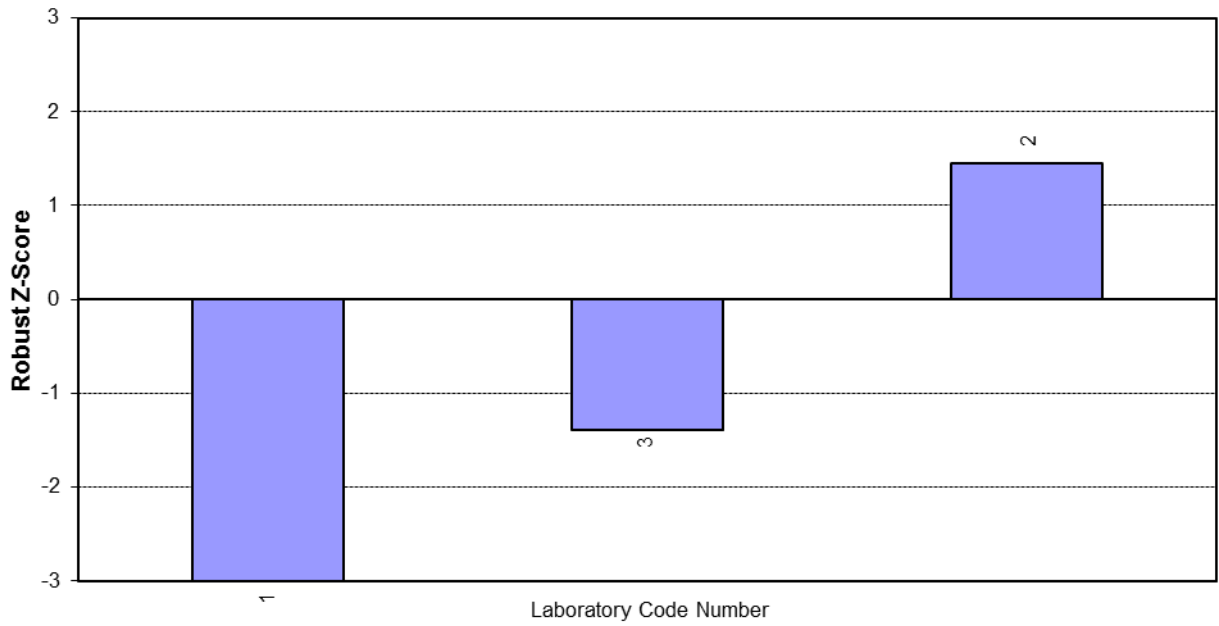
Note:

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).

