

**REPORT NO. 1223**

**MICROBIOLOGICAL WATERS**

**PROFICIENCY TESTING PROGRAM**

**ROUND 69**

**NOVEMBER 2020**

**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.

© **COPYRIGHT PROFICIENCY TESTING AUSTRALIA 2020**

PO Box 1122 Archerfield BC QLD 4108 AUSTRALIA



CONTENTS	PAGE
1. Foreword	1
2. Features of the Program	1
3. Format of the Appendices	2
4. Statistical Design of the Program	3
5. Outlier Results	3
Table A - Summary Statistics	4
Table B - Summary of Outlier Results	5
6. PTA and Technical Adviser's Comments	5
7. References	19

## APPENDIX A

### Tables of Results and Z-Scores, Summary Statistics and Graphical Displays

<i>E. coli</i> MF	A1.1
<i>E. coli</i> MPN	A2.1
Thermotolerant Coliforms MF	A3.1
Thermotolerant Coliforms MPN	A4.1
Total Coliforms MF	A5.1
Total Coliforms MPN	A6.1
Enterococci MF	A7.1
<i>Pseudomonas aeruginosa</i> MF	A8.1
<i>Clostridium perfringens</i> (vegetative cells) MF	A9.1
Plate Count All Techniques	A10.1

## APPENDIX B

Sample Preparation, Homogeneity and Stability Testing	B1
---	----

## APPENDIX C

Instructions to Participants	C1
Instructions for Re-hydration of Sample	C2
Results Sheet	C3

## 1. **FOREWORD**

This report summarises the results of a microbiological proficiency testing program on water.

The program was conducted in September 2020 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 sixty-ninth round in a series of on-going water proficiency testing programs. This program is accredited to ISO/IEC 17043:2010 “*Conformity assessment - General requirements for proficiency testing*”, by International Accreditation New Zealand (IANZ). This report was authorised by Mrs K Cividin, 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 five separate laboratories received samples for the program with four 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 reported more than one set of results, 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 potable water samples.

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

*Escherichia coli* (*E. coli*), Thermotolerant Coliforms, Total Coliforms, Enterococci, *Pseudomonas aeruginosa*, *Clostridium perfringens* (vegetative cells) and 36°C (or 35-37°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 Coliforms, Total Coliforms, Enterococci and 36°C (or 35-37°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) technique, the tables contain 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 5. 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>	MF cfu/100mL	1	34	1.730	0.124
		2	36	1.680	0.133
	MPN MPN/100mL	1	66	1.800	0.195
		2	66	1.720	0.172
Thermotolerant Coliforms	MF cfu/100mL	1	37	1.760	0.133
		2	37	1.710	0.156
	MPN MPN/100mL	1	14	1.590	0.337
		2	14	1.605	0.241
Total Coliforms	MF cfu/100mL	1	31	1.760	0.082
		2	31	1.850	0.126
	MPN MPN/100mL	1	65	1.820	0.170
		2	65	1.900	0.178
Enterococci	MF cfu/100mL	1	24	1.400	0.104
		2	23	1.360	0.107
<i>Pseudomonas aeruginosa</i>	MF cfu/100mL	1	14	1.890	0.172
		2	15	1.690	0.093
<i>Clostridium perfringens</i> (vegetative cells)	MF cfu/100mL	1	19	1.360	0.140
		2	19	1.080	0.096
Plate Count	All cfu/mL	1	25	1.650	0.104
		2	25	1.860	0.082

All statistics (including No. of Results) are calculated from Global Proficiency Ltd's (GP) 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	-	-	-
Thermotolerant Coliforms	MF	1	-	-
	MPN	-	-	-
Total Coliforms	MF	1, 2B	-	-
	MPN	-	-	-
Enterococci	MF	4	-	-
<i>Pseudomonas aeruginosa</i>	MF	4	-	-
<i>Clostridium perfringens</i>	MF	-	2A, 2B	-
Plate Count	All	1	-	-

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* as the only coliform organism, whereas sample PTA 2 contained *E. coli* and *Citrobacter freundii* as the coliform organisms in the sample. *Enterococcus faecalis* was included as a member of the enterococci group in both samples. *Pseudomonas aeruginosa* and *Clostridium perfringens* were included in both samples as indicator organisms. Other mesophilic organisms, which did not interfere with the indicator organism tests, were included in the samples to contribute to the Plate Count at 36°C (or 35-37°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.

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

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 for a range of tube and dilution combinations to be 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.



**Total Coliforms:**

For Total Coliforms, four laboratories reported results for the MF technique and three laboratories reported results for the MPN technique. Laboratory code 2 reporting two sets of results for both techniques (2A and 2B) and laboratory code 5 reported a result for sample PTA 1 only using the MPN technique. Laboratory codes 1 and 2B recorded an outlier for Sample PTA 1 using the MF technique with results lower than expected.

All results classified as outliers should be investigated further.

The methods used by laboratories were mainly AS/NZS and APHA.

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.

<b>Total Coliforms via:</b>	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/MPN/100mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/MPN /100mL)
Membrane Filtration	1.760 $\pm$ 0.018	1.850 $\pm$ 0.028
Most Probable Number	1.820 $\pm$ 0.027	1.900 $\pm$ 0.028

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

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

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values. The laboratory may wish 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 Total Coliform 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.

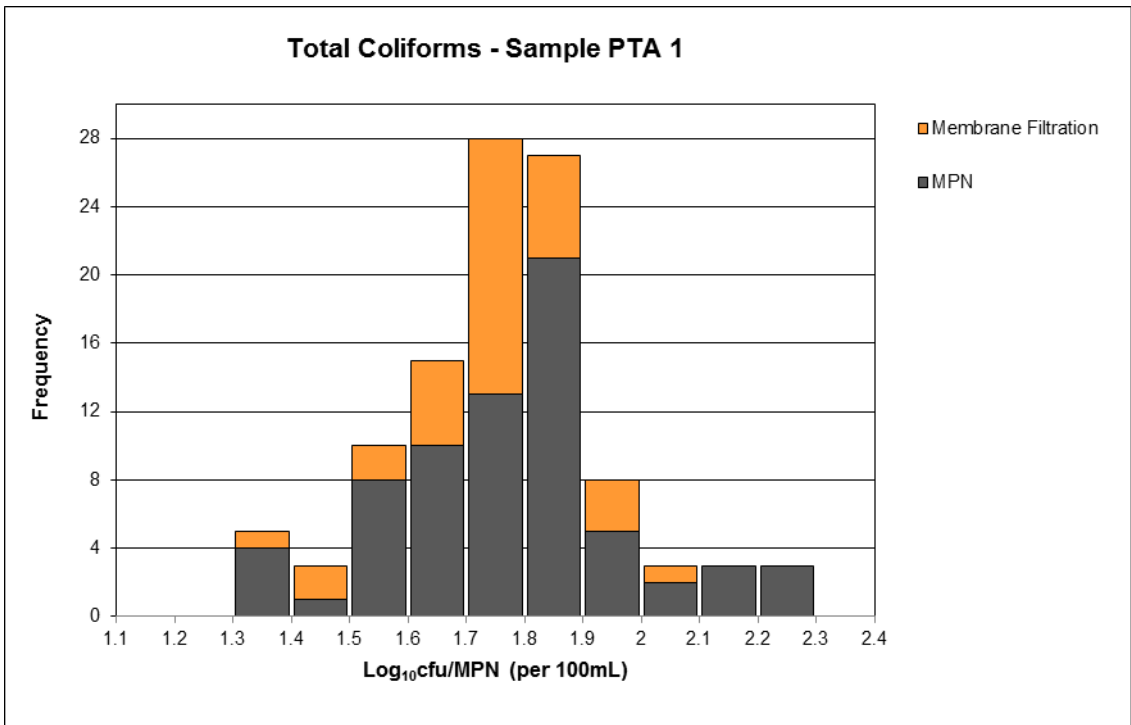


Figure TA-1. Total Coliform results for Sample PTA 1

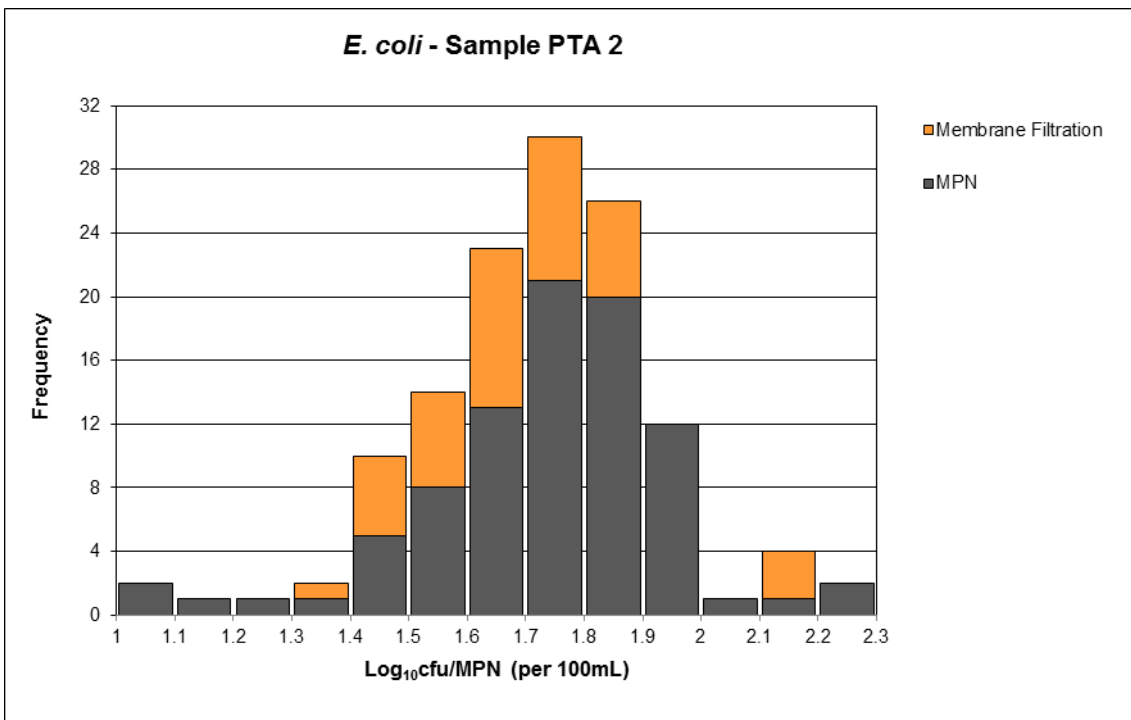


Figure TA-2. Total Coliform results for Sample PTA 2

***E. coli*:**

For *E. coli*, four laboratories reported results for the MF technique and three laboratories reported results for the MPN technique. Laboratory code 2 reporting two sets of results for both techniques (2A and 2B) and laboratory code 5 reported a result for sample PTA 1 only using the MPN technique. No outliers were reported for this test.

