

**Amended Report -  
REPORT NO. 1135B  
(Replaces Report 1135 issued May 2019)**

**MICROBIOLOGICAL WATERS ROUND 64**

**PROFICIENCY TESTING PROGRAM**

**JUNE 2019**

**ACKNOWLEDGMENTS**

PTA wishes to gratefully acknowledge the technical assistance provided for this program by Ms S Mott, Global Proficiency Ltd (New Zealand). This assistance included providing input into the design of the program, technical advice and discussion of the final report. PTA would also like to thank Ms S Giannoulidis, Global Proficiency Pty Ltd (Australia), who arranged for the supply of the samples and Global Proficiency Ltd (New Zealand) for the production of the samples.

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## 1. **FOREWORD**

This report summarises the results of a microbiological proficiency testing program on water. This report replaces report number 1135 due to an error in the Enterococci Membrane Filtration technique result for Laboratory Code 1B in Sample PTA 2. It constitutes the sixty-fourth 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 March 2019 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 seven separate laboratories received samples for the program with 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 (where a lab as reported more than one results for a test, letters have been placed after their code number).

Participants included laboratories from Australia 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 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) and Most Probable Number (MPN) tests, each of these tables contains the results returned by each laboratory, including the transformed log values and the z-score calculated for each sample. For the coliform tests via MPN, results from both traditional and enzyme substrate methods were analysed together (pooled).

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.  $z\text{-score} \leq -3.0$  or  $z\text{-score} \geq 3.0$ ). 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
<i>E. coli</i> orgs/100mL	MF	1	26	4.775	0.143
		2	26	4.450	0.134
	MPN	1	38	4.795	0.240
		2	38	4.530	0.227
Thermotolerant (Faecal) Coliforms orgs/100mL	MF	1	33	4.720	0.104
		2	32	4.440	0.100
	MPN	1	12	4.790	0.315
		2	11	4.540	0.259
Total Coliforms orgs/100mL	MF	1	17	4.780	0.145
		2	16	4.595	0.140
	MPN	1	33	4.800	0.240
		2	32	4.665	0.233
Enterococci orgs/100mL	MF	1	21	4.820	0.145
		2	20	4.490	0.135
	Enterolert	1	17	4.610	0.138
		2	17	4.300	0.129
Plate Count orgs/mL	All	1	10	3.565	0.100
		2	10	3.330	0.111

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<sub>10</sub> 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
<i>E. coli</i>	MF	-	-	-
	MPN	4, 7	-	6
Faecal Coliforms	MF	-	-	-
	MPN	4	-	6
Total Coliforms	MF	-	-	-
	MPN	4	-	6
Enterococci	MF	-	-	-
	Enterolert	1A	-	6
Plate Count	All	-	-	6

Note:

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

**6. PTA AND TECHNICAL ADVISER'S COMMENTS**

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

*E. coli* was the only coliform organism included in Sample PTA 1. Sample PTA 2 contained two coliform organisms; *E. coli* and *Citrobacter freundii* (*C. freundii*). *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.

For the Total Coliforms and *E. coli* tests, the Most Probable Number and Colilert tests were combined into one data set in this round and it is the intent that this continues for all subsequent rounds. There are several reasons this decision has been made and these are included below for your reference.

- Colilert Quanti-Tray is a multi-well method utilising MPN tables. Although the methodology is based on the use of proprietary defined-substrate technology to reduce the number of false-negative (and false-positive) results, it is Global Proficiency's experience that Coliform / *E. coli* results obtained from testing proficiency samples from both traditional MPN and Colilert Quanti-Tray methods compare well.
- Multiple-tube procedures allow a range of tube and dilution combinations read from different tables, and the choice is dependent on the water type and expected level of contamination. APHA Standard Methods for the Examination of Water and Wastewater 23<sup>rd</sup> Edition, Chapters 9221 (Multiple-tube Fermentation Technique for Members of the Coliform group) and 9223 (Enzyme Substrate Coliform test) both allow combinations, some of which are listed below:
  - 5 tubes x 20mL sample (*Traditional & Enzyme Substrate*)
  - 10 tubes x 10mL sample (*Traditional & Enzyme Substrate*)
  - 15 tubes (5 tubes x 3 dilutions) (10mL, 1mL, 0.1mL) (*Traditional & Enzyme Substrate*)
  - 51-well Quanti-Tray (*IDEXX products only*)
  - 97-well Quanti-Tray/2000 (*IDEXX products only*)

In addition, there are several different types and brands of both traditional and enzyme-hydrolysable substrate media available for use with the first three combinations listed above.

So, a laboratory may choose to apply one of the combinations above using traditional methodology or may decide to utilise enzyme substrate methodology instead. Upon submission of results into a proficiency round, it is not always apparent which methodology or tube combination has been used, unless the method referenced is specific, which also supports combining all MPN results for Coliform / *E. coli* testing.

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:**

For Total Coliforms, one laboratory reported results for the MF technique and six laboratories reported results for the MPN technique with laboratory code 1 reporting two sets of results for the MPN technique i.e. MPN (1A) and Colilert (1B). One participant (Laboratory cod 4) recorded outliers using the MPN technique with results lower than expected for both samples. It is recommended that results classified as outliers are investigated further.

Laboratory code 6 reported incomplete results for the MPN method for both samples, submitted as ">2005 MPN/100mL" (via Colilert-18, 51 wells). Given the medians were 4.800 (63,000) MPN/100mL and 4.665 (46,000) MPN/100mL for sample PTA 1 and 2 respectively, it is evident insufficient dilutions were performed for a simulated effluent sample.

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 following equation:

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

<b>Total Coliforms via:</b>	<b>Sample PTA 1</b> Median ± Uncertainty (Log <sub>10</sub> cfu/100mL)	<b>Sample PTA 2</b> Median ± Uncertainty (Log <sub>10</sub> cfu/100mL)
Membrane Filtration	4.780 ± 0.044	4.595 ± 0.044
Most Probable Number	4.800 ± 0.052	4.665 ± 0.052

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

**Measurement Uncertainty: Total Coliforms**

Two laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as ± log values by both laboratories.

Graphs showing the differentiation of methods used for Total Coliform testing are included on the following page. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data for the methods listed above.