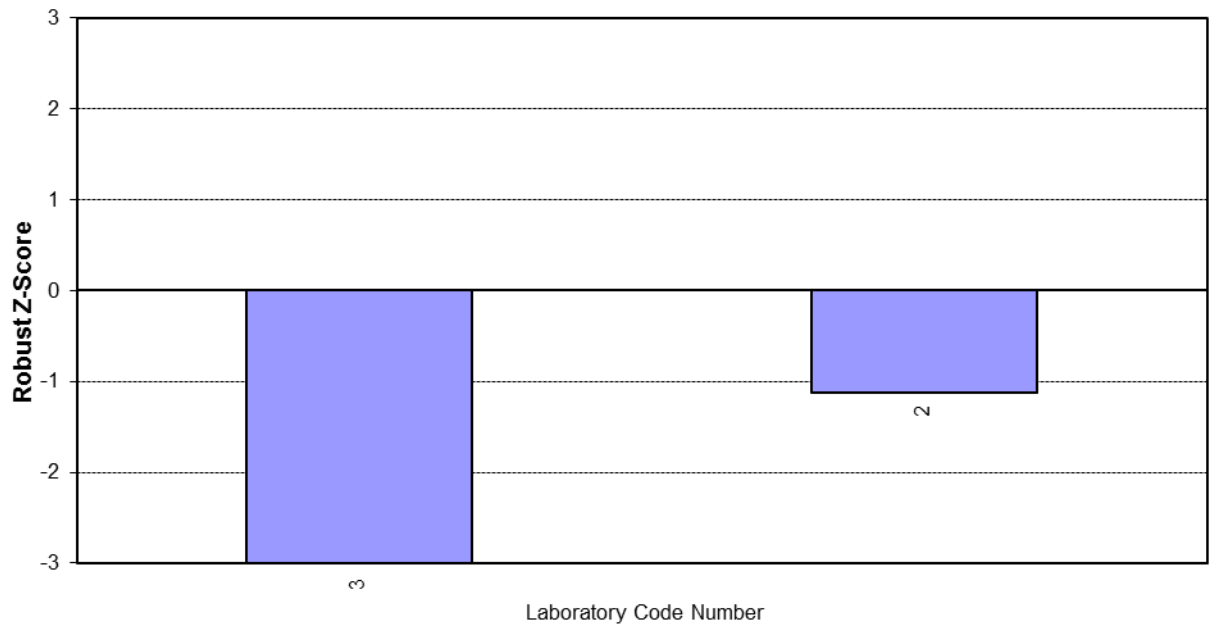
A7.2

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
2	30	±4	17	±2	1.48	1.23	-2.03	-2.37
3	46	31 - 65	30	19 - 45	1.66	1.48	-0.30	-1.04
5	33	27-41	21	17-25	1.52	1.32	-1.64	-1.88

Summary Statistics

Sample - PTA 1

No. of Results	26
Median	1.695
Norm IQR	0.107
Robust CV	6.3%
Minimum	1.48
Maximum	1.98
Range	0.50
Uncertainty (Median)	0.026

Sample - PTA 2

No. of Results	29
Median	1.670
Norm IQR	0.185
Robust CV	11.1%
Minimum	1.23
Maximum	2.12
Range	0.89
Uncertainty (Median)	0.043

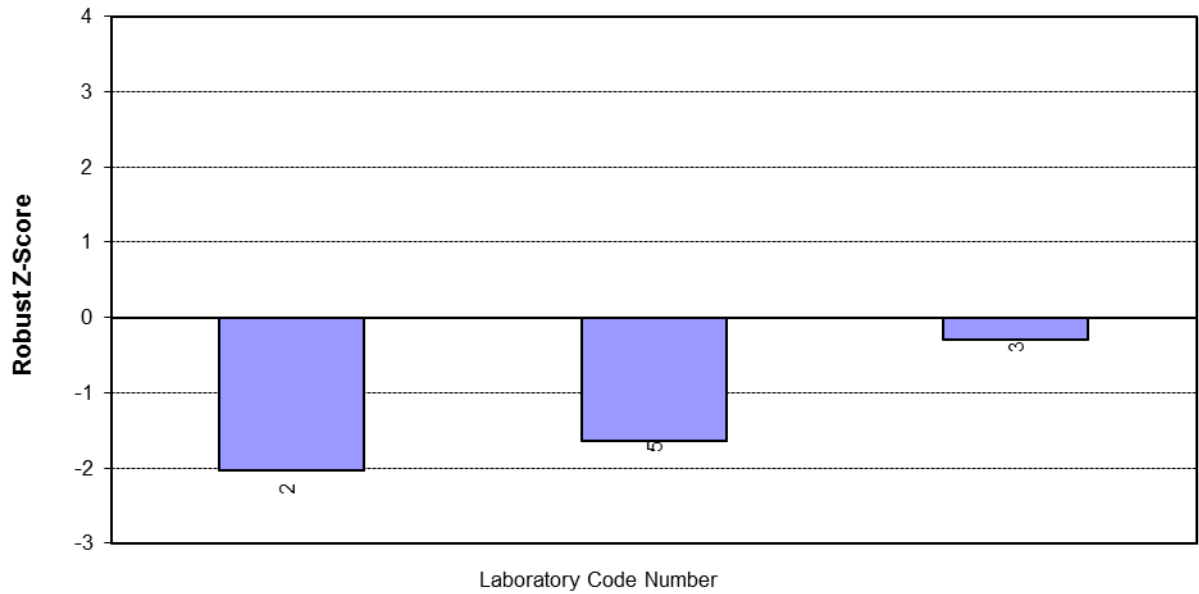
Note:

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).