The methods used by laboratories were mainly AS/NZS and APHA.

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.

<b><i>E. coli</i> via:</b>	<b>Sample PTA 1</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/MPN/100mL)	<b>Sample PTA 2</b> Median $\pm$ Uncertainty (Log <sub>10</sub> cfu/MPN /100mL)
Membrane Filtration	1.730 $\pm$ 0.027	1.680 $\pm$ 0.028
Most Probable Number	1.800 $\pm$ 0.030	1.720 $\pm$ 0.027

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

**Measurement Uncertainty - *E. coli* via Membrane Filtration (MF):**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values. The laboratory may wish 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 two methods used in this round and include Global Proficiency Ltd and PTA data for methods indicated above.

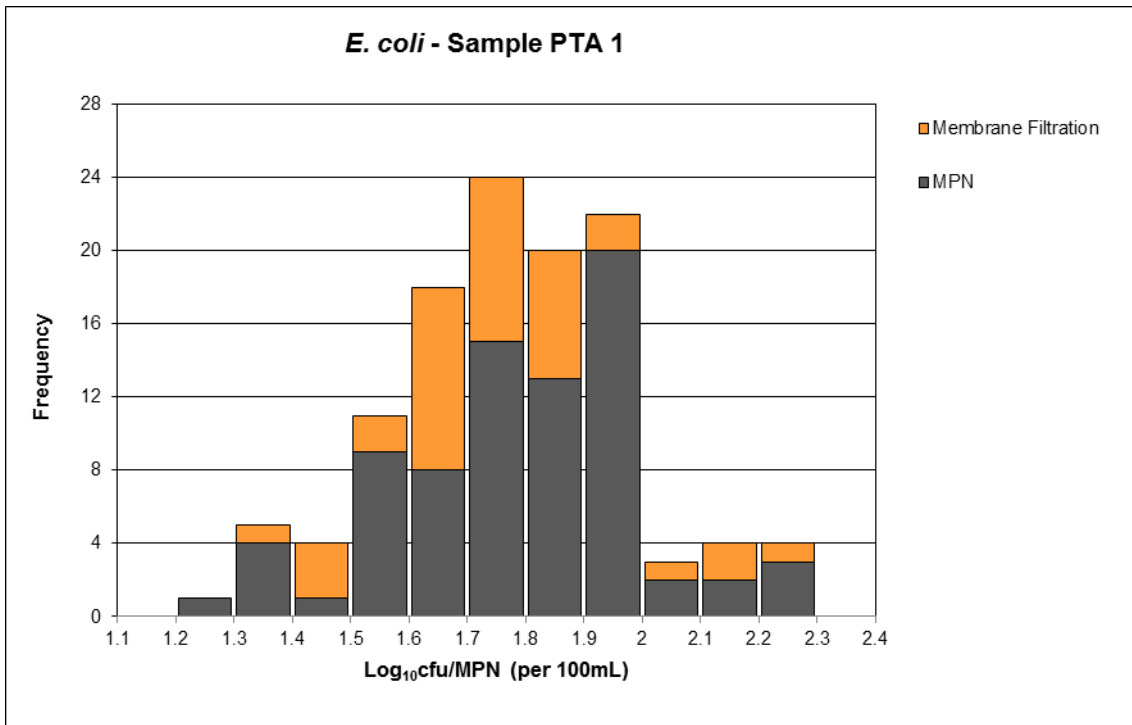


Figure TA-3. *E. coli* results for Sample PTA 1.

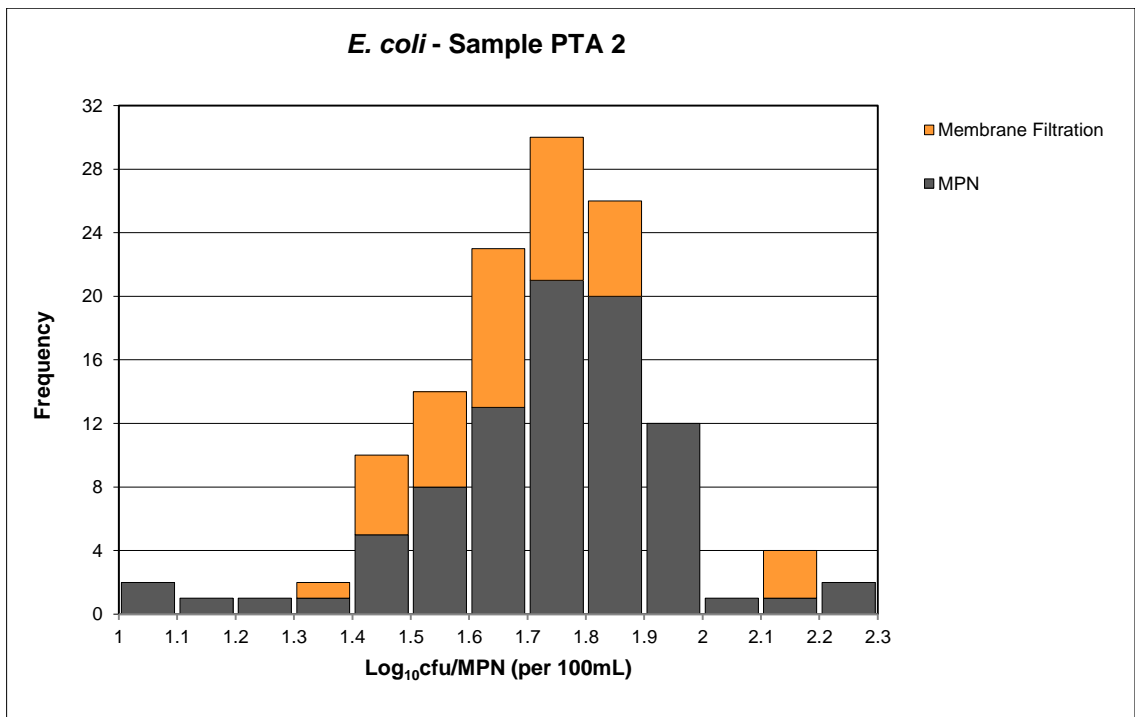


Figure TA-4. *E. coli* results for Sample PTA 2.

**Thermotolerant (Faecal) Coliforms:**

For Thermotolerant Coliforms, three laboratories reported results for the MF technique and two laboratories reported results for the MPN technique with laboratory code 2 reporting two sets of results for both techniques (2A and 2B). Laboratory code 1 recorded outliers for both samples using the MF technique with results lower than expected.

Interestingly, the medians obtained for this test for both samples were lower than the medians obtained for the *E. coli* MPN test both samples. Given the only thermotolerant coliform present in the samples was *E. coli*, the medians were expected to be closer than what they were. However, this is most likely attributable to the significant difference in the number of results contributing to the calculation of the medians for both samples, and the proportion of results obtained from Colilert QuantiTray for *E. coli* (73%) vs other methods for the Thermotolerant coliforms group. The table below summarises the data for comparison.

Test	Technique	Sample (PTA)	No. of Results	Median Log <sub>10</sub> / <b>MPN</b> per 100mL	Normalised IQR
<i>E. coli</i> orgs/100mL	MPN	1	66	1.800 / <b>63</b>	0.195
		2	66	1.720 / <b>52</b>	0.172
Thermotolerant Coliforms orgs/100mL	MPN	1	14	1.590 / <b>39</b>	0.337
		2	14	1.605 / <b>40</b>	0.241

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.

<b>Thermotolerant 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	1.760 ± 0.027	1.710 ± 0.032
Most Probable Number	1.590 ± 0.113	1.605 ± 0.081

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

**Measurement Uncertainty - Thermotolerant Coliforms via Membrane Filtration (MF):**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values. The laboratory may wish 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 Thermotolerant Coliform testing are included below. These graphs show the distribution of results from the two methods used in this round.