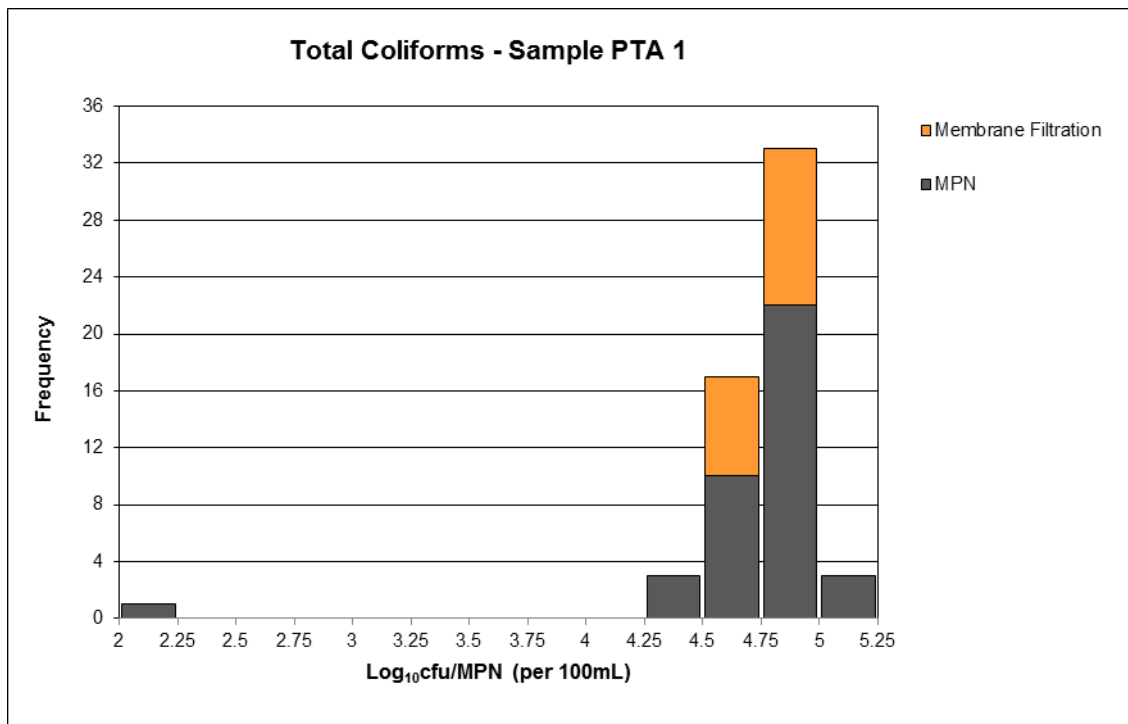


Figure TA-1. Total Coliform results for Sample PTA 1

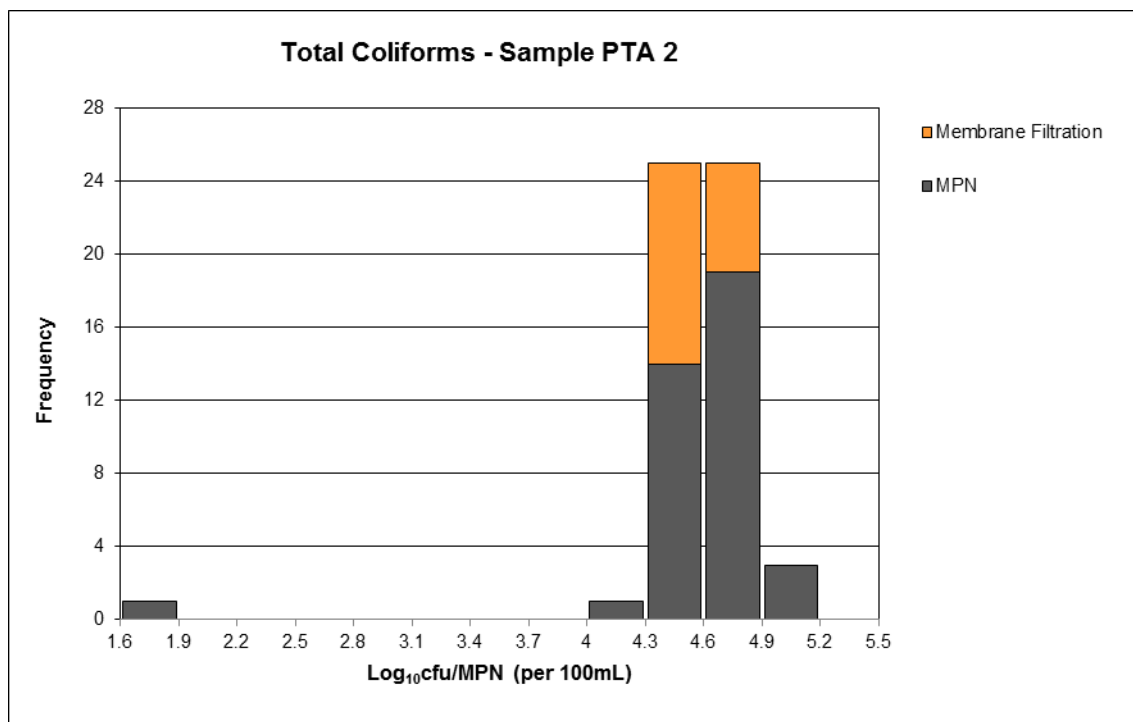


Figure TA-2. Total Coliform results for Sample PTA 2

***E. coli:***

For *E. coli*, two laboratories reported results for the MF technique and six laboratories reported results for the MPN technique with laboratory code 1 reporting two sets of results for the MPN technique i.e. MPN (1A) and Colilert (1B). Two participants (Laboratory codes 4 and 7) recorded outliers for the MPN technique. Laboratory code 4 recorded an outlier for both samples with results lower than expected. Laboratory code 7 recorded an outlier for Sample PTA 1 with results lower than expected.

Laboratory code 6 reported incomplete results for the MPN method for both samples, submitted as “>2005 MPN/100mL” (via Colilert-18, 51 wells). Given the medians were 4.795 (62,000) MPN/100mL and 4.530 (34,000) MPN/100mL for sample PTA 1 and 2 respectively, it is evident insufficient dilutions were performed for a simulated effluent sample.

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 7 of this report), which was calculated for each test and/or method within a test.

<b><i>E. coli</i> via:</b>	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)
Membrane Filtration	4.775 $\pm$ 0.035	4.450 $\pm$ 0.033
Most Probable Number	4.795 $\pm$ 0.049	4.530 $\pm$ 0.046

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

**Measurement Uncertainty: *E. coli***

Two laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values by both laboratories.

Graphs showing the differentiation of methods used for *E. coli* testing are included on the following page. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data for the methods listed above.

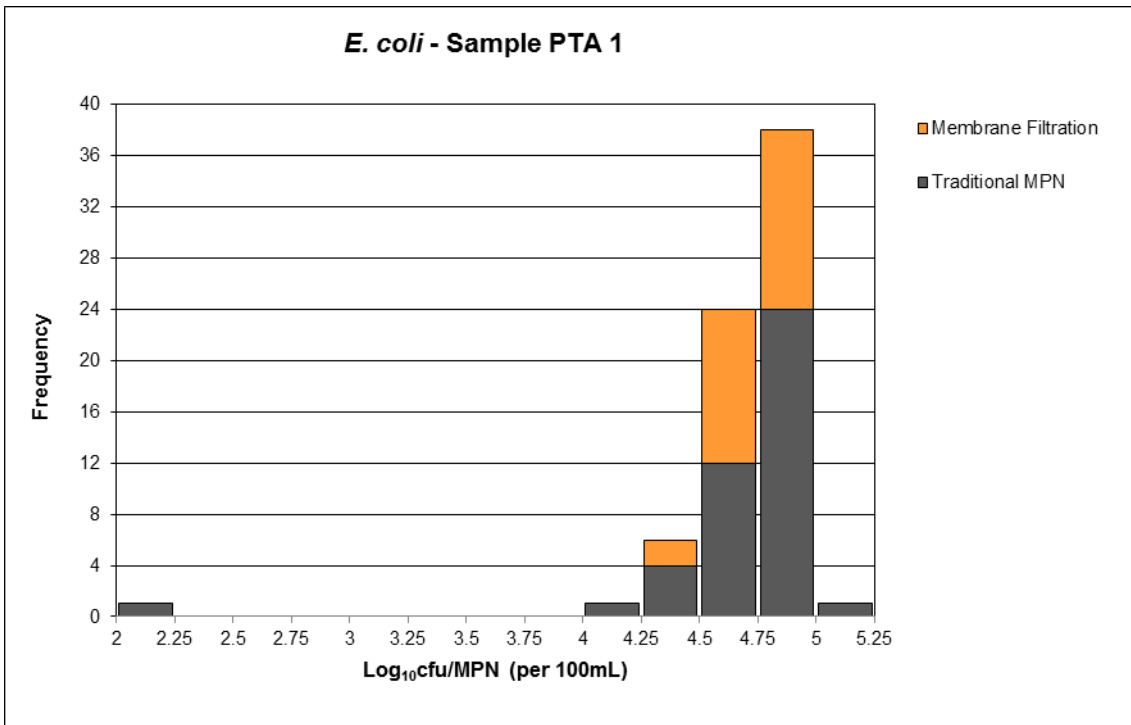


Figure TA-3. *E. coli* results for Sample PTA 1.

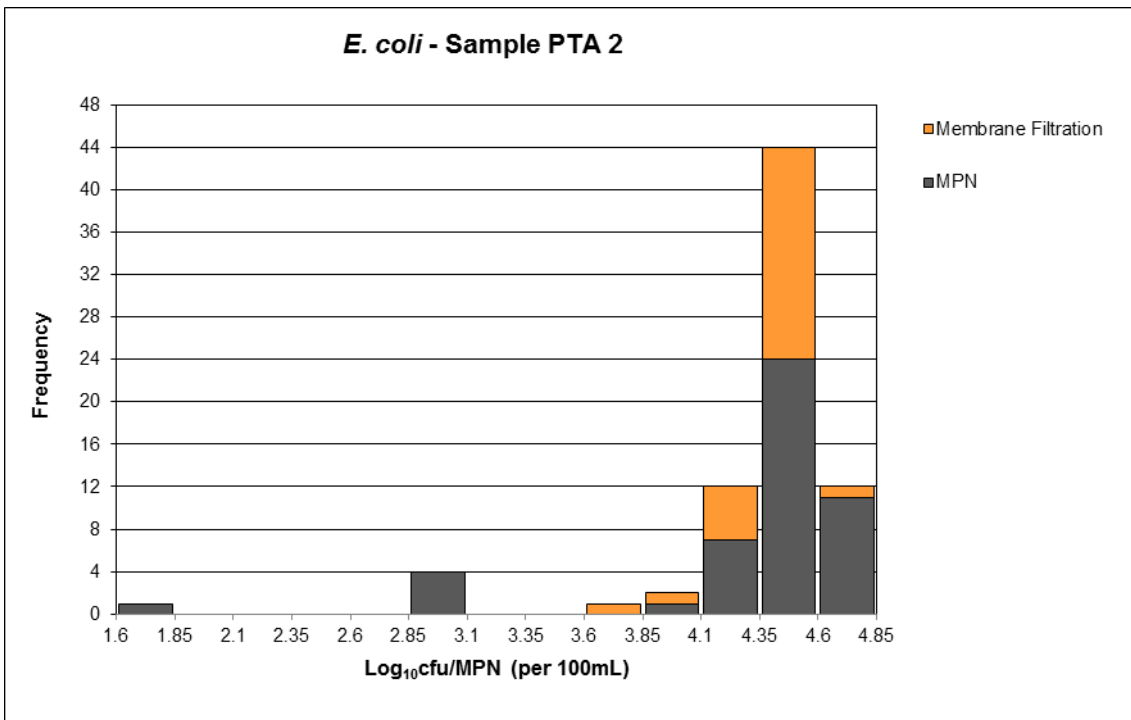


Figure TA-4. *E. coli* results for Sample PTA 2.

**Thermotolerant (Faecal) Coliforms:**

For Faecal Coliforms, two laboratories reported results for the MF technique and five laboratories reported results for the MPN technique. One participant (Laboratory code 4) recorded an outlier for the MPN technique for both samples with results lower than expected.

Laboratory code 6 reported incomplete results for the MPN method for both samples, submitted as “>24196 MPN/100mL” (via Colilert-18, 97 wells). Given the medians were 4.790 (62,000) MPN/100mL and 4.540 (35,000) MPN/100mL for sample PTA 1 and 2 respectively, it is evident insufficient dilutions were performed for a simulated effluent sample.

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 7 of this report), which was calculated for each test and/or method within a test.

<b>Faecal Coliforms via:</b>	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)
Membrane Filtration	4.720 $\pm$ 0.023	4.440 $\pm$ 0.022
Most Probable Number	4.790 $\pm$ 0.114	4.540 $\pm$ 0.098

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

**Measurement Uncertainty: Faecal Coliforms:**

Two laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values in by both laboratories.

Graphs showing the differentiation of methods used for Thermotolerant (Faecal) Coliform testing are included on the following page. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data for the methods listed above.