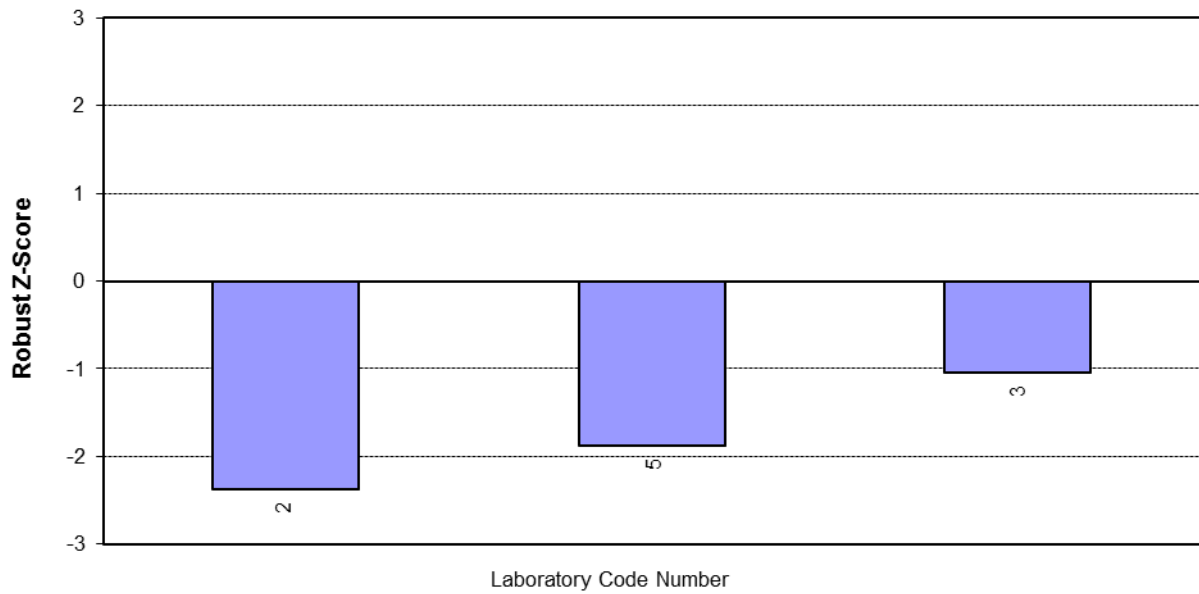
A8.2

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
2	35	±5	32	±4	1.54	1.51	0.07	0.91
3A	48	±0.28 log ₁₀	42	±0.28 log ₁₀	1.68	1.62	2.38	1.71
3B	38	±0.29 log ₁₀	33	±0.29 log ₁₀	1.58	1.52	0.67	1.00
5	48	27-69	25	14-36	1.68	1.40	2.38	0.19
6	40	0.10	22	0.10	1.60	1.34	1.05	-0.19

Summary Statistics

Sample - PTA 1

No. of Results	13
Median	1.540
Norm IQR	0.059
Robust CV	3.9%
Minimum	1.20
Maximum	1.94
Range	0.74
Uncertainty (Median)	0.021

Summary Statistics

Sample - PTA 2

No. of Results	14
Median	1.370
Norm IQR	0.148
Robust CV	10.8%
Minimum	0.95
Maximum	1.94
Range	0.99
Uncertainty (Median)	0.050