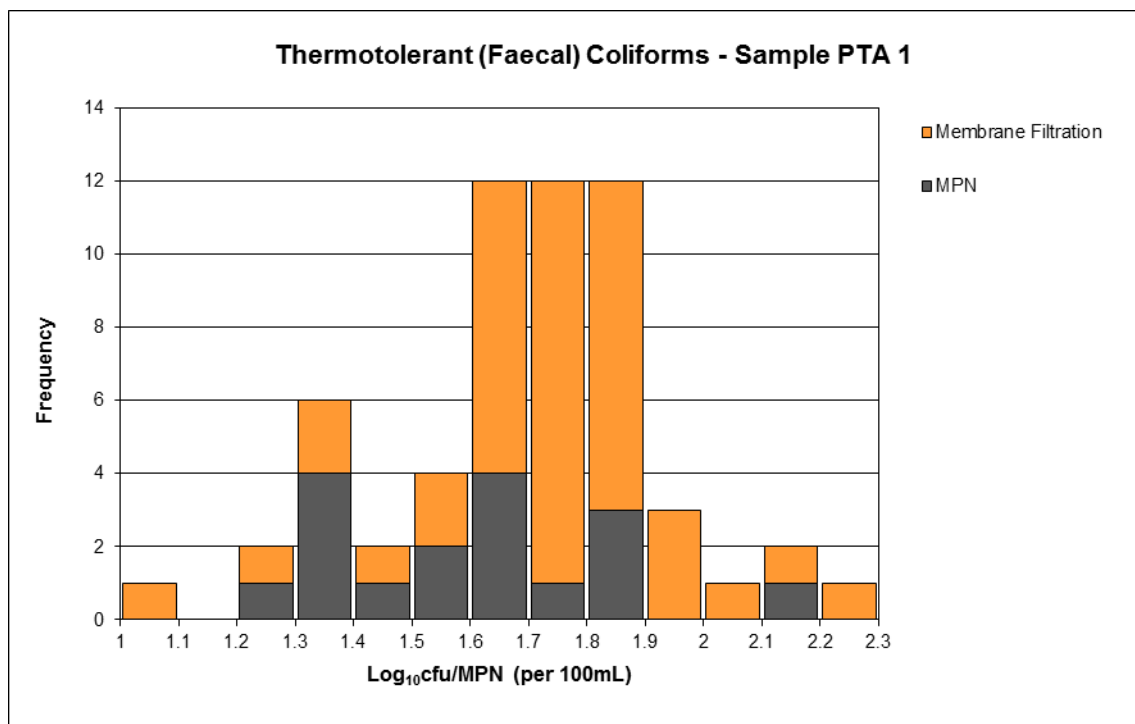


Figure TA-5. Thermotolerant Coliform results for Sample PTA 1

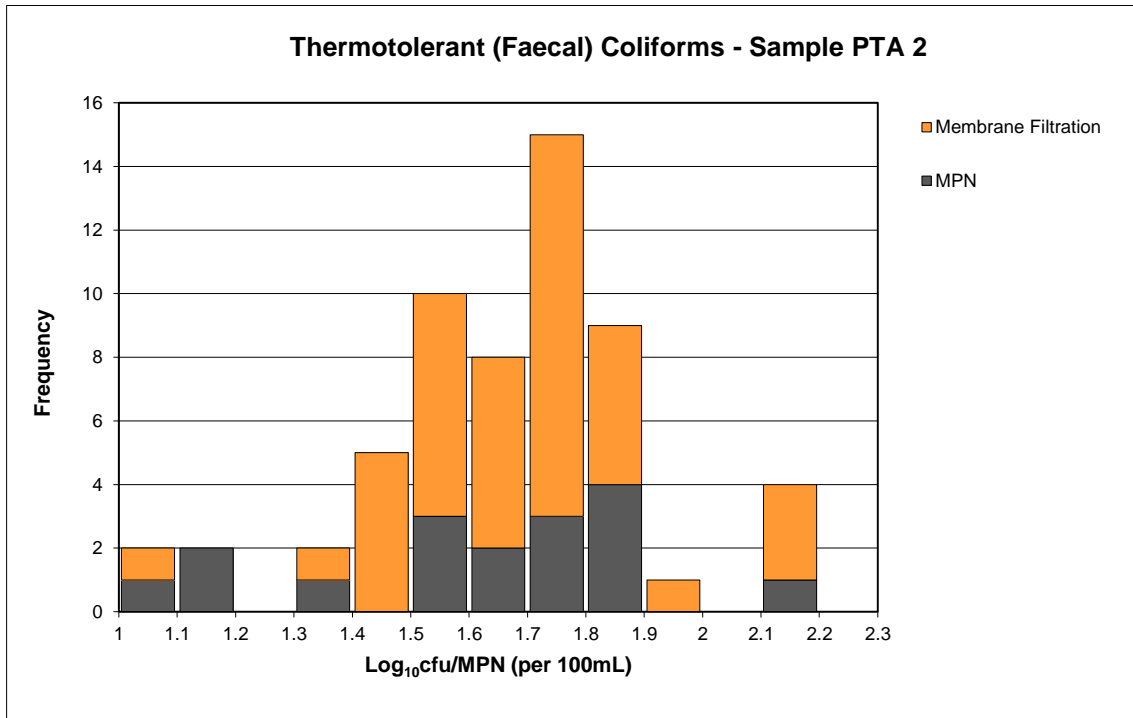


Figure TA-6. Thermotolerant Coliform results for Sample PTA 2

**Enterococci:**

For Enterococci, three laboratories reported results for the MF technique with laboratory code 2 reporting 2 sets of results (2A and 2B). Laboratory code 4 recorded an outlier for PTA sample 1 with a result lower than expected

The majority of laboratories reported using Australian Standard /New Zealand Standard with one laboratory using an APHA Enterolert method. Although results obtained via Enterolert are not usually assessed with those derived from membrane filtration methods, due to there being no other results to compare against, the Enterolert results were assessed against the MF data and appeared to compare well.

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.

<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	1.400 $\pm$ 0.027	1.360 $\pm$ 0.028

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

**Measurement Uncertainty - Enterococci via Membrane Filtration (MF):**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values.



***Pseudomonas aeruginosa:***

For *Pseudomonas aeruginosa*, two laboratories reported results for the MF technique with laboratory code 2 reporting 2 sets of results (2A and 2B). Laboratory code 4 recorded an outlier for PTA sample 2 with a result lower than expected.

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.

<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	1.890 $\pm$ 0.058	1.690 $\pm$ 0.030

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

**Measurement Uncertainty - *Pseudomonas aeruginosa* via Membrane Filtration (MF):**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values.

***Clostridium perfringens* (vegetative cells):**

Members of the sulphite-reducing anaerobes group which includes *Clostridium perfringens* are widespread in the environment, present in soils, wastewaters and faecal material from warm-blooded animals including humans. Their presence in water sources is of concern due to their ability to form spores that are resistant to both chemical disinfection agents and heat treatment processes used to treat water. While many published test methods concentrate on detecting spores, there are those that also examine water samples for the presence of *C. perfringens* vegetative cells - in conjunction with spores.

One of the challenges presented to PT Providers when offering (simulated) water samples containing *C. perfringens* spores, is the ability to produce large numbers of spores that can be harvested in order to prepare a spore suspension for the manufacture of the freeze-dried samples provided for the PT programme. This is compounded by an additional challenge where *C. perfringens* strains have a tendency to lose the ability to produce spores in the laboratory over time – a process known as “strain degeneration” (Kutzenok and Aschner 1952; Kashket and Cao 1995). Due to these occurrences, this round could only offer testing for *C. perfringens* vegetative cells and did not include spores. Although the challenge of undertaking the heat treatment step was not included in the proficiency testing process, good information on other aspects of the testing procedures can still be gained by participation.

For *Clostridium perfringens*, one laboratory (laboratory code 2) reported two sets of results (2A and 2B) for the MF technique. Laboratory codes 2A and 2B recorded identification outliers for both samples with false negatives for *Clostridium perfringens* vegetative cells.

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.

<b>Enterococci 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	1.360 ± 0.040	1.080 ± 0.028

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

**Measurement Uncertainty - *Clostridium perfringens* via Membrane Filtration (MF):**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as ± log values.

**Plate Count:**

Five sets of results (from four laboratories) were submitted for the Plate Count test. Laboratory Code 5 reported results for sample PTA 1 only. Four laboratories reported using a Pour Plate technique and one reported using Spread Plate technique. Laboratory code 1 recorded an outlier for PTA sample 1 with a result higher than expected.

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.

	<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>	1.650 $\pm$ 0.026	1.860 $\pm$ 0.020

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

**Measurement Uncertainty - Plate Count:**

Only one laboratory reported Measurement Uncertainty (MU) estimations associated with their test results in this round. MU was reported as  $\pm$  log values.

### **General Comments**

A total of 75 results were submitted for analysis in this round. Of these results, eleven (15%) were outlier results. This is higher than the 8% of outlier results reported in Round 65.

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* (2019). (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 4276.5-2019: *Water microbiology - Coliforms, Escherichia coli and thermotolerant 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/NZS 4276.13-2008: *Water Microbiology – Pseudomonas aeruginosa – Membrane filtration method*
- [11] AS 4276.17.1-2016: *Water Microbiology – Spores of Clostridium perfringens – Membrane filtration method*
- [12] AS 4276.21-2005: *Water Microbiology - Examination for coliforms and Escherichia coli - Determination of most probable number (MPN) using enzyme hydrolysable substrates*
- [13] APHA 9221 – *Multiple-Tube Fermentation Technique for Members of the Coliform Group. American Public Health Association: Standard methods for the examination of water and wastewater, 23<sup>rd</sup> Edition (2017)*
- [14] APHA 9223 – *Enzyme Substrate Coliform Test. American Public Health Association: Standard methods for the examination of water and wastewater, 23<sup>rd</sup> Edition (2017)*

- [15] 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)*
- [16] 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* (cfu/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	31		70		1.49	1.85	-1.92	1.24
2A	42	±0.15	61	±0.15	1.62	1.79	-0.86	0.79
2B	27	±0.15	52	±0.15	1.43	1.72	-2.41	0.27
4	32		22		1.51	1.34	-1.81	-2.53

### **Summary Statistics**

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

No. of Results	34
Median	1.730
Norm IQR	0.124
Robust CV	7.2%
Minimum	1.36
Maximum	2.23
Range	0.87
Uncertainty (Median)	0.027

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

No. of Results	36
Median	1.680
Norm IQR	0.133
Robust CV	7.9%
Minimum	1.43
Maximum	1.86
Range	0.43
Uncertainty (Median)	0.028