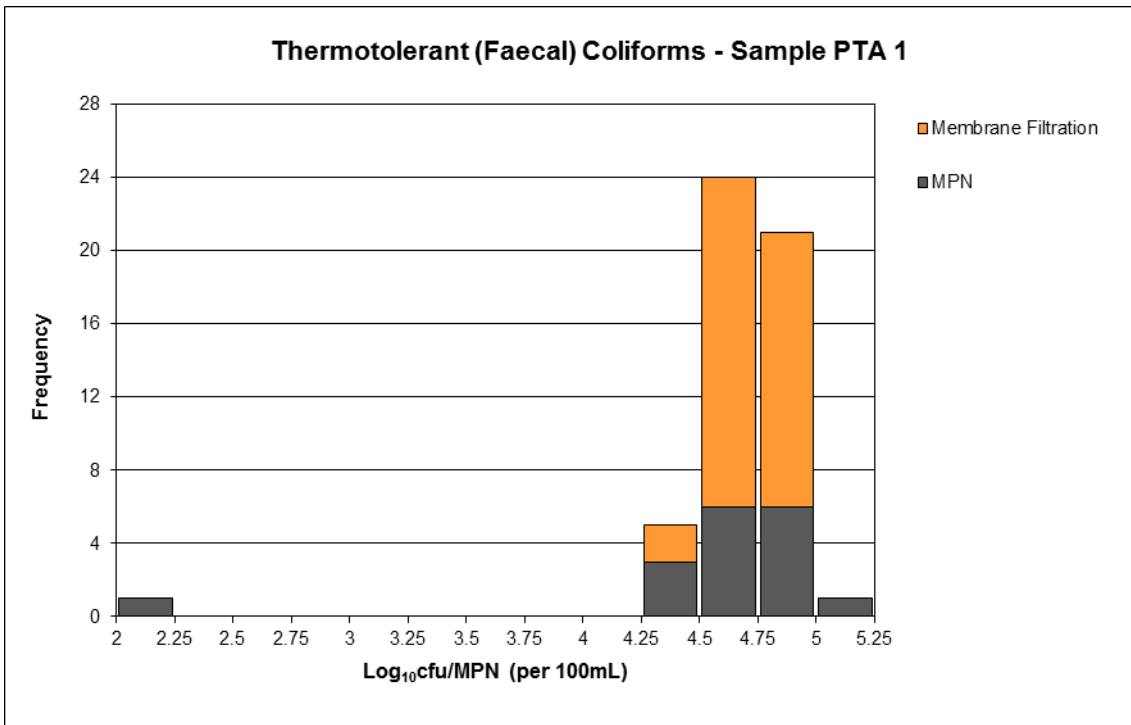


Figure TA-5. Thermotolerant (Faecal) Coliforms results for Sample PTA 1.

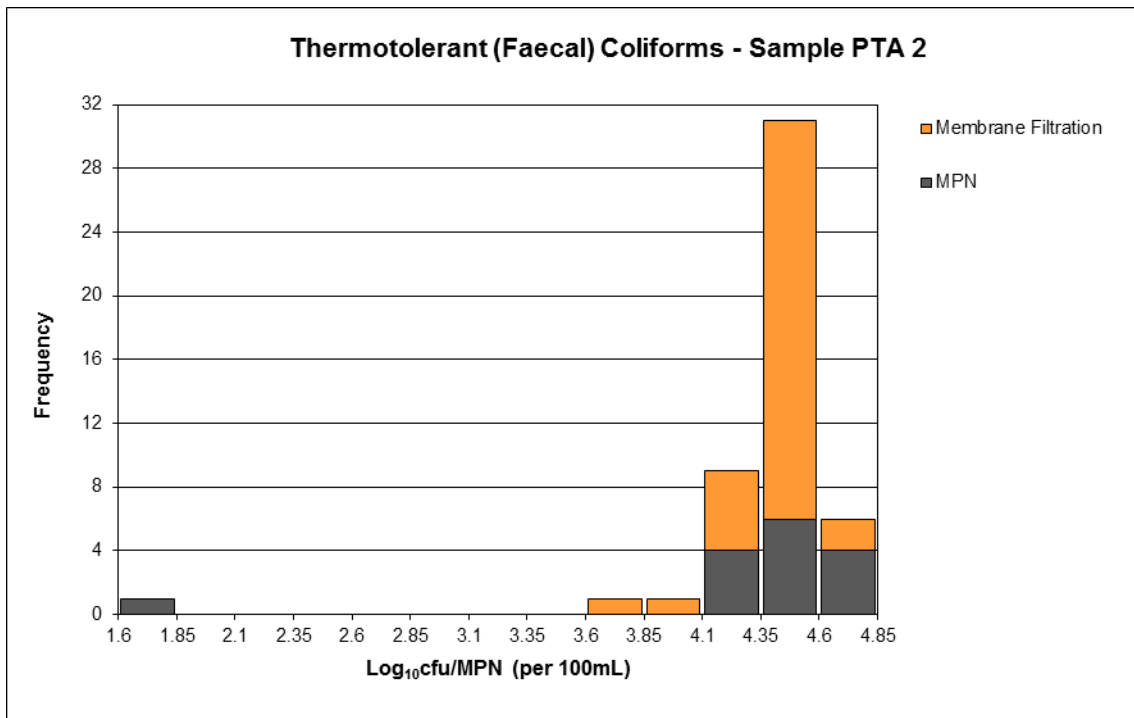


Figure TA-6. Thermotolerant (Faecal) Coliforms results for Sample PTA 2.

**Enterococci:**

For Enterococci, three laboratories reported results for the MF technique with laboratory 1 reporting 2 sets of results using two different methods (1A) and (1B), and two laboratories reported results for the Enterolert technique. One participant (Laboratory code 1) recorded outliers for the Enterolert technique for both samples with results lower than expected.

Laboratory code 6 reported incomplete results for the MPN method for both samples, submitted as ">2005 MPN/100mL" (via Enterolert, 51 wells). Given the medians were 4.300 (20,000) MPN/100mL and 4.610 (41,000) MPN/100mL for sample PTA 1 and 2 respectively, it is evident insufficient dilutions were performed for a simulated effluent sample.

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 7 of this report), which was calculated for each test and/or method within a test.

<b>Enterococci via:</b>	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/100mL)
Membrane Filtration	4.820 $\pm$ 0.040	4.490 $\pm$ 0.038
Enterolert	4.610 $\pm$ 0.042	4.300 $\pm$ 0.039

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

**Measurement Uncertainty: Enterococci:**

Two laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values by both laboratories.

Of the reported MUs for the Enterococci tests, 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 code 1A may need to re-examine their test results or their MU calculations for the Enterolert 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 Enterococci testing are included below. These graphs show the distribution of results from the two methods used in this round and include Global Proficiency Ltd and PTA data for the methods listed above.

