



Report No. 1031

***Legionella* Proficiency Testing**

Round 54

July 2017

Acknowledgments

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Also thank you to Ms N Patel from the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) who supplied the samples and Ms S Giannoulidis of Global Proficiency Pty Ltd who distributed the samples.

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1. FOREWORD

This report summarises the results of a proficiency testing program on the microbiological analysis of water samples for *Legionella*. It constitutes the fifty-fourth round of an ongoing series of programs.

The program was conducted in March 2017 by Proficiency Testing Australia (PTA). The aim of the program was to assess laboratories' abilities to competently perform the prescribed analyses.

The Program Coordinators were Ms Y Christie and Dr M Li, and the Technical Adviser was Mr F Lagala of SA Pathology Food and Environmental Laboratory. This report was authorised by Mrs F Watton, PTA Quality Manager.

2. FEATURES OF THE PROGRAM

- (a) Participants were provided with three lenticule discs, labelled G100B, G100C and G100D.
- (b) Participants were requested to test the samples for the presence of *Legionella* species, giving quantitative results in (CFU/mL) for:
 - a) total *Legionella*;
 - b) *Legionella pneumophila* (*L. pneumophila*) Serogroup 1;
 - c) *L. pneumophila* other than Serogroup 1; and
 - d) other *Legionella* species.
- (c) A total of seven laboratories received samples, comprising:
 - six Australian participants; and
 - one overseas participant from South Africa.All laboratories returned results for inclusion in the final report.
- (d) Laboratories were provided with the *Instructions to Participants* and *Results Sheet* (see Appendix C). Laboratories were requested to perform the tests according to their routine methods and to record their results on the *Results Sheet*.
- (e) Each laboratory was randomly allocated a unique code number for the program to ensure confidentiality of results. Reference to each laboratory in this report is by code number only.
- (f) For each test per sample, single results were reported by the laboratories, along with details of the test method used. These 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.

- (g) The document entitled *Guide to Proficiency Testing Australia*, 2016 (reference [1]) defines the statistical terms and details the statistical procedures referred to in this report.

3. FORMAT OF THE APPENDICES

- (a) Appendix A contains the following:

- tables of the results reported by laboratories;
- the transformed results, calculated z-scores and table of summary statistics;
- ordered z-score charts; and
- tables of the 'Number of Colonies' data received.

A description of the statistics and graphical displays used can be found at the beginning of Appendix A. Further details about the statistics and graphical displays, including guidance on their interpretation, may be found in the *Guide to Proficiency Testing Australia* [1].

- (b) Appendix B contains details of the homogeneity and stability testing.
- (c) Appendix C contains copies of the *Instructions to Participants* and *Results Sheet*.

4. STATISTICAL DESIGN OF THE PROGRAM

For this proficiency testing program, samples were provided by the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) in the form of lenticule discs.

Sample G100B contained *Legionella pneumophila* serogroup 1, *Ralstonia pickettii* and *Escherichia coli*.

Sample G100C contained *Legionella jordanis*, *Morganella morganii* and *Roseomonas aestuarii*.

Sample G100D contained *Legionella pneumophila* serogroup 2-14, *Escherichia coli* and *Acinetobacter junii*.

5. OUTLIER RESULTS AND SUMMARY STATISTICS

In order to achieve the aim of the program - assessing laboratories' testing performance - use has been made of a robust z-score technique. These z-scores are used to detect excessively large variations between the individual laboratory and the overall consensus.

Any result which has an absolute z-score value greater than or equal to 3.0 (i.e. $Z \leq -3.0$ or $Z \geq 3.0$) is classified as an outlier. For further details on the calculation and interpretation of the robust z-scores, please see the *Guide to Proficiency Testing Australia* [1].

In addition to statistical outliers, other types of outliers are false positives/negatives - i.e. when a laboratory erroneously reports the presence/absence of an organism or species which is/is not present. In this round, there were two Z - scores > 3.0 and zero false negative/positive results were reported. Tables listing the outlier results are on page 4.

The following is a summary of the results for the program.