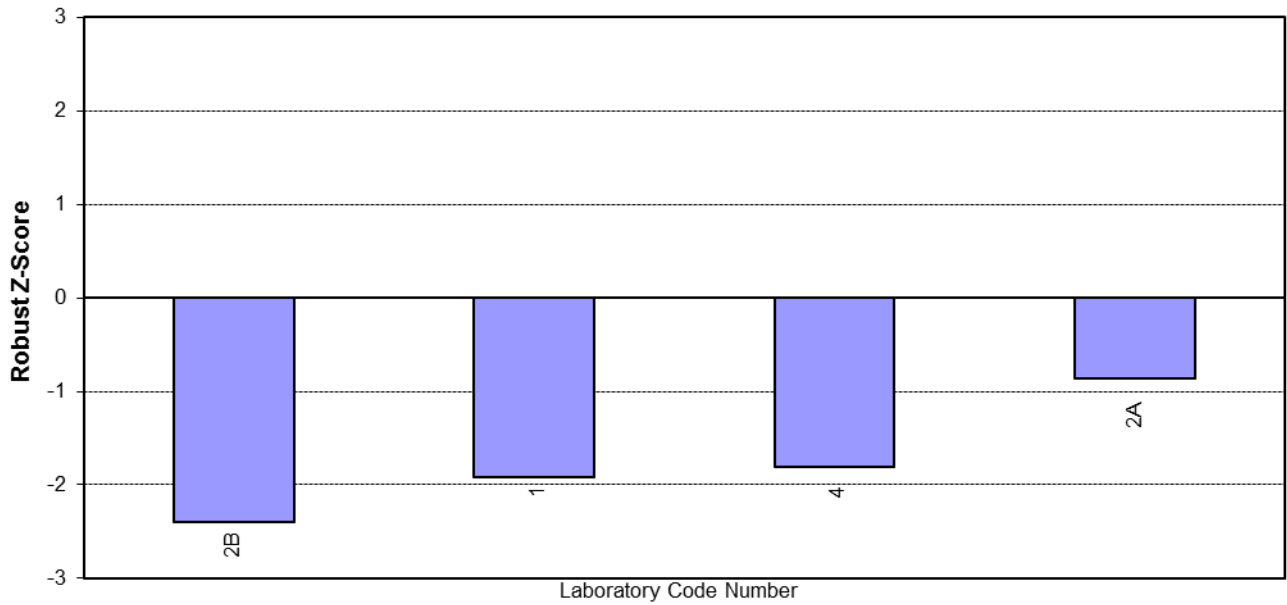
**Note:**

1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

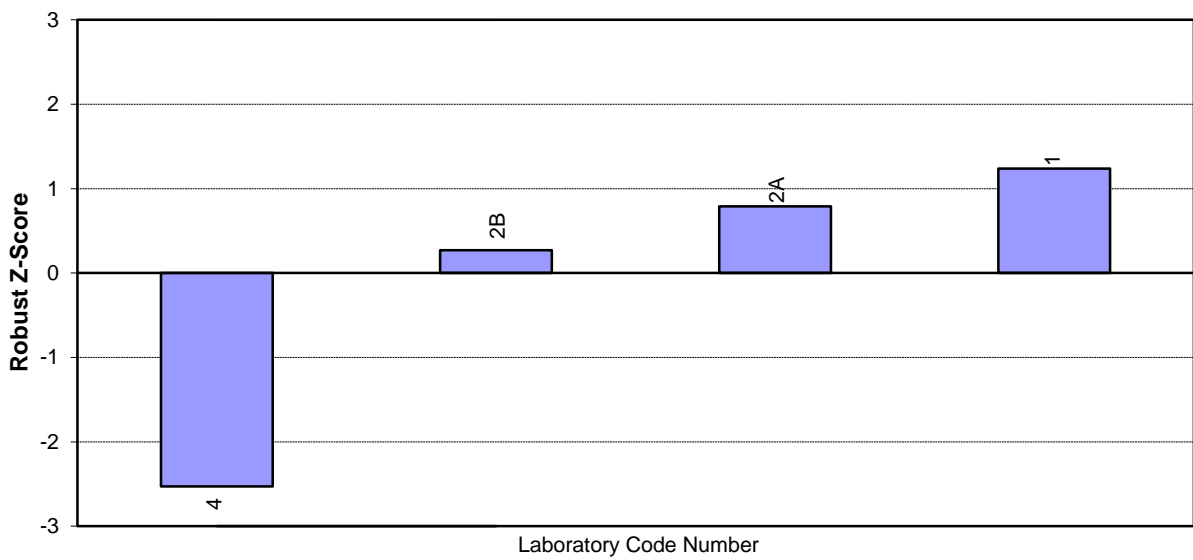
A1.2

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

***Sample - PTA 1***



***Sample - PTA 2***



## A2.1

### ***E. coli* (MPN/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	53		64		1.72	1.81	-0.39	0.50
2A	62	±0.15	69	±0.15	1.79	1.84	-0.04	0.69
2B	48	±0.15	69	±0.15	1.68	1.84	-0.61	0.69
4	50		50		1.70	1.70	-0.52	-0.12
5	79				1.90	N/A	0.50	N/A

**Note:**

1. N/A - not applicable

### Summary Statistics

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

No. of Results	66
Median	1.800
Norm IQR	0.195
Robust CV	10.8%
Minimum	1.20
Maximum	2.23
Range	1.03
Uncertainty (Median)	0.030

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

No. of Results	66
Median	1.720
Norm IQR	0.172
Robust CV	10.0%
Minimum	1.04
Maximum	2.26
Range	1.22
Uncertainty (Median)	0.027

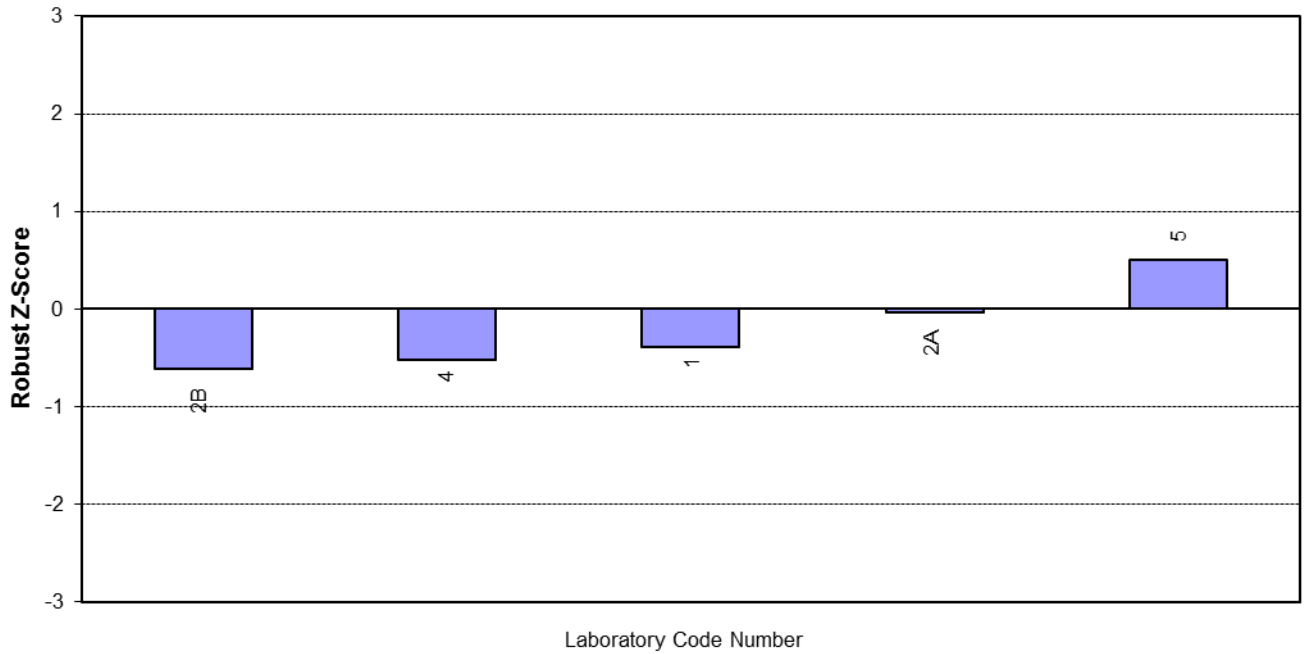
**Note:**

1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

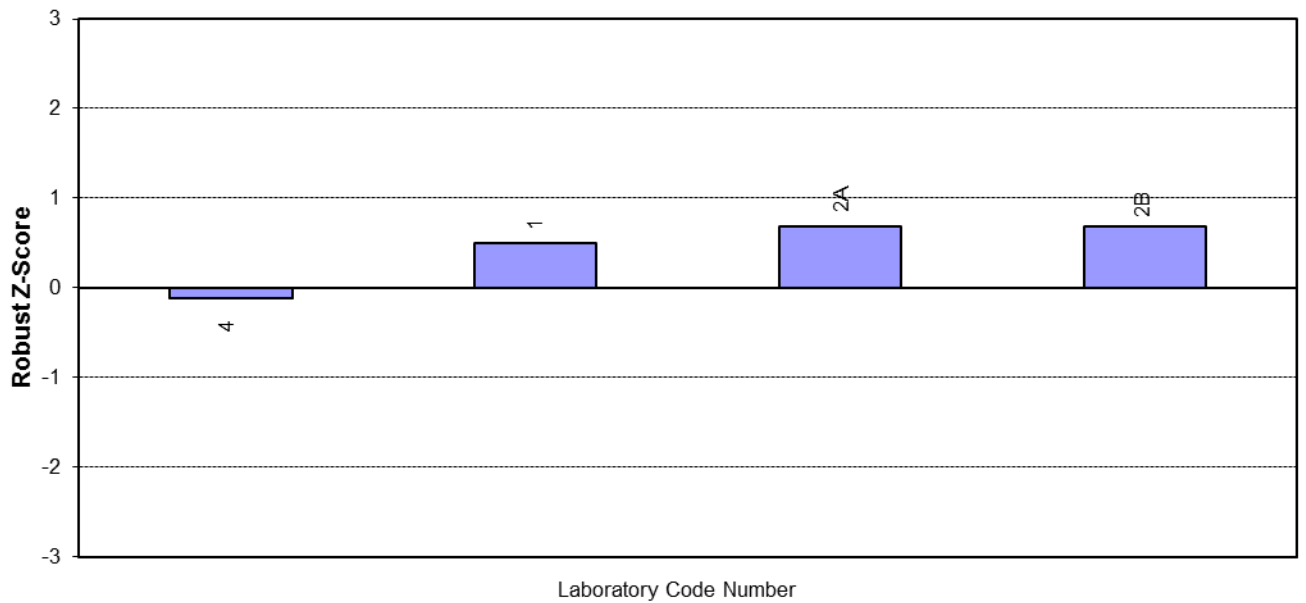
A2.2

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

***Sample - PTA 1***



***Sample - PTA 2***



**SECTIONS A3 to A4**

**Thermotolerant Coliforms**

### A3.1

#### Thermotolerant Coliforms (cfu/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	11		12		1.04	1.08	-5.39 §	-4.05 §
2A	42	±0.15	61	±0.15	1.62	1.79	-1.02	0.48
2B	27	±0.15	52	±0.15	1.43	1.72	-2.46	0.04
4	32		22		1.51	1.34	-1.91	-2.36

**Note:**

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

#### Summary Statistics

##### Sample - PTA 1

No. of Results	37
Median	1.760
Norm IQR	0.133
Robust CV	7.6%
Minimum	1.23
Maximum	2.23
Range	1.00
Uncertainty (Median)	0.027

##### Sample - PTA 2

No. of Results	37
Median	1.710
Norm IQR	0.156
Robust CV	9.1%
Minimum	1.43
Maximum	2.18
Range	0.75
Uncertainty (Median)	0.032