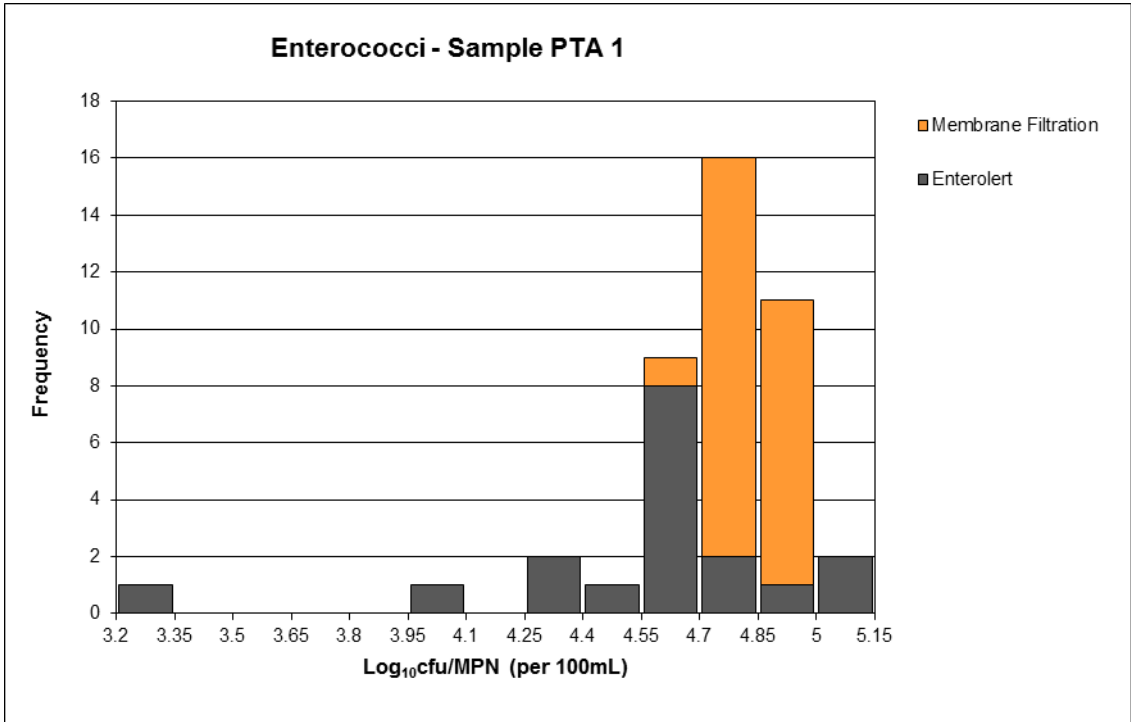


Figure TA-7. Enterococci results for Sample PTA 1

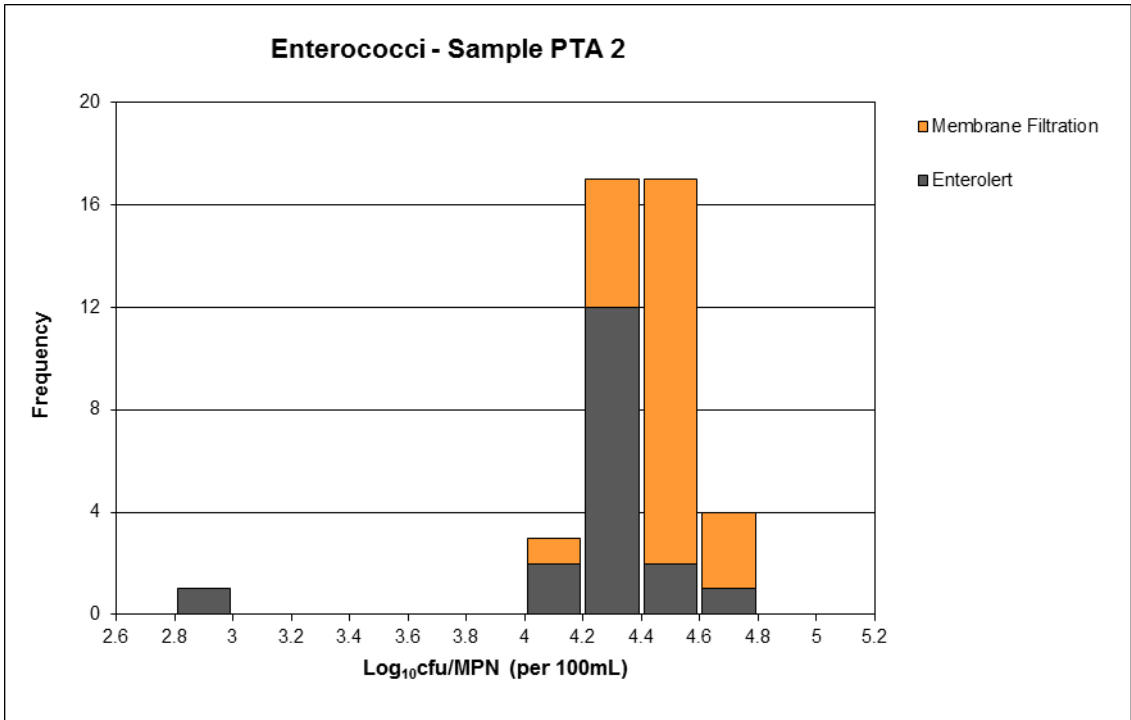


Figure TA-8. Enterococci results for Sample PTA 2



**Plate Count:**

Six sets of results were submitted for the Plate Count test. Two sets of results were obtained using the Australian/New Zealand Standard method (AS/NZS 4276.3.1); one set of results was obtained using an APHA method; one a BAM method, one used SimPlate and one laboratory used Standard Methods with R2A filtration.

Laboratory code 6 reported incomplete results for the SimPlate method (84 wells) for both samples, submitted as ">738 MPN/mL". Given the medians were 3.565 (3,700) cfu/mL and 3.330 (2,100) cfu/mL for sample PTA 1 and 2 respectively, it is evident insufficient dilutions were performed for a simulated effluent sample.

No outliers were recorded for this test.

Confidence in the medians can be expressed as the Uncertainty of the Median (as defined on page 7 of this report), which was calculated for each test and/or method within a test.

	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/mL)
<b>Plate Count:</b>	3.565 $\pm$ 0.040	3.330 $\pm$ 0.044

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

**Measurement Uncertainty: Plate Count:**

Two laboratories reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values by both laboratories.

Of the reported MUs for the Plate Count test, 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 code 3 may need to re-examine their test results or their MU calculations for the pour plate method as their results for PTA 1 and the stated uncertainty was outside the expected range of the median and its associated uncertainty.

### **General Comments**

A total of 72 results were submitted for analysis in this round. Of these results 9 (13%) were outlier results. This is higher than the 8% of results which were outlier results in the previous effluent water round (Round 62).

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](http://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, 23<sup>rd</sup> 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, 23<sup>rd</sup> Edition (2017)*

# **APPENDIX A**

**Tables of Results and Z-Scores,**

**Summary Statistics**

**and**

**Graphical Displays**

# **SECTIONS A1 to A2**

***E. coli***

## A1.1

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

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	54000	±0.12 log <sub>10</sub>	22000	±0.12 log <sub>10</sub>	4.73	4.34	-0.30	-0.80
3	41000	0.20	27000	0.20	4.61	4.43	-1.13	-0.14

### **Summary Statistics**

#### ***Sample - PTA 1***

No. of Results	26
Median	4.775
Norm IQR	0.143
Robust CV*	3.0%
Minimum	4.28
Maximum	4.90
Range	0.62
Uncertainty (Median)	0.035

#### ***Sample - PTA 2***

No. of Results	26
Median	4.450
Norm IQR	0.134
Robust CV*	3.0%
Minimum	3.78
Maximum	4.66
Range	0.88
Uncertainty (Median)	0.033

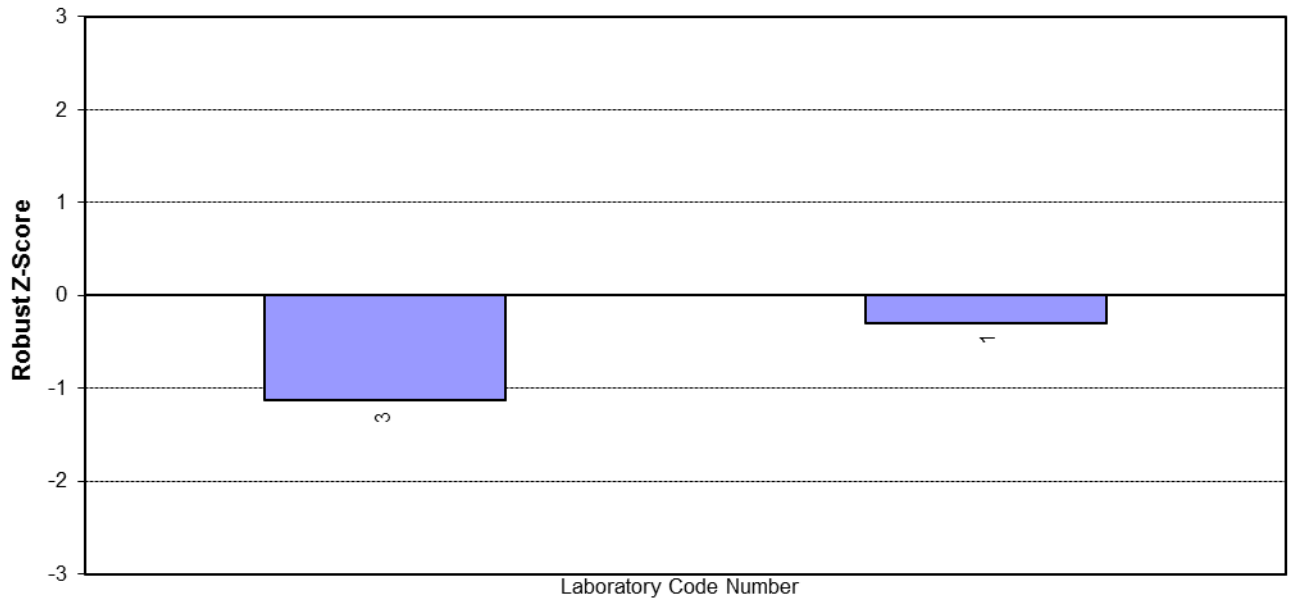
**Note:**

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 = 2.1% for sample PTA 1 and 1.8% 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].

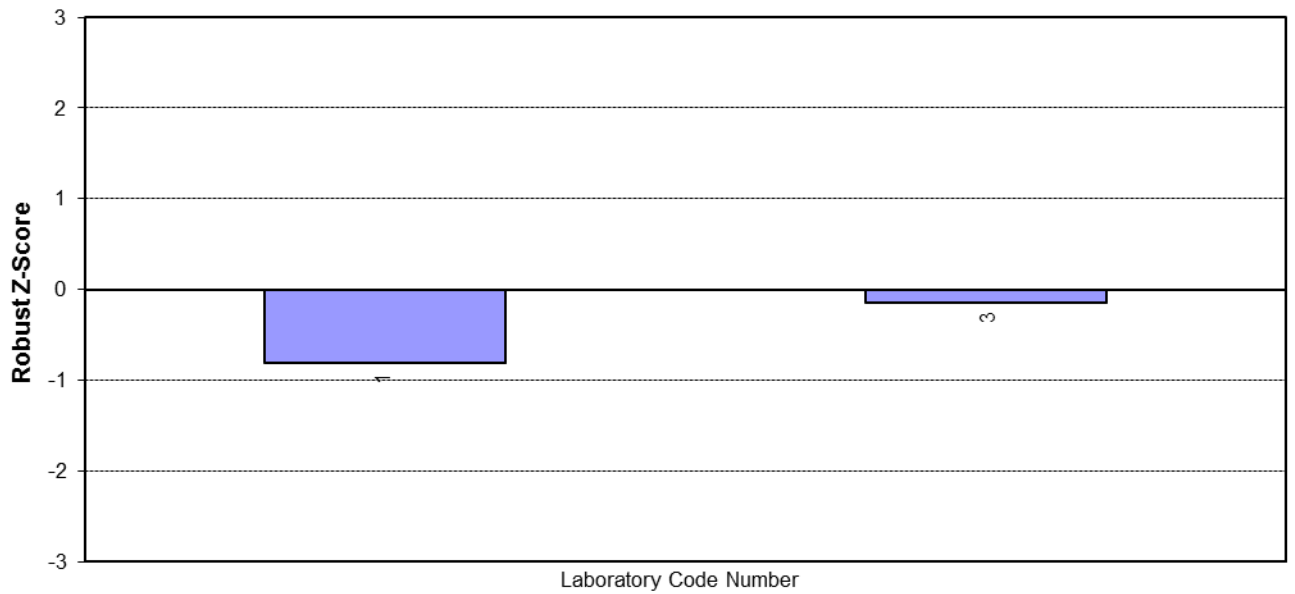
A1.2

***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 <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1A	24000	±0.59 log <sub>10</sub>	13000	±0.59 log <sub>10</sub>	4.38	4.11	-1.73	-1.83
1B	52000	±0.17 log <sub>10</sub>	26000	±0.17 log <sub>10</sub>	4.72	4.41	-0.33	-0.51
3	35000	0.44	17000	0.44	4.54	4.23	-1.05	-1.32
4	110		46		2.04	1.66	-11.47 §	-12.63 §
5	63000		49000		4.80	4.69	0.02	0.71
6	>2005		>2005		N/A	N/A	N/A	N/A
7	11000		17000		4.04	4.23	-3.14 §	-1.32