TABLE A – TOTAL *LEGIONELLA* - $\text{LOG}_{10}(\text{CFU/mL})$ SUMMARY STATISTICS

Statistic	Sample G100B	Sample G100C	Sample G100D
Median	2.000	3.176	3.690
Normalised IQR	0.282	0.193	0.469
Robust CV	14.1%	6.1%	12.7%
Uncertainty (Median)	0.134	0.091	0.222

SUMMARY OF OUTLIER RESULTS

Code numbers of the laboratories whose results have been identified as outliers appear in Tables B and C, i.e. these laboratories have either statistical outliers (identified by the robust z-scores technique) and/or false positives/negatives.

TABLE B - FALSE POSITIVE AND FALSE NEGATIVE RESULTS

Test	<u>Sample G100B</u> Laboratory Code Number	<u>Sample G100C</u> Laboratory Code Number	<u>Sample G100D</u> Laboratory Code Number
<i>L. pneumophila</i> serogroup 1 FALSE NEGATIVE	-	-	-
<i>L. pneumophila</i> serogroup 1 FALSE POSITIVE	-	-	-
<i>L. pneumophila</i> <u>Other than</u> serogroup 1 FALSE NEGATIVE	-	-	-
<i>L. pneumophila</i> <u>Other than</u> serogroup 1 FALSE POSITIVE	-	-	-
Other <i>Legionella</i> species FALSE POSITIVE	-	-	-
Other <i>Legionella</i> species FALSE NEGATIVE	-	-	-

TABLE C - STATISTICAL OUTLIERS - TOTAL *LEGIONELLA*

Total <i>Legionella</i>	Sample G100B	Sample G100C	Sample G100D
Robust Z-Score	-	#	#

Note: # See comments in Section 6.

6. PTA AND TECHNICAL ADVISER'S COMMENTS

A brief summary of the results appear in Table A. Outlier and false results identified appear in Tables B and C. Complete details of the results received and the statistical analyses appear in Appendix A. Commentary on each of the tests is presented below.

Of the seven sets of results, one laboratory reported two Z-scores > 3.0 for the enumeration of *Legionella* (laboratory 3). (See comments below.) No laboratories reported false results for the detection of *Legionella*.

***Legionella* Enumeration Results**

(refer Appendix A: pages A1 to A9)

Six *Legionella* results were obtained using the standard method AS/NZS 3896:2008 (method code 1), while one result was obtained using the standard method ISO 11731:1998 (method code 2).

Histograms were used to check for the normal distribution of data and upon inspection of the histograms it was found that the data was normally distributed.

Laboratories were also requested to report Measurement Uncertainty (MU) for each reported result. These values are tabulated in Appendix A as reported by the participants.

Sample G100B contained *Legionella pneumophila* serogroup 1, *Aeromonas hydrophila* and *Escherichia coli*. Results reported were evaluated as being satisfactory.

Sample G100C contained *Legionella jordanis*, *Morganella morganii* and *Roseomonas aestuarii*. One laboratory (laboratory 3) obtained a Z-scores > 3.0.

Sample G100D contained *Legionella pneumophila* serogroup 2-14, *Escherichia coli* and *Acinetobacter junii*. One laboratory (laboratory 3) reported a Z-score > 3.0.

It is noted that laboratory 3 received their samples late due to transport delays. As the correct storage of samples (and therefore continued viability) during this time period can not be confirmed, it was considered unfair to classify the aberrant results of laboratory 3 as outliers. Further, technical review deemed the results submitted by laboratory 3 as unusual, with the possibility of a dilution or calculation error occurring. Laboratory 3 should investigate this possibility.

Results of Serotyping (refer Appendix A: page A1)

The serotyping results for all laboratories were considered satisfactory, with the exception of one laboratory (code 3). This laboratory reported two outliers for Total *Legionella* with regard to samples G100C and G100D.

Number of Colonies

(refer Appendix A: page A9)

Laboratories were requested to report the number of colonies picked for confirmation and confirmed as *Legionella*, for each plate and dilution. These counts provide a summary of the level of examination performed by laboratories on the growth seen on plates.