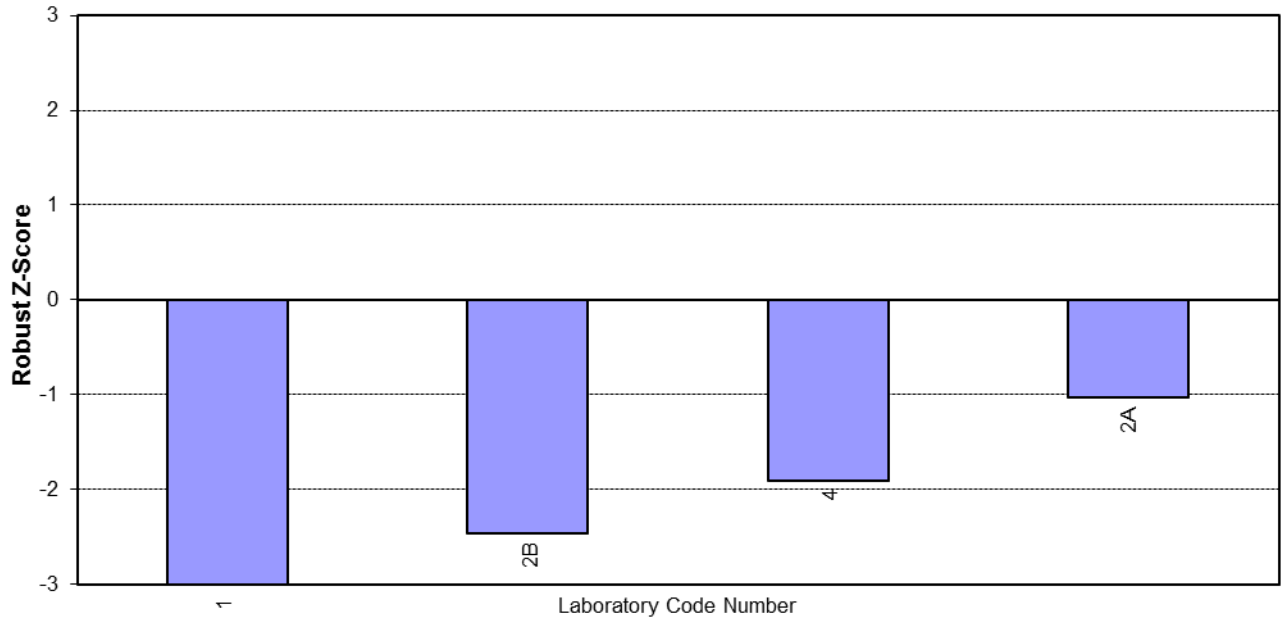
**Note:**

1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

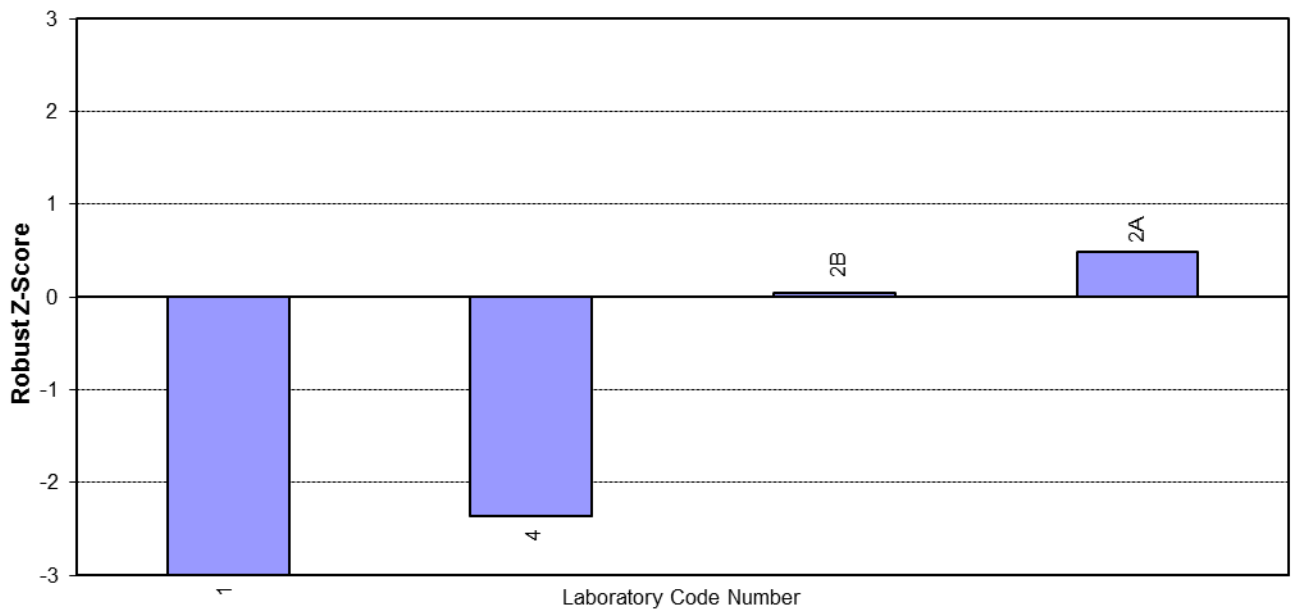
A3.2

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

Sample - PTA 1



Sample - PTA 2



## A4.1

### Thermotolerant Coliforms (MPN/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	31		63		1.49	1.80	-0.29	0.81
2A	46	±0.16	69	±0.16	1.66	1.84	0.22	0.97
2B	48	±0.16	69	±0.16	1.68	1.84	0.27	0.97

### Summary Statistics

#### *Sample - PTA 1*

No. of Results	14
Median	1.590
Norm IQR	0.337
Robust CV	21.2%
Minimum	1.23
Maximum	2.11
Range	0.88
Uncertainty (Median)	0.113

#### *Sample - PTA 2*

No. of Results	14
Median	1.605
Norm IQR	0.241
Robust CV	15.0%
Minimum	0.89
Maximum	2.11
Range	1.22
Uncertainty (Median)	0.081

**Note:**

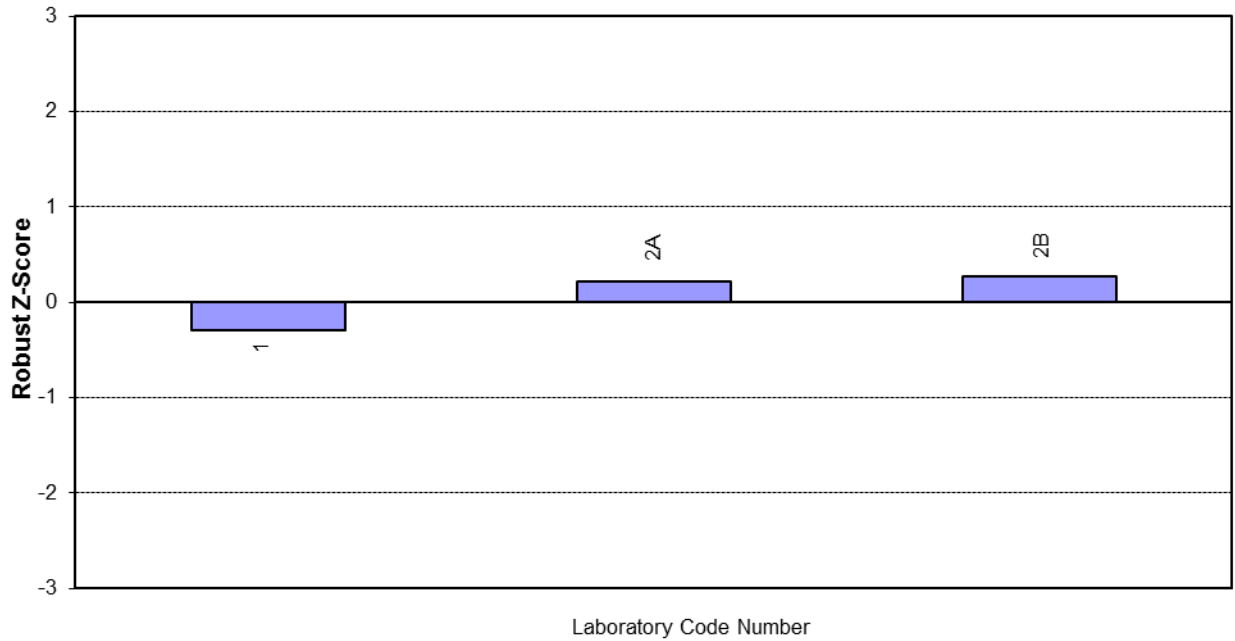
1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.



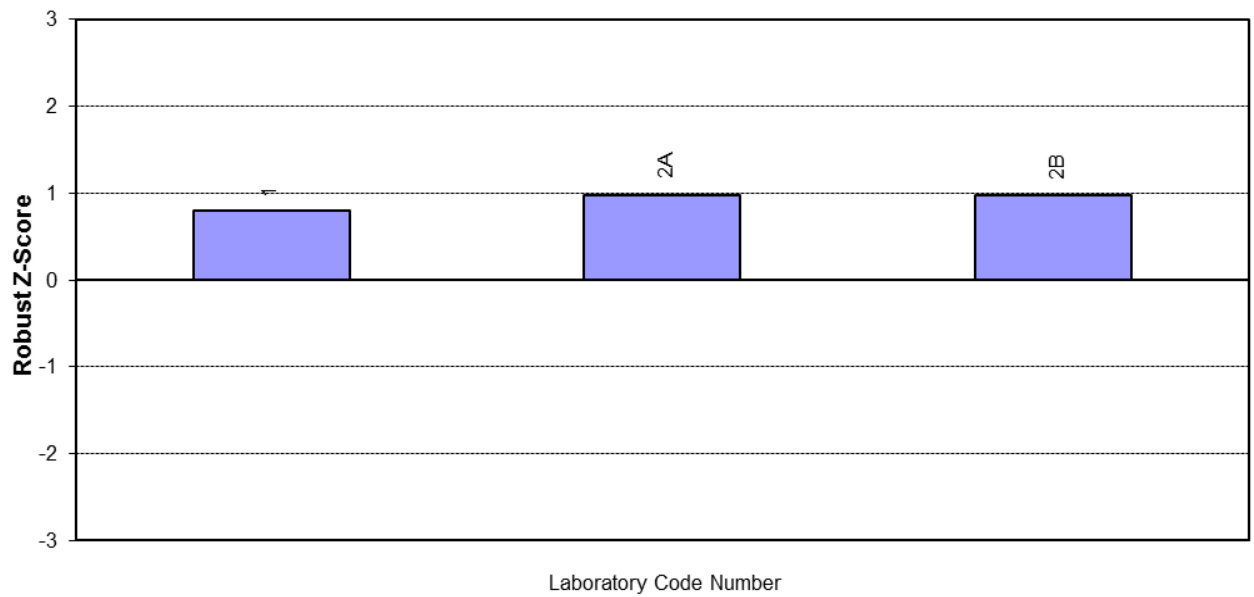
A4.2

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

*Sample - PTA 1*



*Sample - PTA 2*



# **SECTIONS A5 to A6**

## **Total Coliforms**

## A5.1

### Total Coliforms (cfu/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	31		70		1.49	1.85	-3.29 §	-0.04
2A	43	±0.23	70	±0.23	1.63	1.85	-1.55	-0.04
2B	30	±0.23	73	±0.23	1.48	1.86	-3.47 §	0.11
4	45		42		1.65	1.62	-1.31	-1.80