**Notes:**

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. N/A - Not Applicable

**Summary Statistics**

**Sample - PTA 1**

No. of Results	38
Median	4.795
Norm IQR	0.240
Robust CV*	5.0%
Minimum	4.35
Maximum	5.23
Range	0.88
Uncertainty (Median)	0.049

**Sample - PTA 2**

No. of Results	38
Median	4.530
Norm IQR	0.227
Robust CV*	5.0%
Minimum	4.04
Maximum	4.83
Range	0.79
Uncertainty (Median)	0.046

**Notes:**

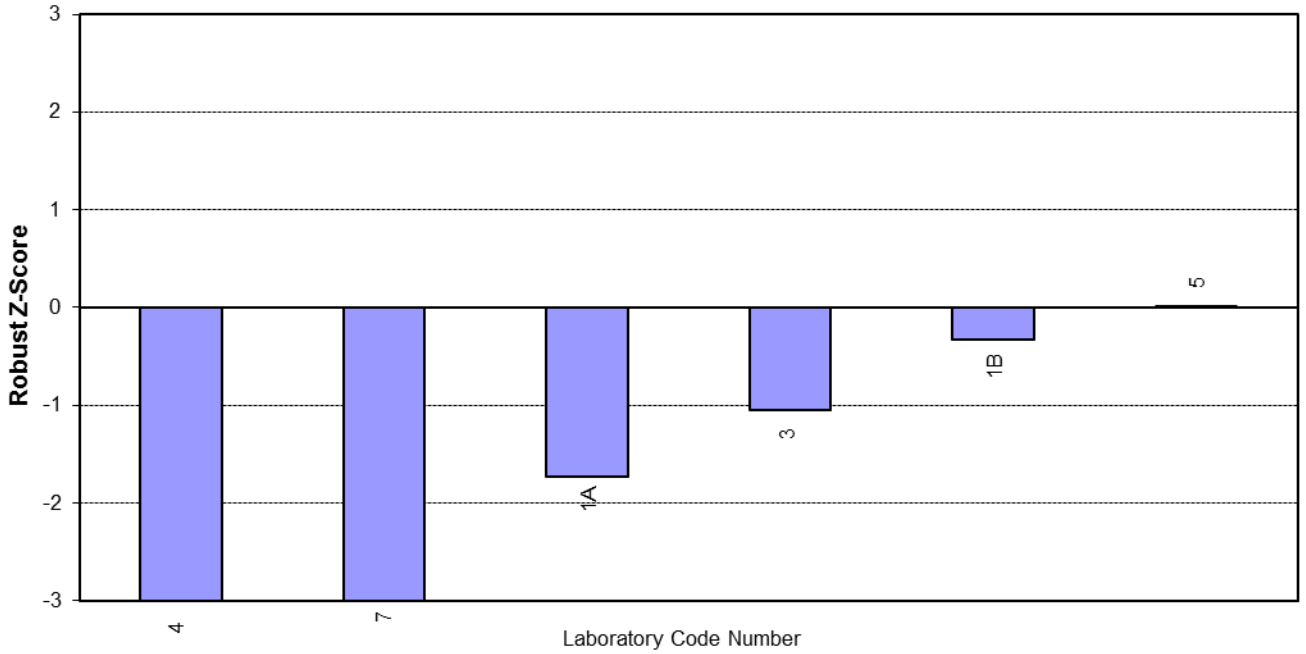
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).
2. \*The robust CV's achieved for both samples were considered low for an MPN test (robust CV = 3.9% for sample PTA 1 and 3.4% for sample PTA 2) so in this case a target robust CV of 5.0% 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].



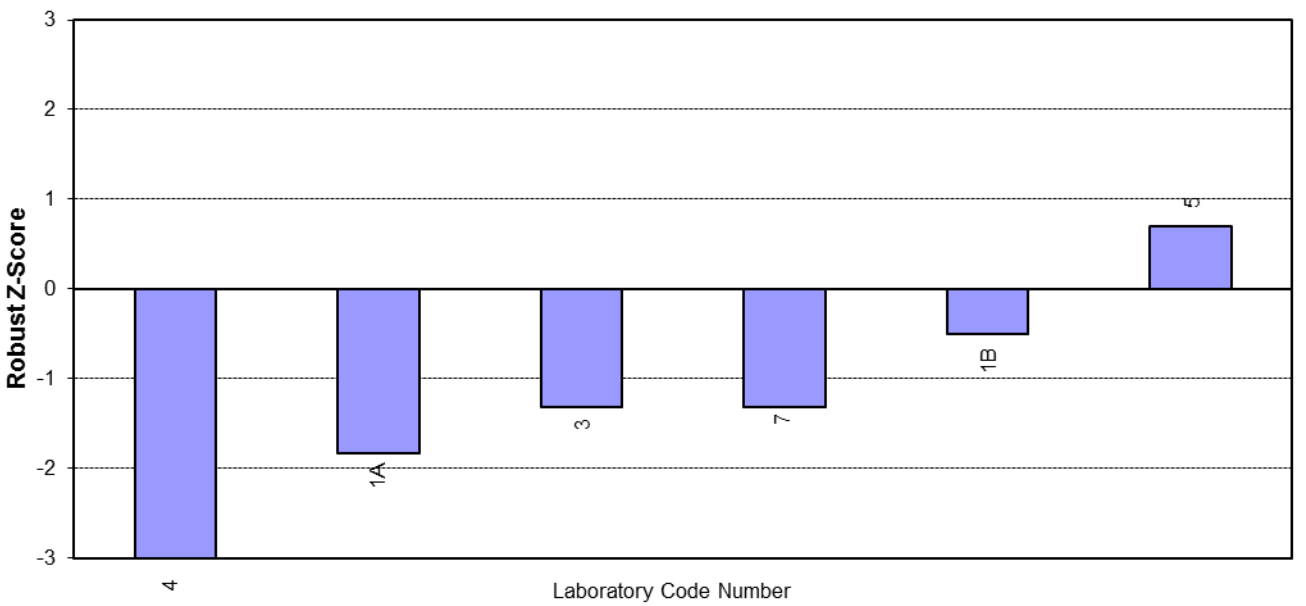
A2.2

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

**Sample - PTA 1**



**Sample - PTA 2**



## **SECTIONS A3 to A4**

### **Thermotolerant (Faecal) Coliforms**

### A3.1

#### Thermotolerant (Faecal) Coliforms (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	54000	±0.12 log <sub>10</sub>	22000	±0.12 log <sub>10</sub>	4.73	4.34	0.12	-0.98
3	41000	0.13	27000	0.13	4.61	4.43	-1.03	-0.09

#### Summary Statistics Sample - PTA 1

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

#### Sample - PTA 2

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

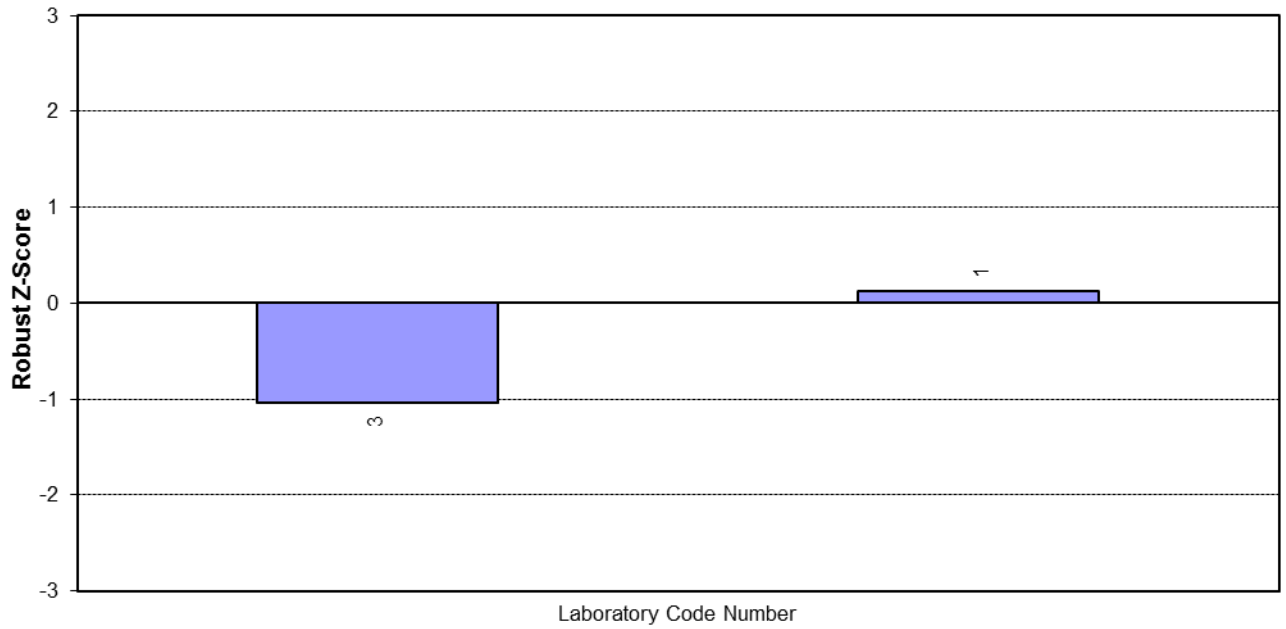
**Note:**

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).