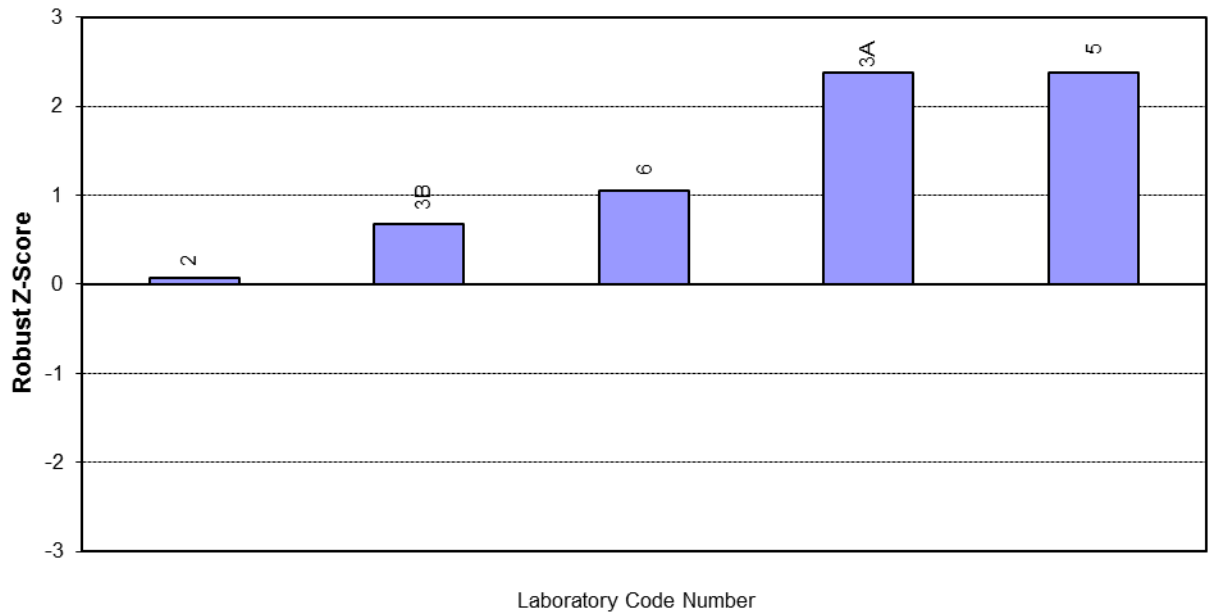
Note:

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).