**Note:**

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

### Summary Statistics

#### *Sample - PTA 1*

No. of Results	31
Median	1.760
Norm IQR	0.082
Robust CV	4.6%
Minimum	1.34
Maximum	2.01
Range	0.67
Uncertainty (Median)	0.018

#### *Sample - PTA 2*

No. of Results	31
Median	1.850
Norm IQR	0.126
Robust CV	6.8%
Minimum	1.53
Maximum	2.20
Range	0.67
Uncertainty (Median)	0.028

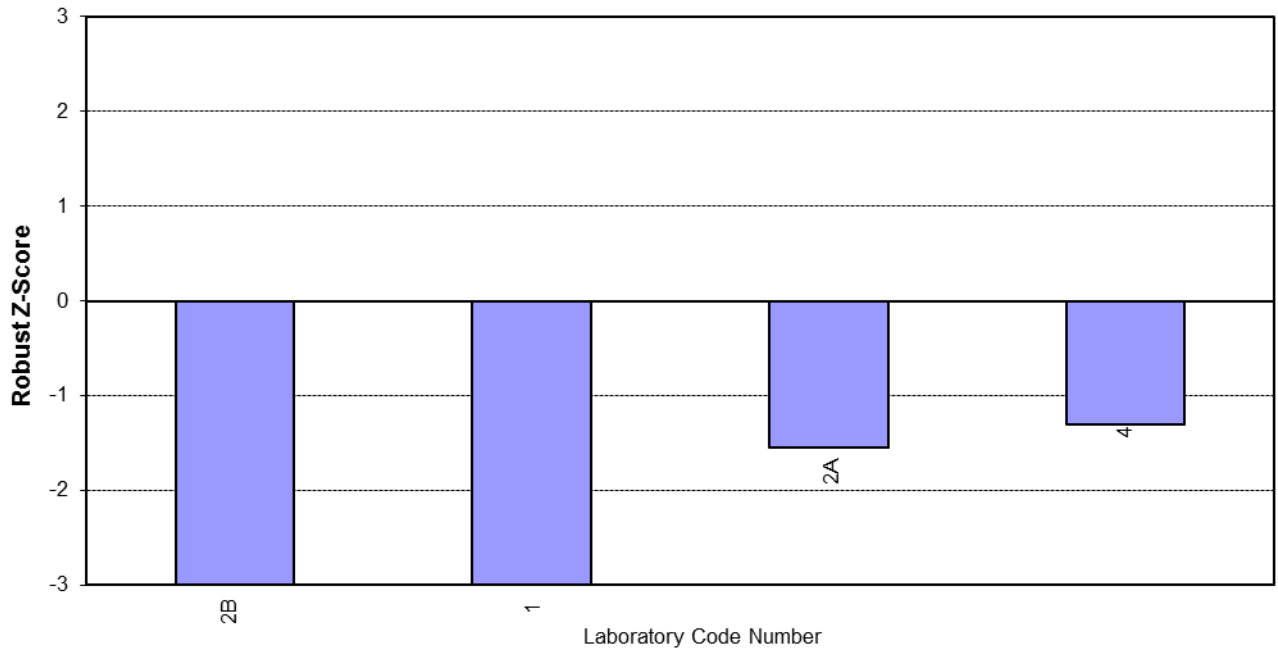
**Note:**

1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

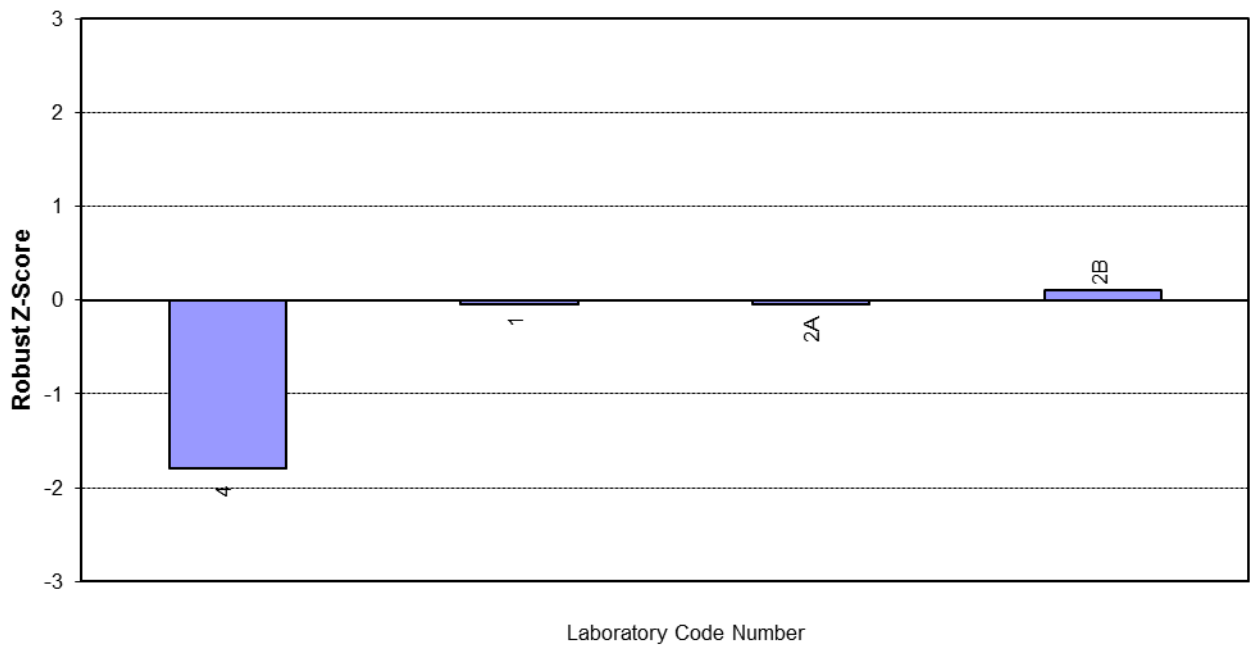
A5.2

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

Sample - PTA 1



Sample - PTA 2



## A6.1

### Total Coliforms (MPN/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	53		64		1.72	1.81	-0.56	-0.53
2A	62	±0.14	86	±0.14	1.79	1.93	-0.16	0.19
2B	50	±0.14	85	±0.14	1.70	1.93	-0.71	0.17
4	50		88		1.70	1.94	-0.71	0.25
5	79				1.90	N/A	0.46	N/A

**Note:**

1. N/A - not applicable

### Summary Statistics

#### *Sample - PTA 1*

No. of Results	65
Median	1.820
Norm IQR	0.170
Robust CV	9.4%
Minimum	1.34
Maximum	2.30
Range	0.96
Uncertainty (Median)	0.027

#### *Sample - PTA 2*

No. of Results	65
Median	1.900
Norm IQR	0.178
Robust CV	9.4%
Minimum	1.23
Maximum	2.44
Range	1.21
Uncertainty (Median)	0.028

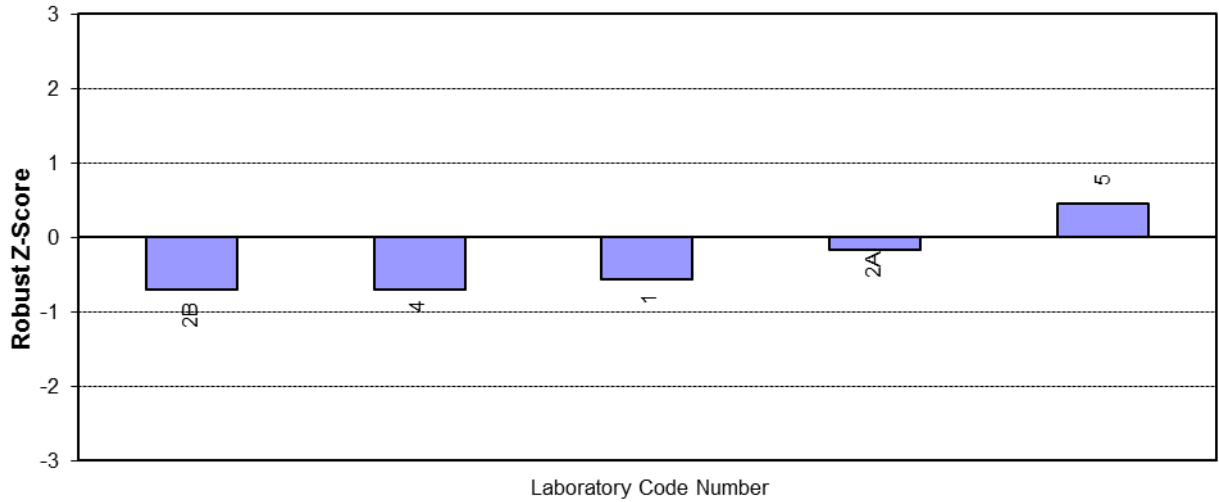
**Note:**

1. Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

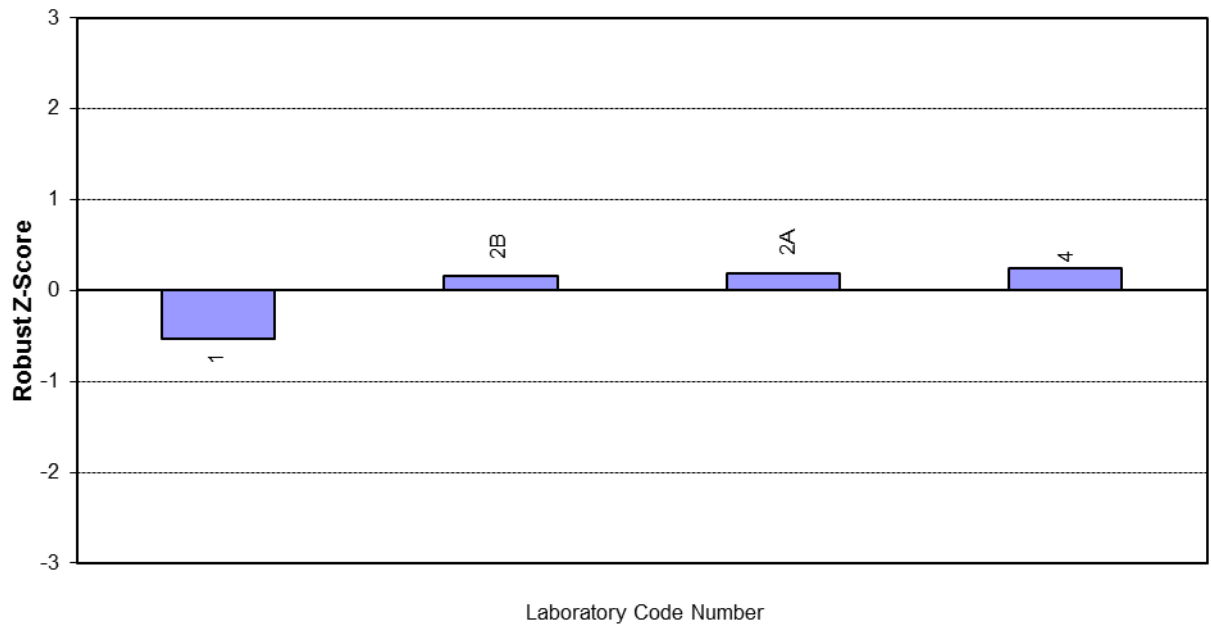
A6.2

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

Sample - PTA 1



Sample - PTA 2



# **SECTION A7**

## **Enterococchi**

**A7.1**  
**Enterococci (cfu/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	31		31		1.49	1.49	0.88	1.22
2A	27	±0.32	22	±0.32	1.43	1.34	0.30	-0.16
2B	31	±0.32	25	±0.32	1.49	1.40	0.88	0.35
4	11		13		1.04	1.11	-3.46 §	-2.29