The tables indicate that most laboratories appear to be examining sufficient representative colonies as described in AS/NZS 3896:2008 (reference [2]). The AS standard requires up to three colonies are to be confirmed from each plate. Participants are reminded to complete the *Results Sheet* in its entirety.

Analysis of Grouped Methods

In order for methods to be grouped for analysis, PTA requires at least 11 sets of results from the same method group. As there were less than 11 sets of results submitted, and due to the statistical analysis employed, the analysis of grouped methods does not apply for this round.

Metrological Traceability

Consensus values (median) derived from participants' results are used in this program. These values are not metrologically traceable to an external reference.

Prepared simulated samples (lenticule discs) used for this program were provided by the FEPTU of PHE and were prepared according to their standard operating procedures. FEPTU is accredited to ISO/IEC 17043:2010 by the United Kingdom Accreditation Service (UKAS).

As the assigned value for this program is the median of the results submitted by the participants, the uncertainty of the median has been calculated for samples G100B, G100C and G100D and is tabulated in Table A: Total *Legionella* Summary Statistics, and also in the summary statistics tables in Appendix A.

Measurement Uncertainty (MU)

Around 85.7% of participants in this round reported the measurement uncertainty (MU) associated with their results.

Some laboratories may have notably underestimated their MU, as they indicated that their MU was less than two times the uncertainty of the median, and their results were further from the median than this value.

Conversely, laboratories which indicated a MU which was greater than three times the normalised IQR may have overestimated their MU.

7. REFERENCES

- [1] *Guide to Proficiency Testing Australia 2016* (This document can be found on the PTA website, www.pta.asn.au).
- [2] *AS/NZS 3896:2008 Waters – Examination for Legionella spp. Including Legionella pneumophila.*

APPENDIX A

**Tables of Results and Z-scores,
Summary Statistics, Z-score Charts
and
Number of Colonies Confirmed**

APPENDIX FORMAT(a) Results Submitted

These tables contain the results returned by each laboratory, including MU and serotyping results for total *Legionella*.

Summary Statistics

The table of summary statistics consists of:

- (i) the number of results for that test/sample (*No. of Results*);
- (ii) the median of these results – i.e. the middle value (*Median*);
- (iii) the normalised interquartile range of the results (*Normalised IQR*);
- (iv) the robust coefficient of variation, expressed as a percentage (*Robust CV*) – i.e. $100 \times \text{Normalised IQR} \div \text{Median}$;
- (v) the minimum and maximum laboratory results;
- (vi) the range (*Maximum - Minimum*); and
- (vii) the uncertainty of the median; a robust estimate of the standard deviation of the *Median*.

The median is the middle result. It is a measure of the centre of the data and is similar to the mean (or average).

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.

(b) Transformed Results and Z-Scores

These tables contain the transformed (\log_{10}) total *Legionella* results and the calculated z-scores.

Outliers are identified in the table by a marker “§” next to the relevant z-score.

A(II)

Please see the *Guide to Proficiency Testing Australia* [1] for further details on how these z-scores are calculated.

(c) Ordered Z-Score Charts

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

These charts contain solid lines at +3 and -3, so the outliers are clearly identifiable as the laboratories whose "bar" extends beyond these "cut-off" lines.

Further details of the construction and interpretation of these diagrams is given in the *Guide to Proficiency Testing Australia* [1].

(d) Number of Colonies Confirmed

These tables contain the number of colonies picked for confirmation and confirmed as *Legionella*, for each plate and dilution, reported by each laboratory.

***Legionella* Results**

LEGIONELLA - RESULTS SUBMITTED (CFU/mL)

Lab Code	Total <i>Legionella</i>					
	Sample G100B	MU	Sample G100C	MU	Sample G100D	MU
1	400	0.59	1500	0.59	5800	0.59
2	100	0.2 log 10	1200	0.2 log 10	8000	0.2 log 10
3	78	-	10	-	5	-
4	110	73 -167	1500	790 - 2900	3900	1800 - 8100
5	20	±4	700	±135	770	±149
6	300	0.2 log 10	1600	0.2 log 10	4900	0.2 log 10
7	100	0.2 log 10	1500	0.2 log 10	5700	0.2log 10

Method Code: 1 = AS/NZS 3896:2008 (standard method)
2 = ISO 11731:1998

The above results are tabulated as reported on the results sheet.