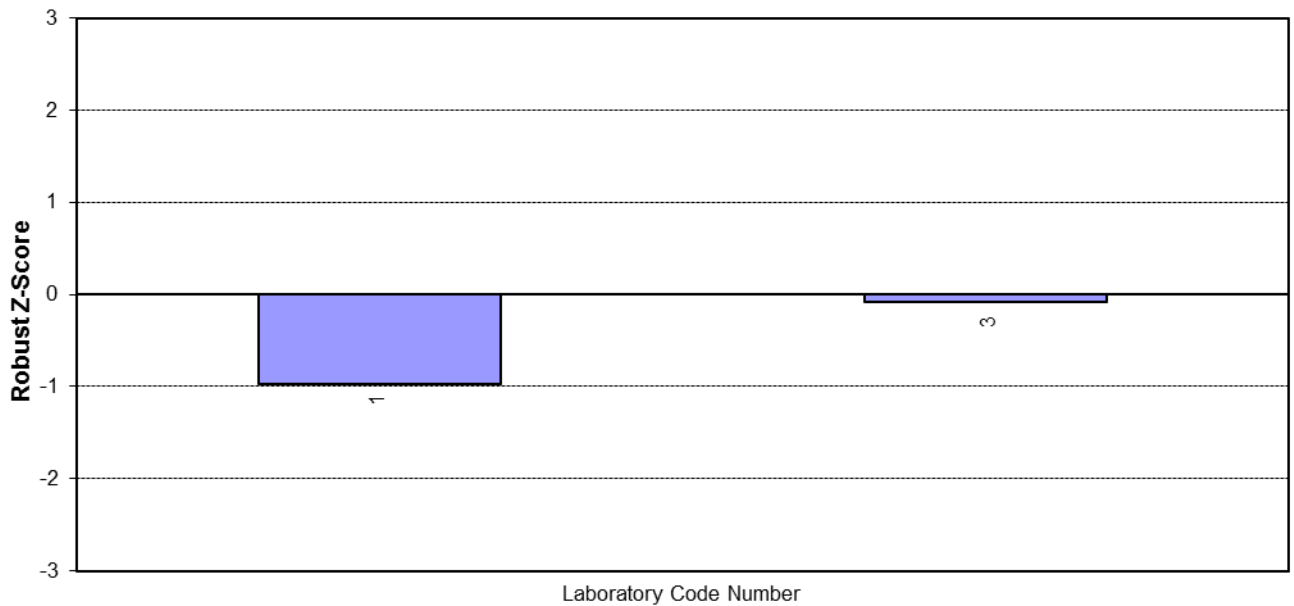
A3.2

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

Sample - PTA 1



Sample - PTA 2



## A4.1

### Thermotolerant (Faecal) Coliforms (orgs/100mL) – MPN Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	24000	±0.59 log <sub>10</sub>	13000	±0.59 log <sub>10</sub>	4.38	4.11	-1.30	-1.64
3	35000	0.32	17000	0.32	4.54	4.23	-0.78	-1.19
4	110		46		2.04	1.66	-8.72 §	-11.09 §
5	56000		25000		4.75	4.40	-0.13	-0.55
6	>24196		>24196		N/A	N/A	N/A	N/A

**Notes:**

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. N/A - Not Applicable

### Summary Statistics

#### Sample - PTA 1

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

#### Sample - PTA 2

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

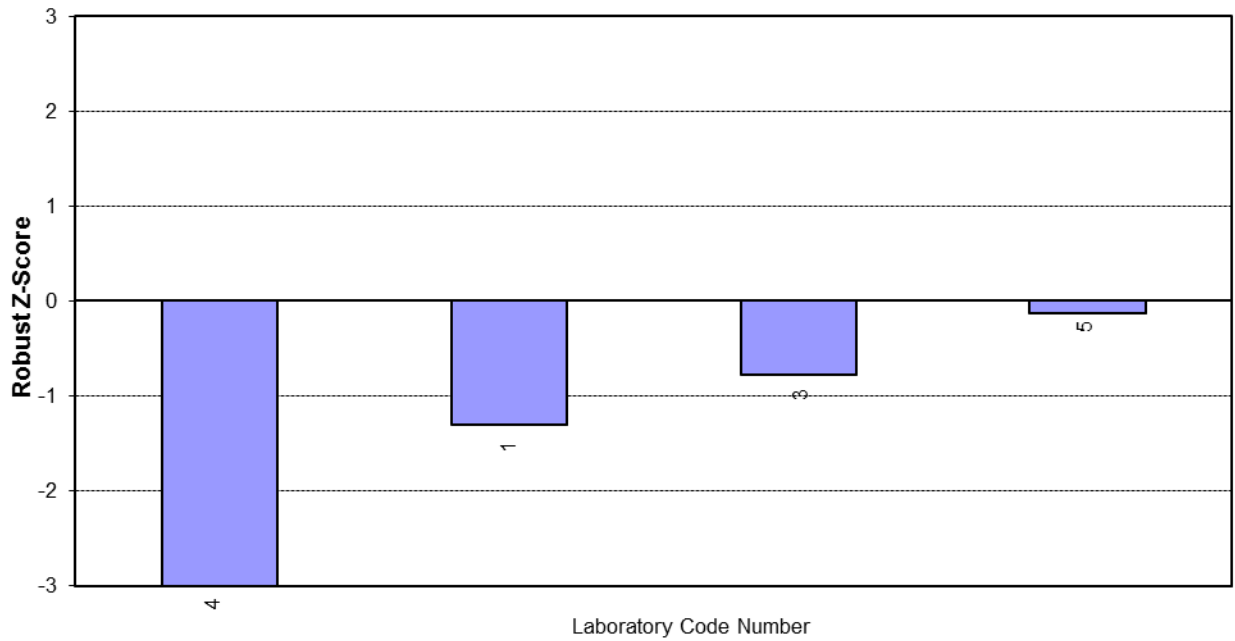
**Note:**

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).

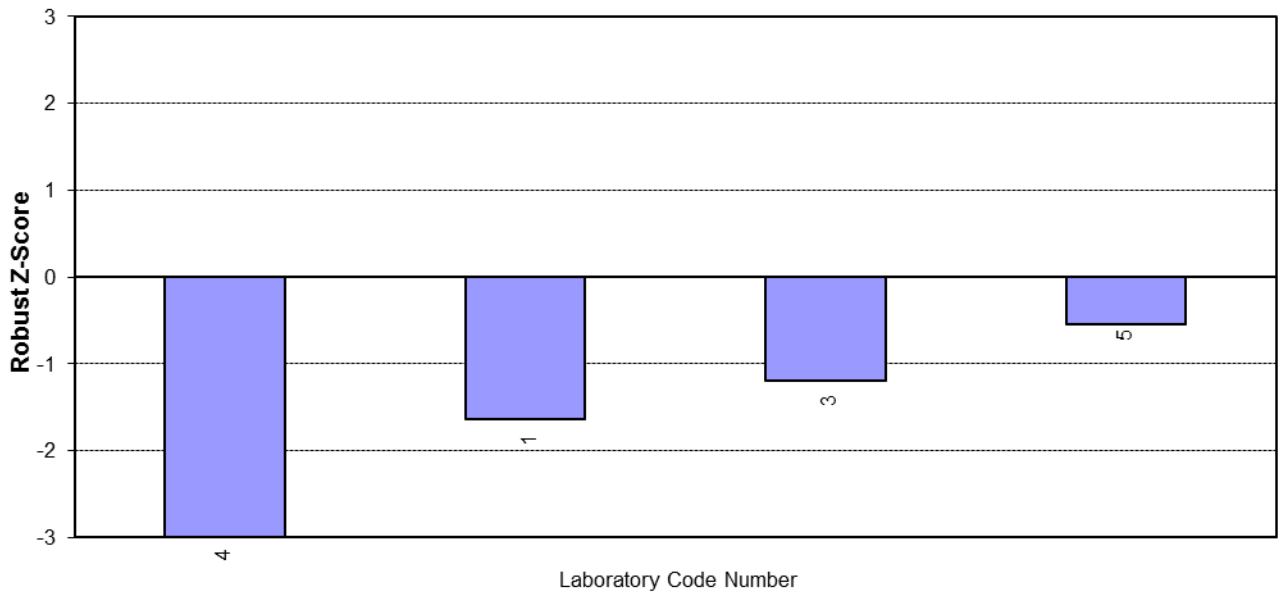
A4.2

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

Sample - PTA 1



Sample - PTA 2



## **SECTIONS A5 to A6**

### **Total Coliforms**

## A5.1

### Total Coliforms (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
3	39000	0.15	23000	0.15	4.59	4.36	-1.30	-1.67

### Summary Statistics

#### *Sample - PTA 1*

No. of Results	17
Median	4.780
Norm IQR	0.145
Robust CV*	3.0%
Minimum	4.72
Maximum	4.96
Range	0.24
Uncertainty (Median)	0.044

#### *Sample - PTA 2*

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

**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].
3. As only one result as been reported, the ordered z-score chart for this test has not been included.



**A6.1**  
**Total Coliforms (orgs/100mL) – MPN Technique**

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1A	24000	±0.71 log <sub>10</sub>	13000	±0.71 log <sub>10</sub>	4.38	4.11	-1.75	-2.37
1B	52000	±0.16 log <sub>10</sub>	44000	±0.16 log <sub>10</sub>	4.72	4.64	-0.35	-0.09
3	35000	0.32	92000	0.32	4.54	4.96	-1.07	1.28
4	110		46		2.04	1.66	-11.49 §	-12.89 §
5	63000		65000		4.80	4.81	0.00	0.63
6	>2005		>2005		N/A	N/A	N/A	N/A
7	160000		54000		5.20	4.73	1.68	0.29

**Notes:**

- § denotes an outlier (i.e. |z-score| ≥ 3.0).
- N/A - Not Applicable.