**Note:**

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

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

No. of Results	24
Median	1.400
Norm IQR	0.104
Robust CV	7.4%
Minimum	0.95
Maximum	1.62
Range	0.67
Uncertainty (Median)	0.027

**Sample - PTA 2**

No. of Results	23
Median	1.360
Norm IQR	0.107
Robust CV	7.9%
Minimum	1.00
Maximum	1.53
Range	0.53
Uncertainty (Median)	0.028

**Note:**

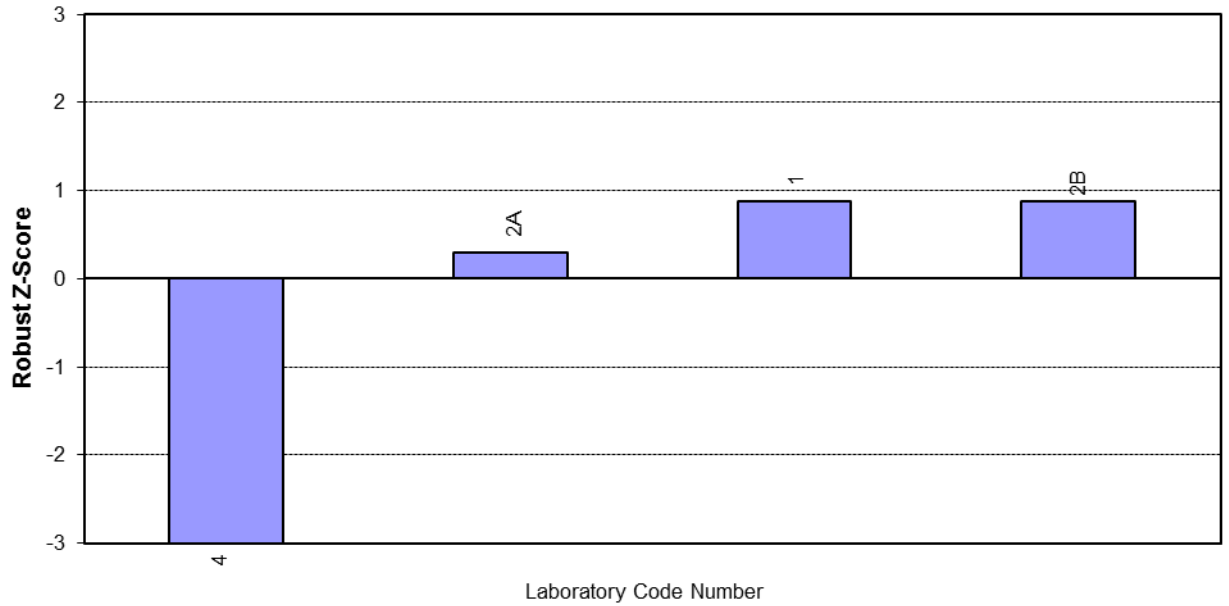
1. Due to an insufficient number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.



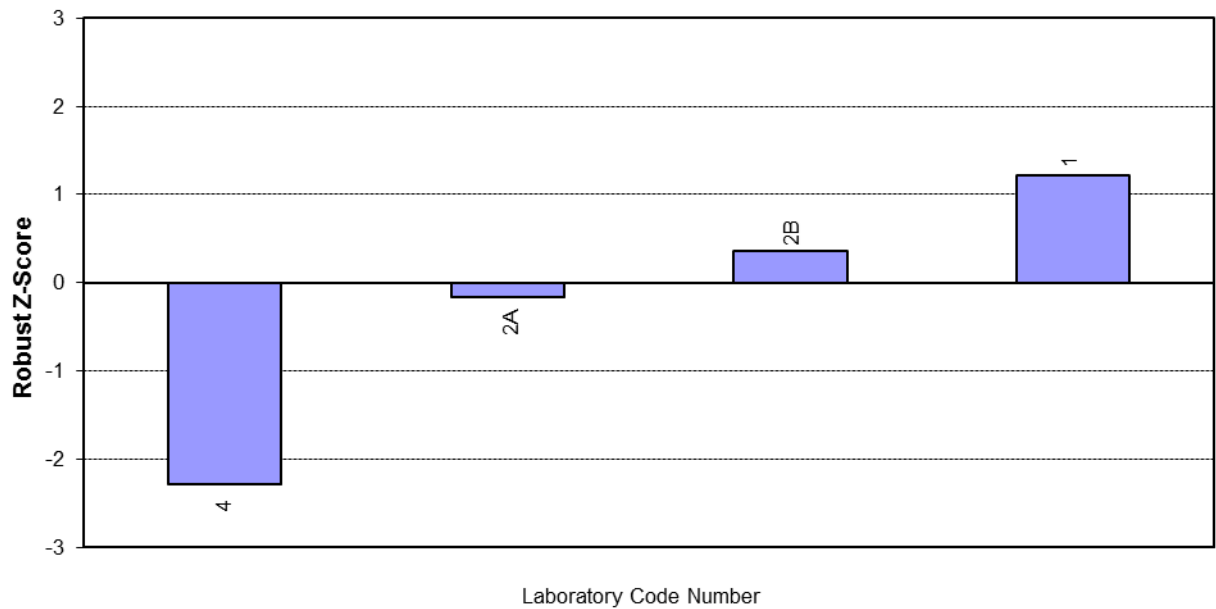
A7.2

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

Sample - PTA 1



Sample - PTA 2



## **SECTION A8**

### ***Pseudomonas Aeruginosa***

**A8.1**  
***Pseudomonas aeruginosa* (cfu/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
2A	70	±0.26	43	±0.26	1.85	1.63	-0.26	-0.61
2B	57	±0.26	39	±0.26	1.76	1.59	-0.78	-1.07
4	45		11		1.65	1.04	-1.37	-7.00 §

**Note:**

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

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

No. of Results	14
Median	1.890
Norm IQR	0.172
Robust CV	9.1%
Minimum	1.41
Maximum	2.11
Range	0.70
Uncertainty (Median)	0.058

***Sample - PTA 2***

No. of Results	15
Median	1.690
Norm IQR	0.093
Robust CV	5.5%
Minimum	1.58
Maximum	2.04
Range	0.46
Uncertainty (Median)	0.030