Lab Code	<i>L. pneumophila</i> Serogroup 1					
	Sample G100B	MU	Sample G100C	MU	Sample G100D	MU
1	400	0.59	<10	0.59	<10	0.59
2	100	0.2 log 10	<10	0.2 log 10	<10	0.2 log 10
3	78	-	0	-	5	-
4	110	73 - 167	<10	-	<10	-
5	20	±4	<10	±2	<10	±2
6	300	0.2 log 10	<10	0.2 log 10	<10	0.2 log 10
7	100	0.2 log 10	<10	0.2 log 10	<10	0.2 log 10

The above results are tabulated as reported on the results sheet.

A2

Lab Code	<i>L. pneumophila</i> other than serogroup 1					
	Sample G100B	MU	Sample G100C	MU	Sample G100D	MU
1	<10	0.59	<10	0.59	5800	0.59
2	<10	0.2 log 10	<10	0.2 log 10	8000	0.2 log10
3	0	-	10	-	0	-
4	<10	-	<10	-	3 900	1880 - 8100
5	<10	±2	<10	±2	770	±149
6	<10	0.2 log 10	<10	0.2 log 10	4900	0.2 log 10
7	<10	0.2 log 10	<10	0.2 log 10	5700	0.2 log 10

The above results are tabulated as reported on the results sheet.

Lab Code	Other <i>Legionella</i> species					
	Sample G100B	MU	Sample G100C	MU	Sample G100D	MU
1	<10	0.59	1500	0.59	<10	0.59
2	<10	0.2 log 10	1200	0.2 log 10	<10	0.2 log 10
3	0	-	10	-	0	-
4	<10	-	1500	790 - 2900	<10	-
5	<10	±2	700	±135	<10	±2
6	<10	0.2 log 10	1600	0.2 log 10	<10	0.2 log 10
7	<10	0.2 log 10	1500	0.2 log 10	<10	0.2 log 10

The above results are tabulated as reported on the results sheet.

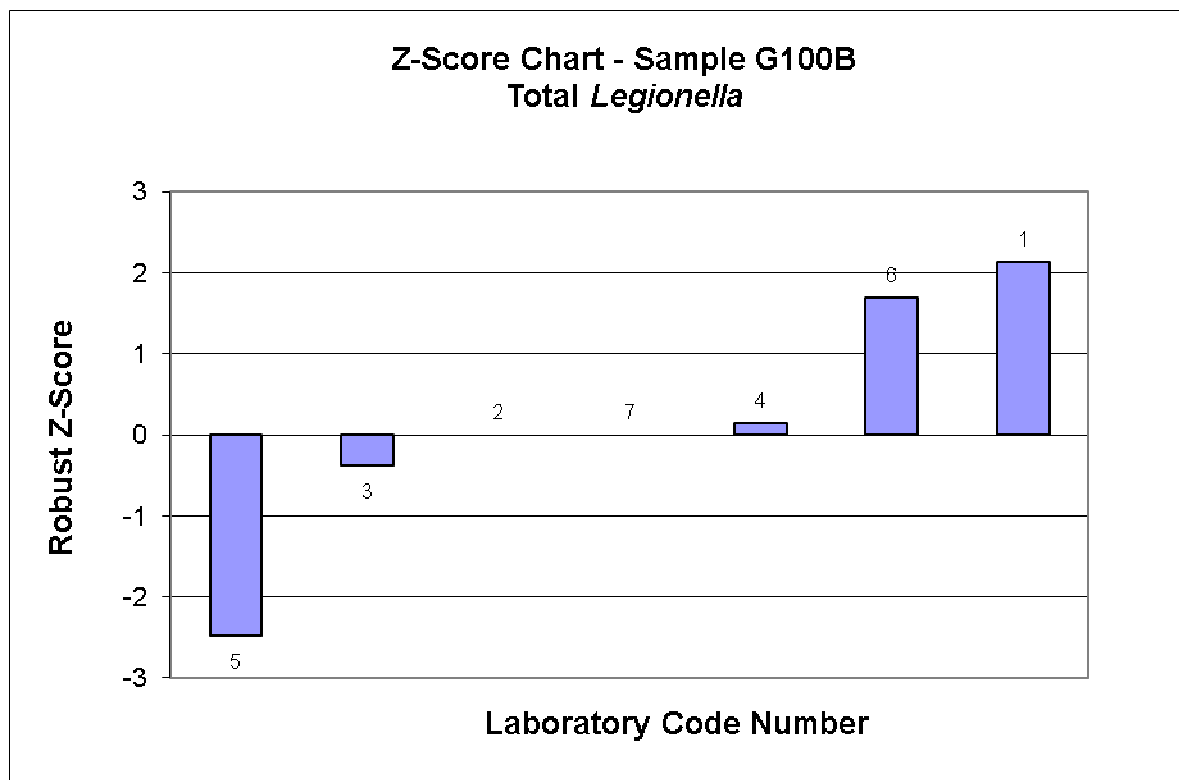
**TOTAL *LEGIONELLA* (Sample G100B)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G100B	Total <i>Legionella</i> [log(CFU/mL)]	Robust z-score	
1	400	2.60	2.13	
2	100	2.00	0.00	
3	78	1.89	-0.38	
4	110	2.04	0.15	
5	20	1.30	-2.48	
6	300	2.48	1.69	
7	100	2.00	0.00	