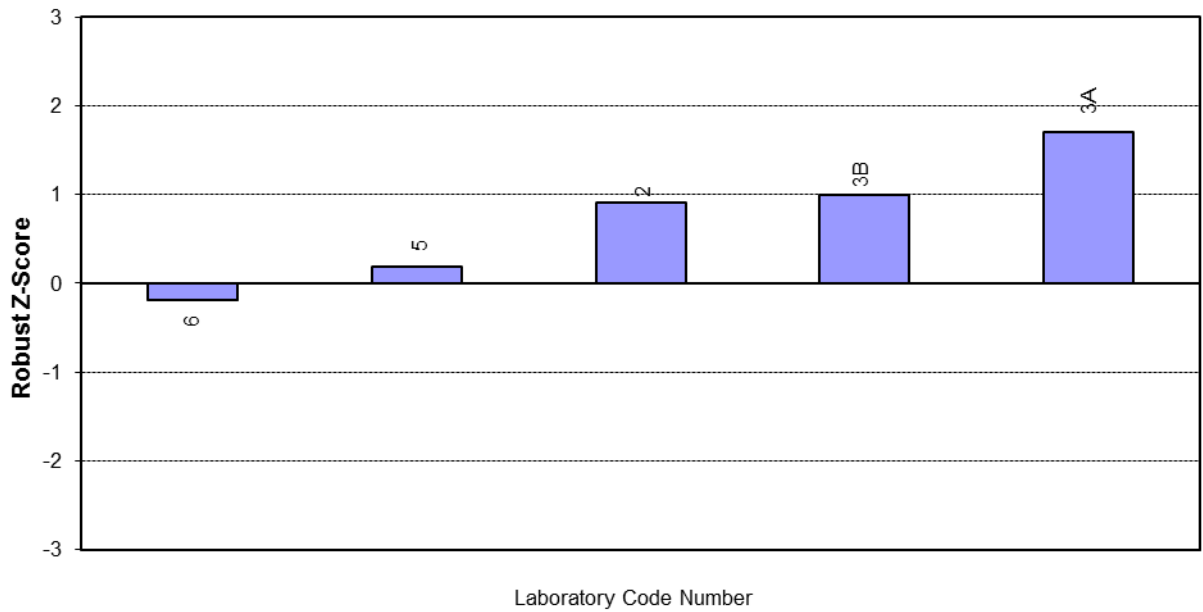
A9.2

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

Sample - PTA 1



Sample - PTA 2



SECTION A10

**Plate Count
All Techniques**

A10.1

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
1	64	Not available	70	Not available	1.81	1.85	-0.59	-1.87
2	65	±13	110	±22	1.81	2.04	-0.51	0.64
3	83	±0.18 log ₁₀	100	±0.18 log ₁₀	1.92	2.00	0.71	0.11
4A	72		78		1.86	1.89	0.00	-1.27
4B	83		98		1.92	1.99	0.71	0.00
5	78	57-108	100	71-141	1.89	2.00	0.40	0.11
6	63	0.07	86	0.07	1.80	1.93	-0.67	-0.73

Summary Statistics
Sample - PTA 1

No. of Results	7
Median	1.857
Norm IQR	0.087
Robust CV	4.7%
Minimum	1.80
Maximum	1.92
Range	0.12
Uncertainty (Median)	0.041

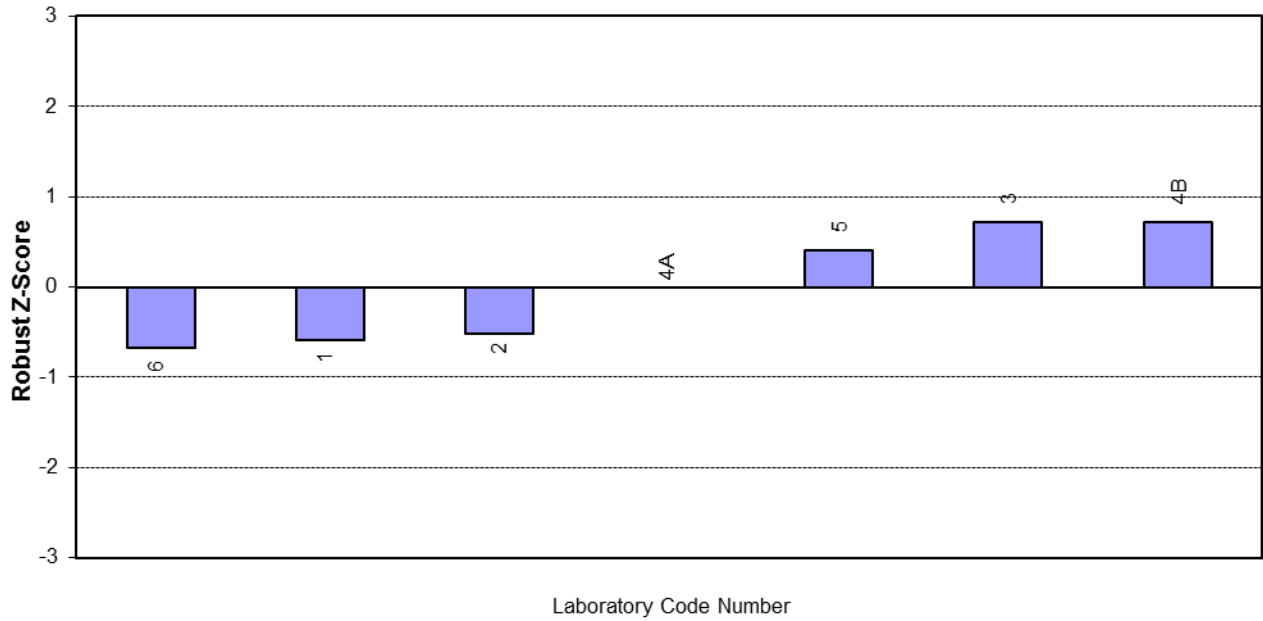
Sample - PTA 2

No. of Results	7
Median	1.991
Norm IQR	0.078
Robust CV	3.9%
Minimum	1.85
Maximum	2.04
Range	0.20
Uncertainty (Median)	0.037