**Summary Statistics**  
**Sample - PTA 1**

No. of Results	33
Median	4.800
Norm IQR	0.240
Robust CV*	5.0%
Minimum	4.38
Maximum	5.23
Range	0.85
Uncertainty (Median)	0.052

**Sample - PTA 2**

No. of Results	32
Median	4.665
Norm IQR	0.233
Robust CV	5.0%
Minimum	4.38
Maximum	4.99
Range	0.61
Uncertainty (Median)	0.052

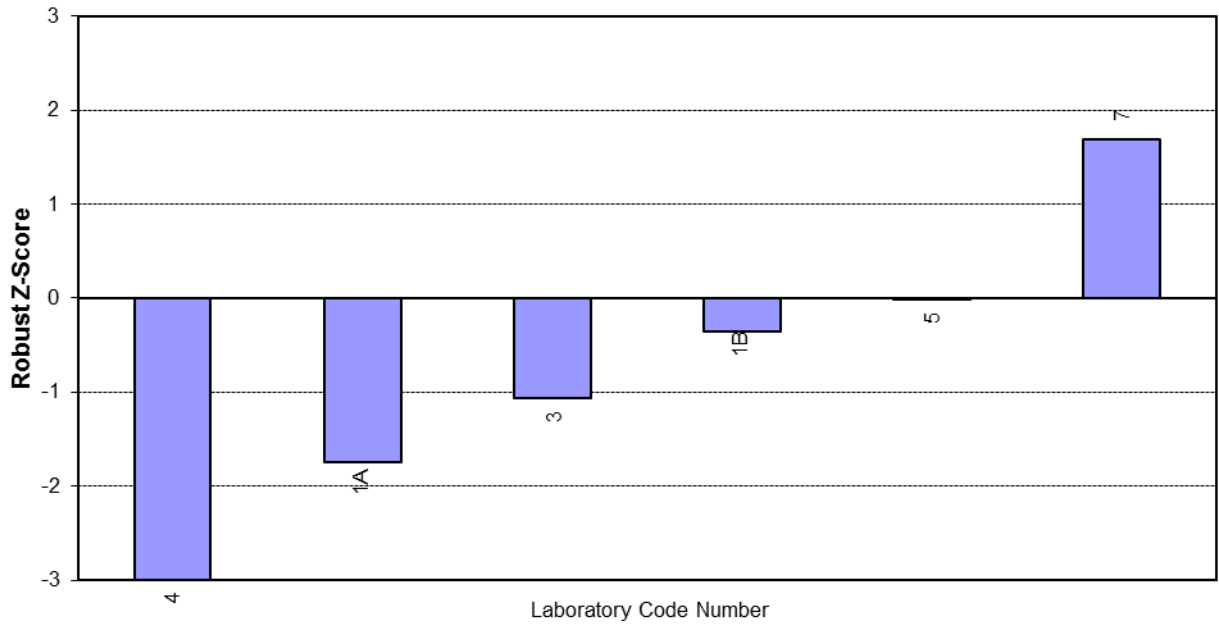
**Notes:**

- 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).
- \*The robust CV's achieved for both samples were considered low for an MPN test (robust CV = 3.4% for sample PTA 1 and 3.0% for sample PTA 2) so in this case a target robust CV of 5.0% 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].

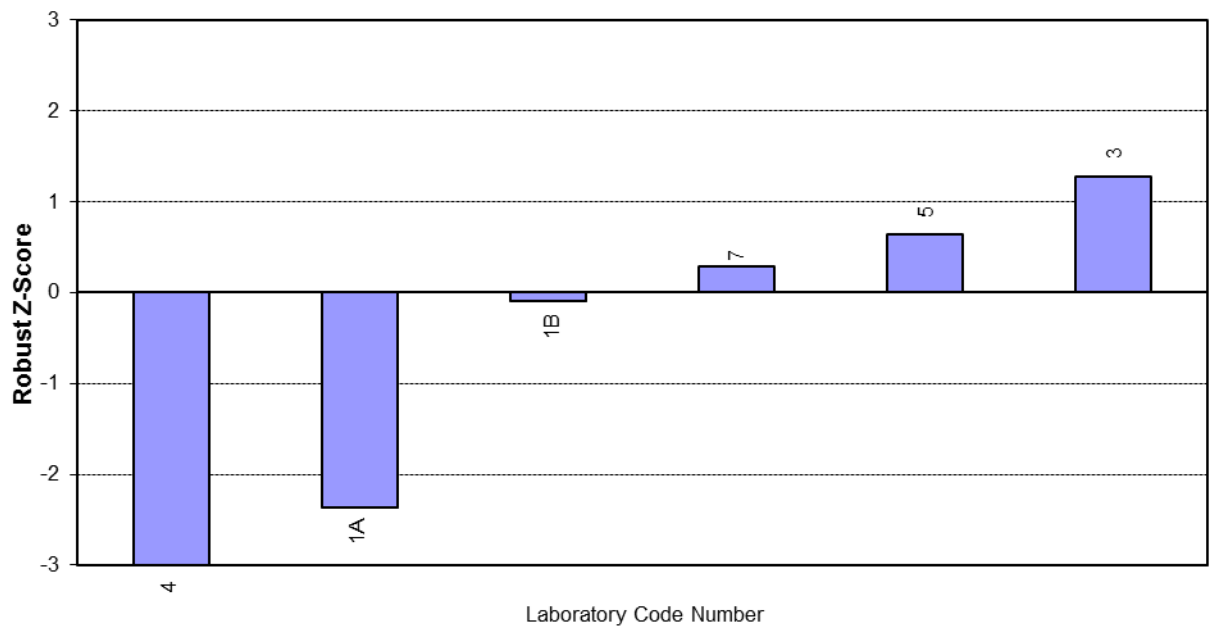
A6.2

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

Sample - PTA 1



Sample - PTA 2



## **SECTION A7 - A8**

### **Enterococci**

## A7.1

### Enterococci (orgs/100mL) – MF Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1A	62000	±0.29 log <sub>10</sub>	25000	±0.29 log <sub>10</sub>	4.79	4.40	-0.19	-0.68
1B	72000	±0.22 log <sub>10</sub>	35000	±0.22 log <sub>10</sub>	4.86	4.54	0.26	0.40
3	53000	0.10	37000	0.10	4.72	4.57	-0.66	0.58
5	64000		33000		4.81	4.52	-0.10	0.21

**Note:**

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

#### Summary Statistics

##### *Sample - PTA 1*

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

##### *Sample - PTA 2*

No. of Results	20
Median	4.490
Norm IQR	0.135
Robust CV*	5.0%
Minimum	4.00
Maximum	4.67
Range	0.67
Uncertainty (Median)	0.038

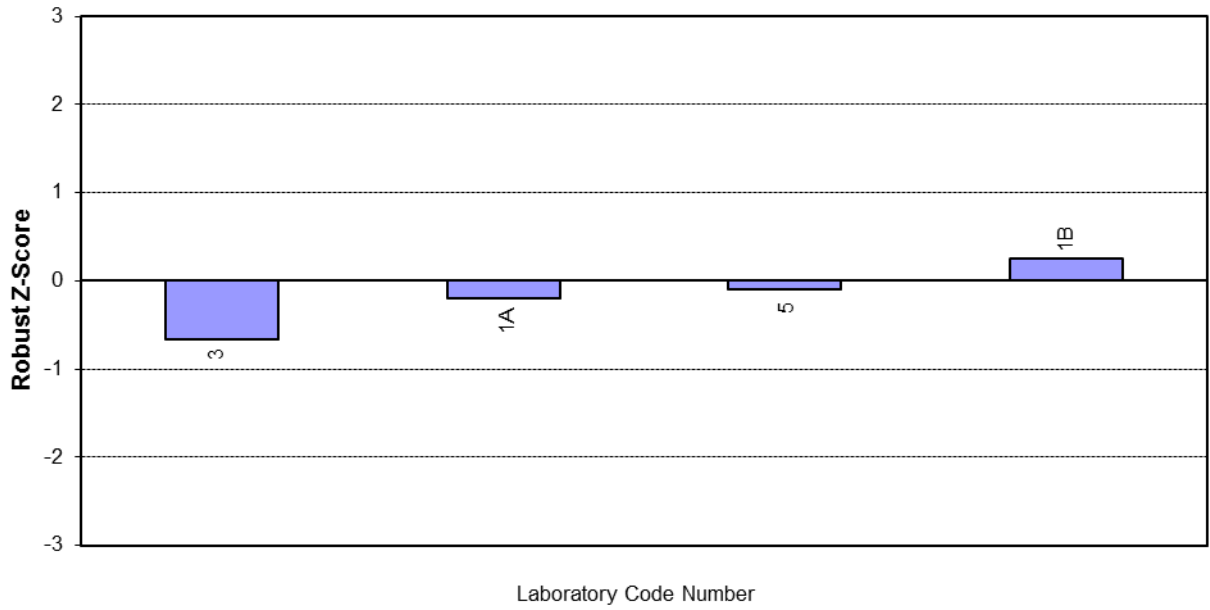
**Note:**

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 = 1.5% for sample PTA 1 and 2.5% 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].

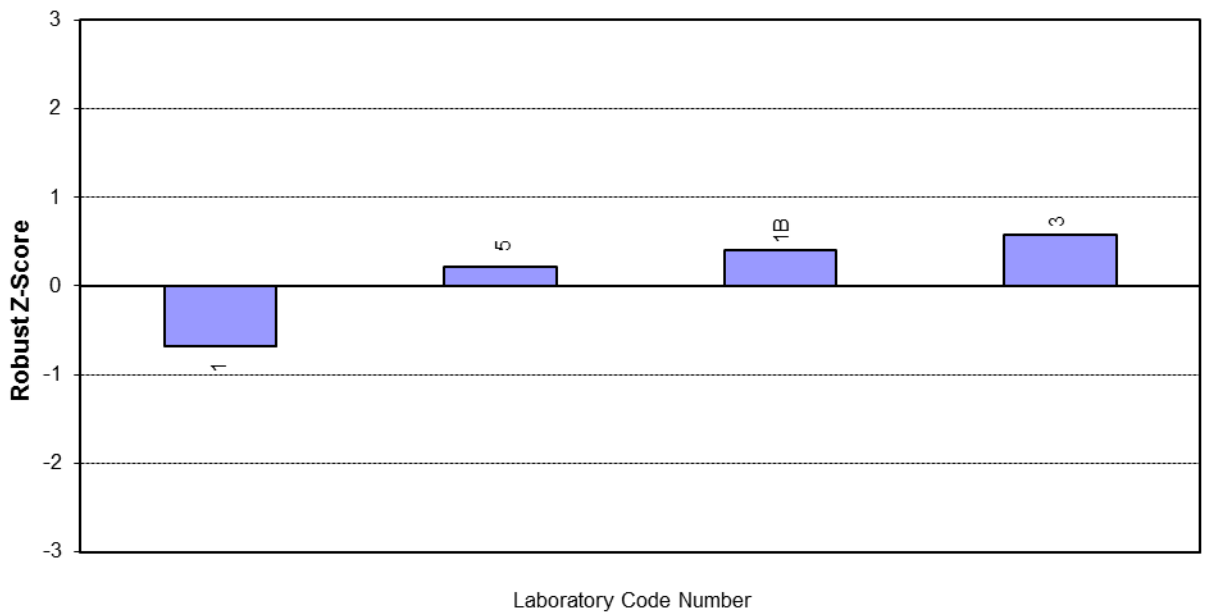
A7.2

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

Sample - PTA 1



Sample - PTA 2



## A8.1

### Enterococci (orgs/100mL) – Enterolert Technique

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	1700	±0.38 log <sub>10</sub>	790	±0.38 log <sub>10</sub>	3.23	2.90	-9.85 §	-10.79 §
6	>2005		>2005		N/A	N/A	N/A	N/A

**Notes:**

1. § denotes an outlier (i.e. |z-score| ≥ 3.0).
2. N/A - Not Applicable.