SUMMARY STATISTICS

No. of Results	7
Median	2.000
Normalised IQR	0.282
Robust CV	14.1%
Minimum	1.30
Maximum	2.60
Range	1.30
Uncertainty (Median)	0.134

**TOTAL *LEGIONELLA* (Sample G100B)
ORDERED ROBUST Z-SCORE CHART**



**TOTAL *LEGIONELLA* (Sample G100C)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

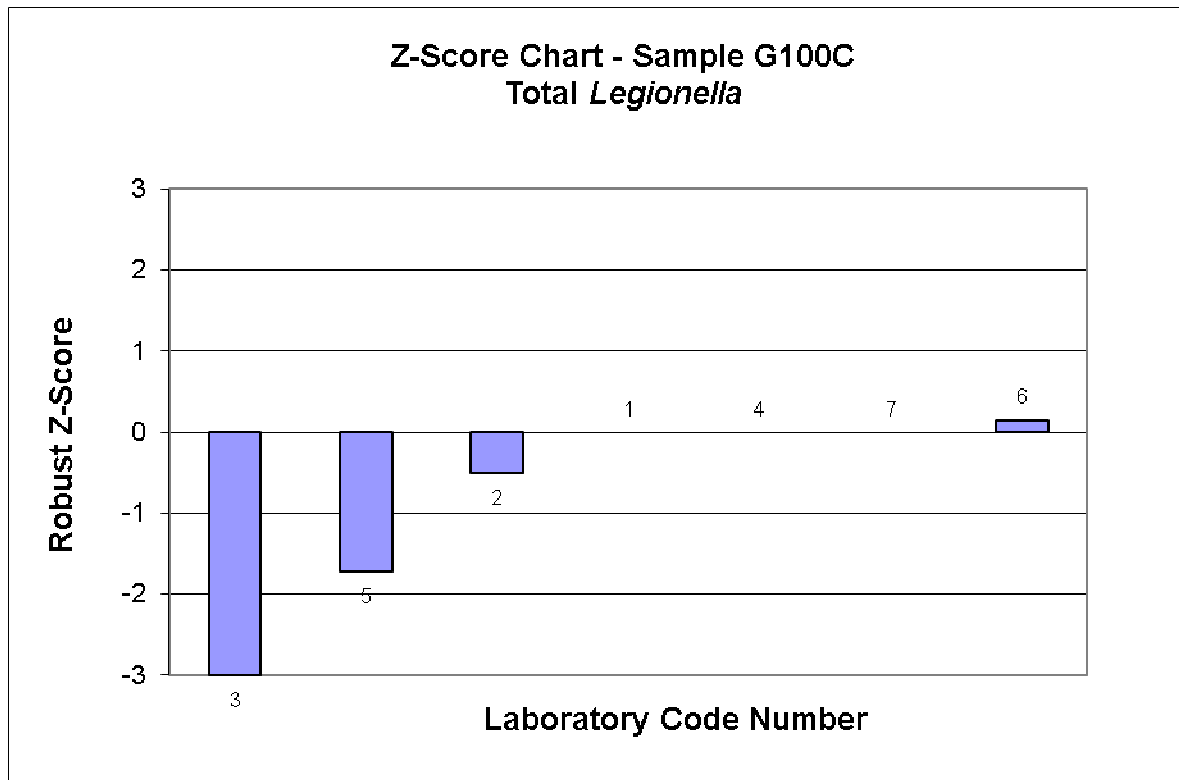
Lab Code	Sample G100C	Total <i>Legionella</i> [log(CFU/mL)]	Robust z-score
1	1500	3.18	0.00
2	1200	3.08	-0.50
3	10	1.00	-11.29 #
4	1500	3.18	0.00
5	700	2.85	-1.72
6	1600	3.20	0.15
7	1500	3.18	0.00