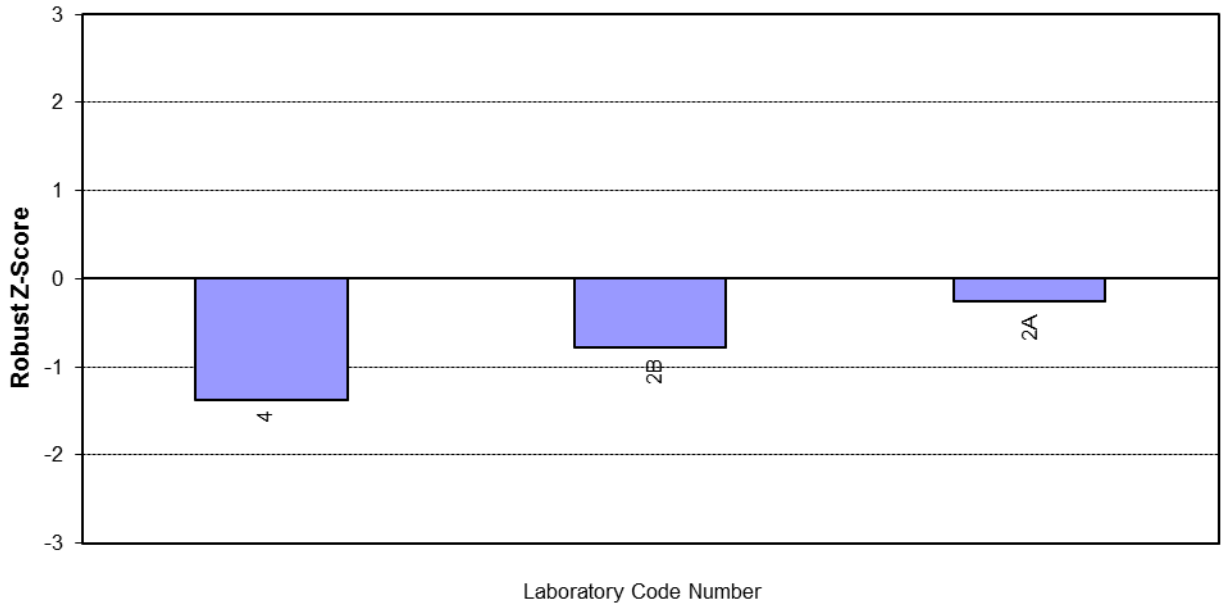
**Note:**

1. Due to an insufficient number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

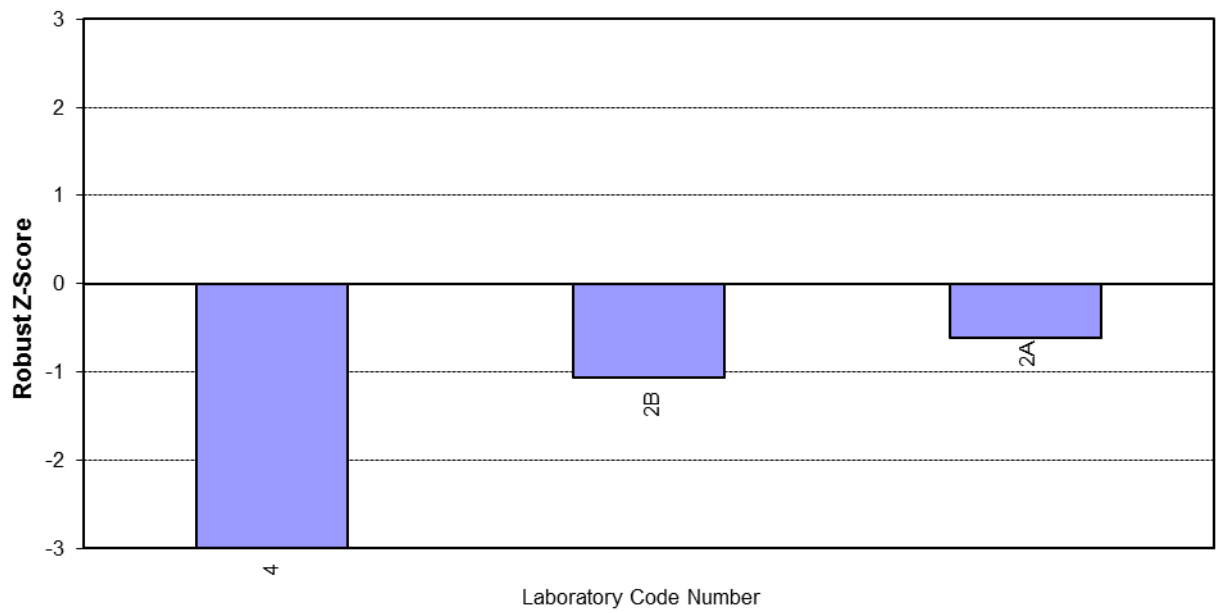
A8.2

***Pseudomonas Aeruginosa* (cfu/100mL) – MF Technique  
Ordered Robust Z-Score Charts**

***Sample - PTA 1***



***Sample - PTA 2***



## **SECTION A9**

***Clostridium perfringens***

**(Vegetative cells)**

**A9.1**  
***Clostridium perfringens* (cfu/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
2A	<1	±0.23	<1	±0.23	N/A	N/A	N/A †	N/A †
2B	<1	±0.23	<1	±0.23	N/A	N/A	N/A †	N/A †

**Note:**

1. † denotes a false negative.

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

No. of Results	19
Median	1.360
Norm IQR	0.140
Robust CV	10.3%
Minimum	1.08
Maximum	1.85
Range	0.77
Uncertainty (Median)	0.040

***Sample - PTA 2***

No. of Results	19
Median	1.080
Norm IQR	0.096
Robust CV	8.9%
Minimum	0.70
Maximum	1.48
Range	0.78
Uncertainty (Median)	0.028

**Note:**

1. Due to an insufficient number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results.

# **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 <sub>10</sub> Result	PTA 2 log <sub>10</sub> Result	PTA 1 Robust z-score	PTA 2 Robust z-score
1	120		120		2.08	2.08	4.14 §	2.69
2A	59	±0.10	89	±0.10	1.77	1.95	1.16	1.10
2B	55	±0.10	99	±0.10	1.74	2.00	0.87	1.66
4	45		66		1.65	1.82	0.03	-0.50
5	54				1.73	N/A	0.79	N/A

**Note:**

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

**Summary Statistics**

**Sample - PTA 1**

No. of Results	25
Median	1.650
Norm IQR	0.104
Robust CV	6.3%
Minimum	1.41
Maximum	2.02
Range	0.61
Uncertainty (Median)	0.026

**Sample - PTA 2**

No. of Results	25
Median	1.860
Norm IQR	0.082
Robust CV	4.4%
Minimum	1.53
Maximum	2.07
Range	0.54
Uncertainty (Median)	0.020

**Note:**

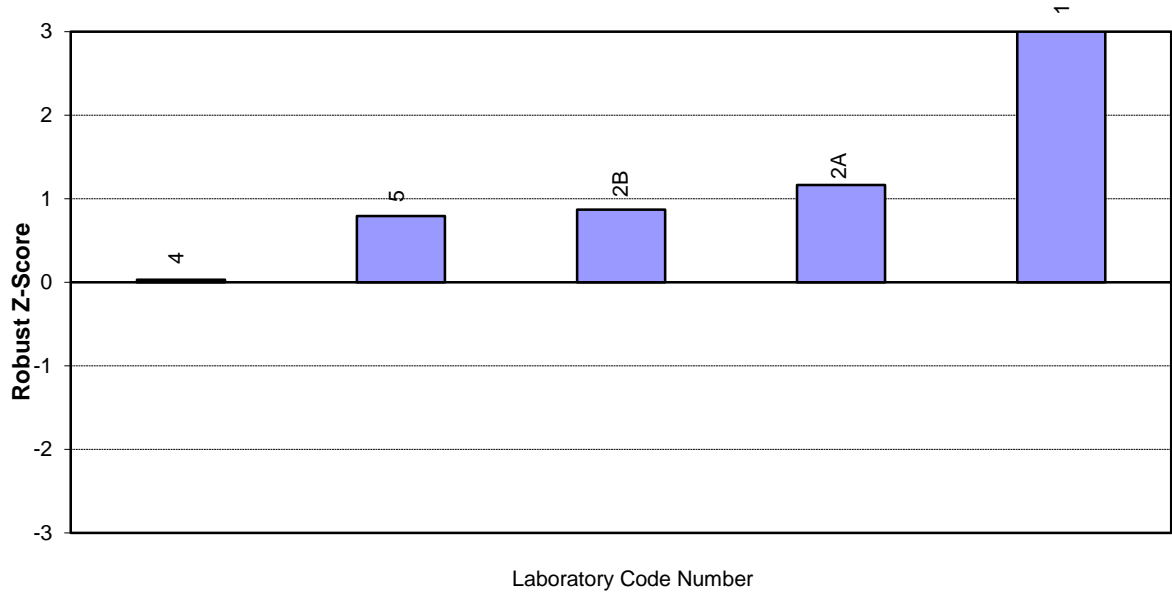
Due to a low number of results reported, robust z-scores were calculated using statistics from Global Proficiency Ltd's results,



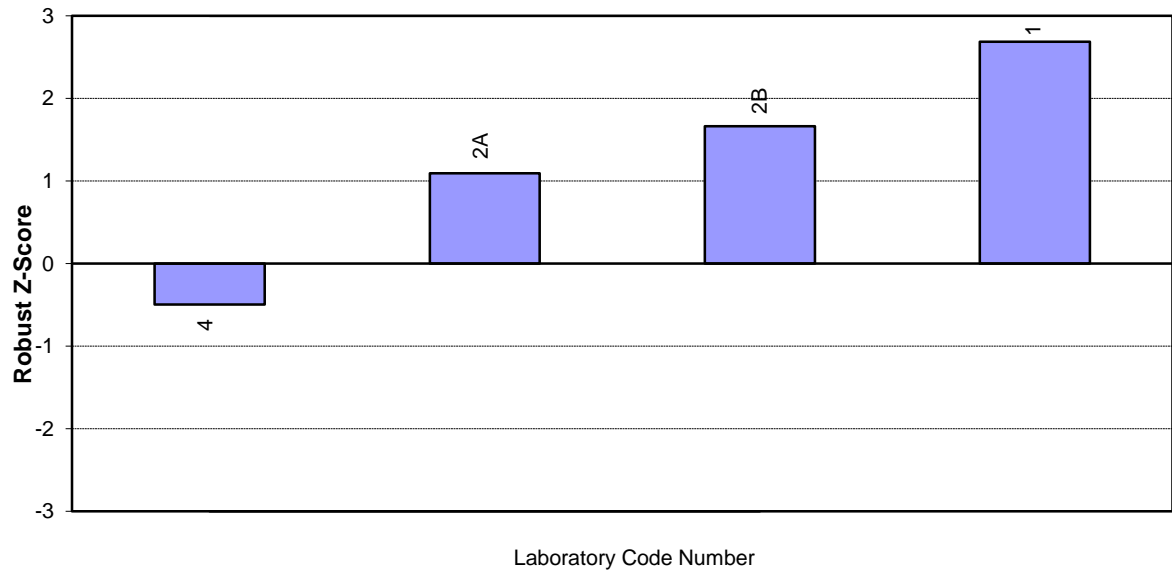
A10.2

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 22 September 2020. When reconstituted and added to the specified volume of sterile water, each sample was representative of a potable water sample.

## **HOMOGENEITY AND STABILITY TESTING**

Global Proficiency Ltd is accredited by International Accreditation New Zealand (IANZ) to ISO/IEC 17043:2010. Global Proficiency Ltd conducted testing on the batches used in this round which found the samples to be homogeneous and stable for the duration of this round.

# **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 69 – SEPTEMBER/OCTOBER 2020

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*, thermotolerant (faecal) coliforms, total coliforms, enterococci, *Pseudomonas aeruginosa*, *Clostridium perfringens* (vegetative cells only\*) and 36°C (or 35-37°C) plate count.

\* **Note:** For the *Clostridium perfringens* (vegetative cells) test, if following AS 4276.17.1 (2016) or any method specific for *C. perfringens* spores, please **omit the sample heat treatment step** (e.g. 60°C for 15min) and proceed to the membrane filtration step as the samples do not contain spores of *C. perfringens*; only vegetative cells.

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 Tuesday 6<sup>th</sup> October 2020 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 69 – SEPTEMBER/OCTOBER 2020****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.

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

**MICROBIOLOGICAL WATERS PROFICIENCY TESTING PROGRAM**

**ROUND 69 SEPTEMBER/OCTOBER 2020**

**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 4276.5-2019 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> AS 4276.21-2019 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 4276.5-2019 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> AS 4276.21-2019 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 4276.5-2019 <input type="checkbox"/> Other:
	MPN					<input type="checkbox"/> AS/NZS 4276.6-2007 <input type="checkbox"/> AS 4276.21-2019 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 4276.9-2019 <input type="checkbox"/> Other:
<i>Pseudomonas aeruginosa</i> (cfu/100mL)	MF					<input type="checkbox"/> AS/NZS 4276.13-2008 <input type="checkbox"/> Other:
<i>Clostridium perfringens</i> <b>(Vegetative cells)</b> (cfu/100mL)	MF					<input type="checkbox"/> AS 4276.17.1-2016 <b>with heat treatment step omitted</b> <input type="checkbox"/> Other:
Plate Count 36°C (or 35- 37°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 --