#### Summary Statistics

##### *Sample - PTA 1*

No. of Results	17
Median	4.610
Norm IQR	0.138
Robust CV*	3.0%
Minimum	3.97
Maximum	5.11
Range	1.14
Uncertainty (Median)	0.042

#### Summary Statistics

##### *Sample - PTA 2*

No. of Results	17
Median	4.300
Norm IQR	0.129
Robust CV*	3.0%
Minimum	4.11
Maximum	4.64
Range	0.53
Uncertainty (Median)	0.039

**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 robust CV's achieved for both samples were low (robust CV = 2.3% for sample PTA 1 and 1.4% 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].
3. As only one numerical result has been reported, the ordered z-score chart for this test has not been included.

## **SECTION A9**

**Plate Count  
All Techniques**

## A9.1

### Plate Count (orgs/mL) – All Techniques

Lab Code	PTA 1 Result	MU	PTA 2 Result	MU	PTA 1 log <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	3600	±0.17 log <sub>10</sub>	2200	±0.17 log <sub>10</sub>	3.56	3.34	-0.09	0.11
3	1900	0.07	1600	0.07	3.28	3.20	-2.86	-1.13
4	3100		2060		3.49	3.31	-0.73	-0.15
5	3600		1800		3.56	3.26	-0.09	-0.67
6	>738		>738		N/A	N/A	N/A	N/A
7	3010		2610		3.48	3.42	-0.86	0.78

### Summary Statistics

#### *Sample - PTA 1*

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

#### *Sample - PTA 2*

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

**Note:**

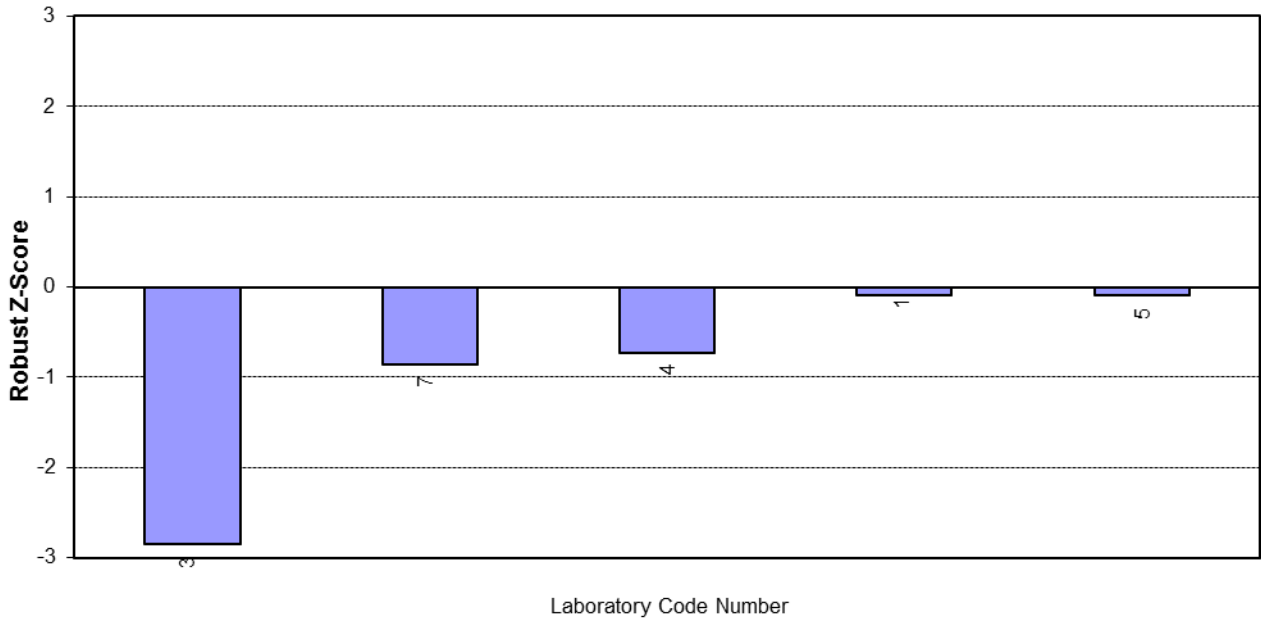
1. Due to insufficient results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results (from another trial using the same samples).



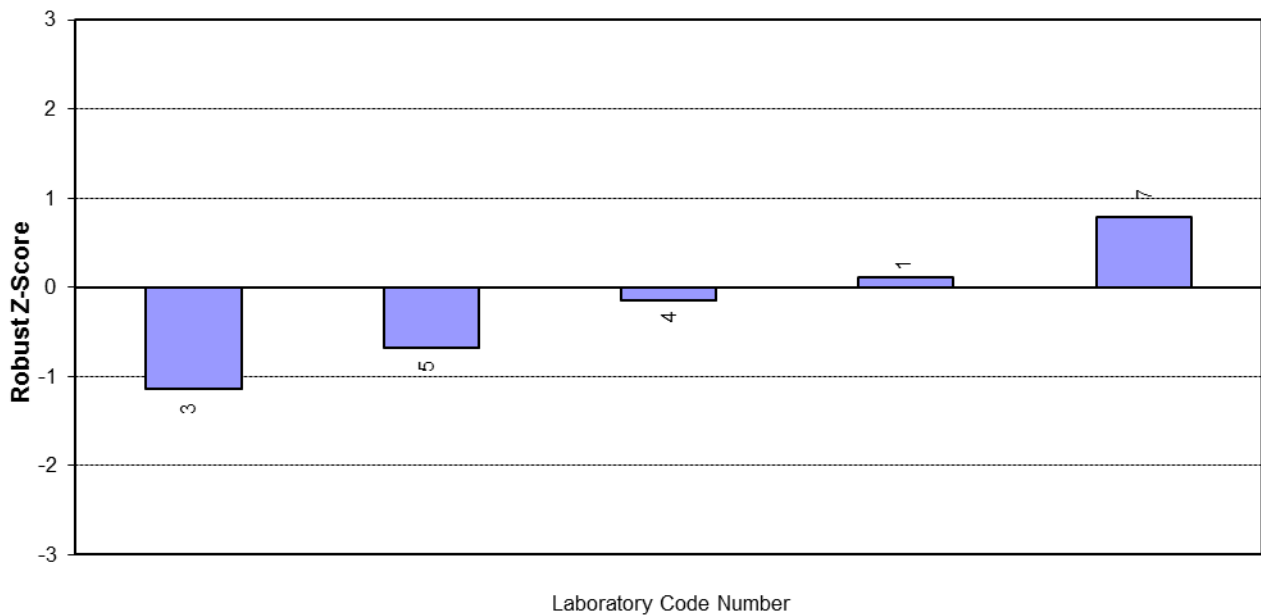
A9.2

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 4 March 2019. 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 1 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
53000	4.7243	52000	4.7160	51000	4.7076	51000	4.7076
50000	4.6990	64000	4.8062	59000	4.7709	67000	4.8261
65000	4.8129	60000	4.7782	64000	4.8062	59000	4.7709
70000	4.8451	70000	4.8451				
65000	4.8129	64000	4.8062				

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 64 – MARCH 2019

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  $<4^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  (or  $35^{\circ}\text{C}$ ) 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.  $6.2 \times 10^1$  cfu/100mL) or if confidence limits from MPN tables are used, or as a  $\text{Log}_{10}$  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 18<sup>th</sup> March 2019 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 64 – MARCH 2019****INSTRUCTIONS FOR RE-HYDRATION OF SAMPLE**

1. For **EACH** sample, re-hydrate the freeze-dried vial by adding 3.0mL of sterile diluent eg (0.1% (w/v) peptone or 0.85% (w/v) NaCl (ISO 6887-1) at room temperature.
2. Allow to stand at room temperature for 10 minutes.
3. Mix the vial contents using a vortex mixer or shake 25 times in about 7 seconds.
4. Aseptically transfer 2.0mL of vial contents to 1000mL sterile deionised (or distilled) water. This will leave 1.0mL remaining in the vial, which may be used to prepare samples for intra-laboratory comparison purposes, if required by the laboratory.
5. Shake the sample bottle 25 times to produce the simulated water sample.
6. Examine the sample using your routine test methods.
7. Repeat steps 1 through 6 for the second sample.

**C3**  
**PROFICIENCY TESTING AUSTRALIA**



**MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM**

**ROUND 64 MARCH 2019**

**RESULTS SHEET**

**Laboratory Code:**

6
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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"/> AS 4276.21-2005 EHS used: <input type="checkbox"/> Colilert <input type="checkbox"/> Colitag™ <input type="checkbox"/> Other: <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"/> AS 4276.21-2005 EHS used (incubation at 44°C): <input type="checkbox"/> Colilert <input type="checkbox"/> Colitag™ <input type="checkbox"/> Other: <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"/> AS 4276.21-2005 EHS used: <input type="checkbox"/> Colilert <input type="checkbox"/> Colitag™ <input type="checkbox"/> Other: <input type="checkbox"/> Other:
Enterococci (cfu/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 --