SUMMARY STATISTICS

No. of Results	7
Median	3.176
Normalised IQR	0.193
Robust CV	6.1%
Minimum	1.00
Maximum	3.20
Range	2.20
Uncertainty (Median)	0.091

Note: # This result was not classified as an outlier (See comments in Section 6).

**TOTAL *LEGIONELLA* (Sample G100C)
ORDERED ROBUST Z-SCORE CHART**



**TOTAL *LEGIONELLA* (Sample G100D)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

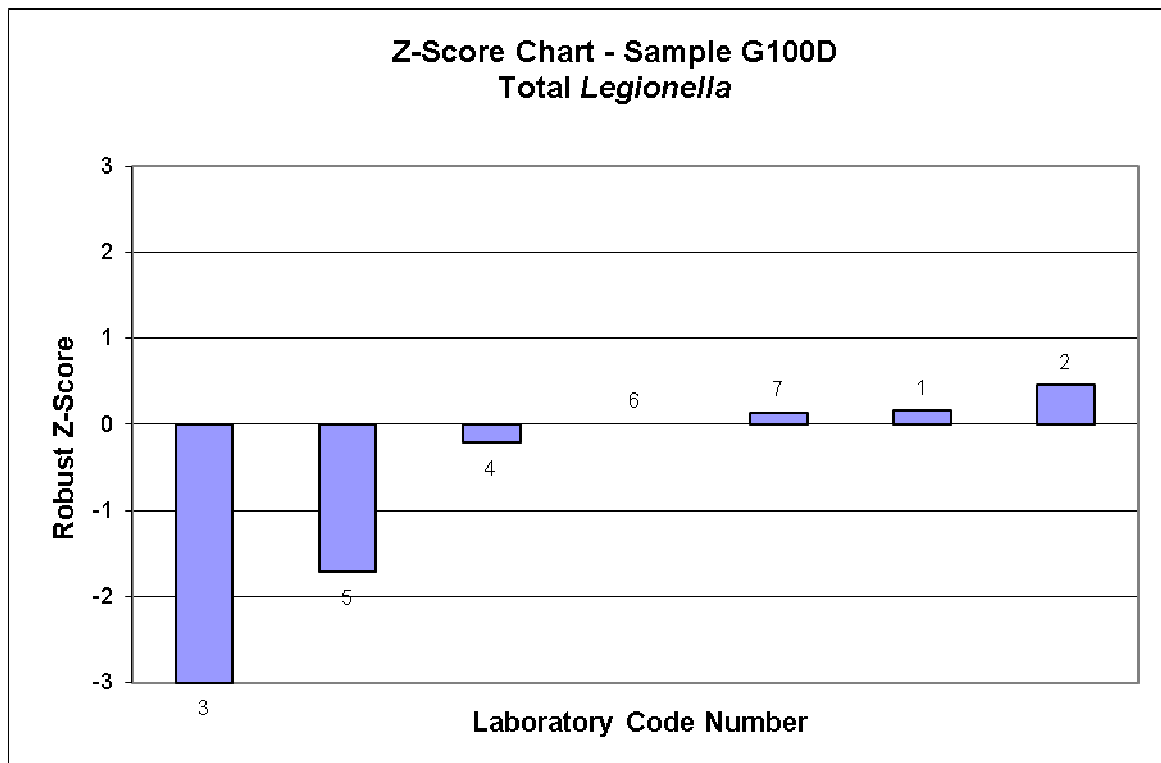
Lab Code	Sample G100D	Total <i>Legionella</i> [log(CFU/mL)]	Robust z-score	
1	5800	3.76	0.16	
2	8000	3.90	0.45	
3	5	0.70	-6.37	#
4	3900	3.59	-0.21	
5	770	2.89	-1.71	
6	4900	3.69	0.00	
7	5700	3.76	0.14	