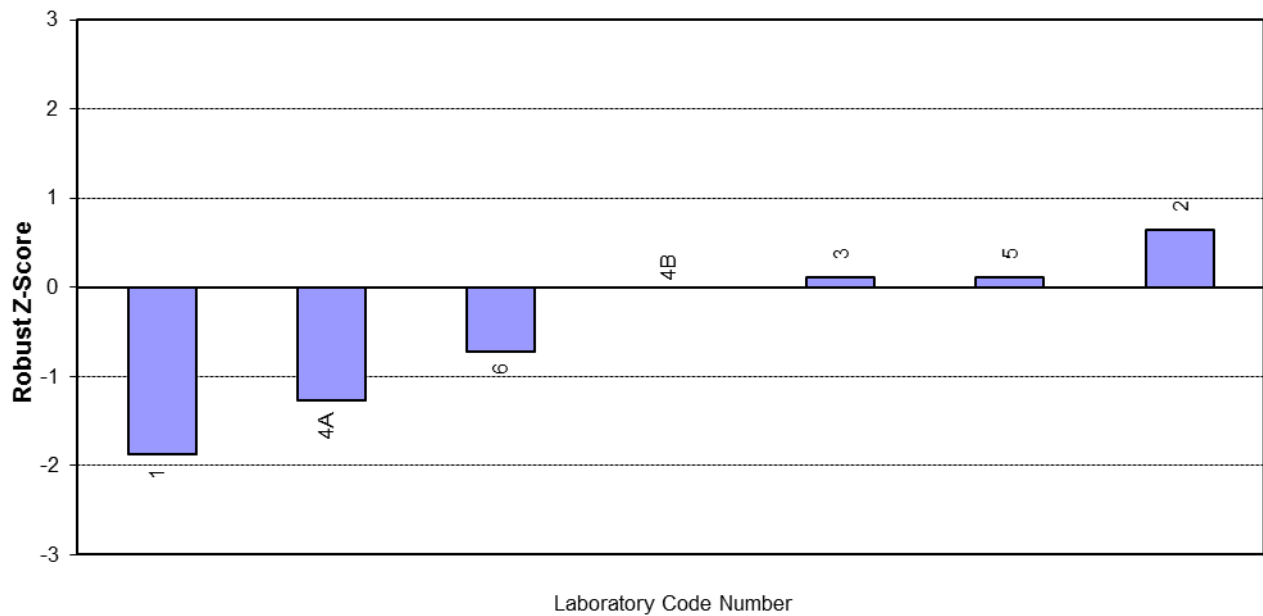
A10.2

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 26 February 2018. When reconstituted and added to the specified volume of sterile water, each sample was representative of a potable water 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, 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
23	1.3617	32	1.5051	16	1.2041	15	1.1761
20	1.3010	17	1.2304	9	0.9542	19	1.2788
23	1.3617	23	1.3617	15	1.1761	17	1.2304
18	1.2553	17	1.2304				
25	1.3979	18	1.2553				

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 61 – MARCH 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 potable 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*, 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 Monday 12th March 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

MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

ROUND 61 – MARCH 2018

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 1.0mL of vial contents to 1000mL sterile deionised (or distilled) water. This will leave 2.0mL 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.

PROFICIENCY TESTING AUSTRALIA



MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM

ROUND 61 MARCH 2018

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:
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)	MF					<input type="checkbox"/> AS/NZS 4276.9-2007 <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 --