SUMMARY STATISTICS

No. of Results	7
Median	3.690
Normalised IQR	0.469
Robust CV	12.7%
Minimum	0.70
Maximum	3.90
Range	3.20
Uncertainty (Median)	0.222

Note: # This result was not classified as an outlier (See comments in Section 6).

**TOTAL *LEGIONELLA* (Sample G100D)
ORDERED ROBUST Z-SCORE CHART**



NUMBER OF COLONIES CONFIRMED

Lab Code	Direct inoculation 0.1 mL						Direct inoculation 0.01 mL					
	G100B		G100C		G100D		G100B		G100C		G100D	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	3	3	3	3	5	5	1	1	0	0	0	0
2	1	0	3	3	3	3	1	1	0	0	3	3
3	-	-	-	-	-	-	-	-	-	-	-	-
4	2/11	2/11	2/148	2/148	2/152	2/152	-	-	-	-	1/3 + 2/36	1/3 + 2/36
5	2	2	1	1	1	1	-	-	-	-	1	1
6	1	0	3	0	3	3	0	0	0	0	3	3
7	1	1	1	1	1	1	1	1	0	0	1	1

NUMBER OF COLONIES CONFIRMED

Lab Code	Heat treated 0.1 mL						Heat treated 0.01 mL					
	G100B		G100C		G100D		G100B		G100C		G100D	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	0	0	3	3	2	2	0	0	2	2	0	0
2	1	0	3	3	3	3	0	0	3	3	0	0
3	-	-	-	-	-	-	-	-	-	-	-	-
4	1/6	1/6	2/141	2/141	2/130	2/130	1/1	1/1	1/9	1/9	1/5	1/5
5	-	-	1	1	-	-	-	-	1	1	-	-
6	0	0	3	3	3	3	0	0	3	3	2	2
7	1	1	1	1	1	1	0	0	1	1	1	1

NUMBER OF COLONIES CONFIRMED

Lab Code	Acid treatment						Other plate/s or dilutions					
	G100B		G100C		G100D		G100B		G100C		G100D	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	0	0	0	0	3	3	0	0	0	0	0	0
2	0	0	0	0	3	3	0	0	1	1	3	3
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	1/8 + 2/28	1/8 + 2/28	N/A	-	N/A	-	N/A	-
5	-	-	-	-	1	1	-	-	-	-	-	-
6	3	3	0	0	3	3	0	0	1	1	3	3
7	0	0	0	0	1	1	0	0	0	0	0	0

The tables above show the quantified results only, as requested in the *Instructions to Participants*. The results are tabulated as reported on the *Results Sheet*.

APPENDIX B

Homogeneity and Stability Testing

Homogeneity and Stability Testing

The PHU- FEPTU - External Quality Assessment Scheme for *Legionella* Isolation from Water Samples is accredited by the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010. Samples provided for this round are simulated samples. PHE conducted testing which found the samples to be homogeneous and stable for the duration of this round.

APPENDIX C

Documentation

Instructions to Participants	C1
Results Sheet	C3



Proficiency Testing Australia

Legionella Proficiency Testing Program
Round 54 (Lenticule discs)

INSTRUCTIONS TO PARTICIPANTS

To ensure that results obtained from this program can be analysed properly, participants are asked to adhere carefully to the following instructions.

1. Each participant is supplied with three LENTICULE discs in screw cap plastic vials (with dessicant). The LENTICULE discs require reconstitution by a process of re-hydration and dispersion prior to examination for *Legionella*.
 2. **Storage:**
 - a) Store the samples at **-20 ± 5°C** on receipt.
 - b) Allow the LENTICULE discs to reach ambient temperature (5 – 10 minutes) before reconstituting in diluent.
 3. **Reconstitution:**
 - a) Open the sample container and transfer the LENTICULE disc into **100mL** of diluent (peptone saline) by inverting the container over the diluent.
 - b) Leave at ambient temperature for a minimum of 10 minutes to re-hydrate. Ensure that the LENTICULE disc has dissolved completely before proceeding.
 - c) Disperse the inoculum by inverting approximately 30 times.
- Each reconstituted LENTICULE disc is equivalent to a 100mL water sample.**
4. Laboratories are requested to test the samples for the presence of *Legionella* species, giving quantitative (in CFU/mL) results for:
 - a) total *Legionella*;
 - b) *L. pneumophila* serogroup 1;
 - c) *L. pneumophila* other than serogroup 1; and
 - d) other *Legionella* species.

Participants are also requested to provide details of the test methods used and the *Legionella* identification kit including the use by date.

If your laboratory is unable to perform any of these tests please note this on your Results Sheet. Note that counts reported as “greater than” (e.g. > 20,000) are NOT acceptable, as they cannot be included in the statistical analysis.

5. 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 $k \approx 2$). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.
6. Laboratories are required to follow their routine test methods.
7. Your laboratory has been allocated the code number shown on the attached Results Sheet. All reference to your laboratory in the final report for this program will be through this code number, thus ensuring the confidentiality of your results.
8. All laboratories must return the Results Sheet no later than **Friday 17th March 2017** to:

Yvette Christie
Proficiency Testing Australia
PO Box 7507, Silverwater NSW 2128 Australia
Phone: +61 2 9739 8295
Fax: +61 2 9743 6664
Email: yvette.christie@pta.asn.au

Proficiency Testing Australia

Legionella Proficiency Testing Program – Round 54

Results Sheet

Lab Code

Samples received at _____ (time) on _____ (date) Temperature of samples on arrival _____

	<u>Sample G100B</u>		<u>Sample G100C</u>		<u>Sample G100D</u>	
<u>RESULTS</u> (CFU/mL)	Result	MU	Result	MU	Result	MU
Total <i>Legionella</i>						
<i>L. pneumophila</i> Serogroup 1						
<i>L. pneumophila</i> <u>Other than</u> serogroup 1						
Other <i>Legionella</i> species						

Testing commenced at _____ (time) on _____ (date) in _____ (city)

Test ☐ 1. AS/NZS 3896:2008

Method: ☐ 2. Other – please specify, outlining the method used: _____

Legionella identification kit and use by date – please specify: _____

NO. OF COLONIES	<u>Sample G100B</u>		<u>Sample G100C</u>		<u>Sample G100D</u>	
Number of colonies...	..picked for confirm.	..confirm.as <i>Legionella</i>	..picked for confirm.	..confirm.as <i>Legionella</i>	..picked for confirm.	..confirm.as <i>Legionella</i>
Direct inoculation plate - 0.1 mL						
Direct inoculation plate - 0.01 mL						
Heat treated sample plate - 0.1 mL						
Heat treated sample plate - 0.01 mL						
Acid treatment plate						
Other plate/s or dilutions						

Signature: _____ Date: _____

Please return results **NO LATER THAN FRIDAY 17th March 2017** to:

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