



Report No. 1141

***Legionella* Proficiency Testing**

Round 58

July 2019

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-eighth 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 April 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 Ms Y Christie and the Technical Adviser was Mr F Lagala of SA Pathology Food and Environmental Laboratory. This report was authorised by Mrs K Cividin, PTA Quality Manager.

2. FEATURES OF THE PROGRAM

- (a) Participants were provided with three lenticule discs, labelled G112A, G112B and G112C.
- (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 nine laboratories received samples, comprising:
 - five Australian participants; and
 - four overseas participants including two from New Zealand, one from South Africa and one from Indonesia.All laboratories returned results for inclusion in the final report, with one laboratory neglecting to submit results for their requested second set of samples.
- (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, where applicable.

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 where appropriate:

- 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 G112A contained *Legionella pneumophila* serogroup 1 and *Brevundimonas vesicularis*.

Sample G112B contained *Legionella pneumophila* serogroup 2-15, *Aeromonas hydrophila* and *Pseudomonas lundensis*.

Sample G112C contained *Legionella bozemanii*, *Acinetobacter junii* and *Pseudomonas lundensis*.

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 three Z - score > 3.0 and three false negative results reported. Tables listing the outlier results are on page 4.

The Robust CV of samples G112A and G112C were high. Target CV's were considered, however were not applied in this round.

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

TABLE A – TOTAL *LEGIONELLA* - LOG₁₀(CFU/mL) SUMMARY STATISTICS

Statistic	Sample G112A	Sample G112B	Sample G112C
Median	2.301	2.301	2.301
Normalised IQR	0.549	0.228	0.417
Robust CV	23.9%	9.9%	18.1%
Uncertainty (Median)	0.229	0.095	0.174

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 G112A</u> Laboratory Code Number	<u>Sample G112B</u> Laboratory Code Number	<u>Sample G112C</u> Laboratory Code Number
<i>L. pneumophila</i> serogroup 1 FALSE NEGATIVE	9	-	-
<i>L. pneumophila</i> serogroup 1 FALSE POSITIVE	-	-	9
<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	9	-	-
Other <i>Legionella</i> species FALSE NEGATIVE	-	-	4, 9

TABLE C - STATISTICAL OUTLIERS - TOTAL *LEGIONELLA*

Total <i>Legionella</i>	Sample G112A	Sample G112B	Sample G112C
Robust Z-Score	5A	5A, 9	-

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 nine sets of results, two laboratories reported a Z-score >3.0 for the enumeration of *Legionella* (laboratory codes 5A and 9). For further information, please see comments below. Two laboratories reported false negative results for the detection of *Legionella* (laboratory codes 4 and 9).

***Legionella* Enumeration Results**

(refer Appendix A: pages A1 to A12)

Four *Legionella* results were obtained using the standard method AS/NZS 3896:2017 [method code 1]. Five results were obtained using other (method code 2) three of which was AS/NZS 3896:2008, while the other two consisted of "detection and enumeration of Legionella (GVPC)" and SM 9260 J - 2017. It is recommended that laboratories using the AS3896 method, should now be using the latest method - AS3896:2017.

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 G112A contained *Legionella pneumophila* serogroup 1 and *Brevundimonas vesicularis*. One participant (laboratory code 5A) obtained an outlier, whilst another participant (laboratory code 9) submitted a false negative result for the analysis of *Legionella pneumophila* serogroup 1 and a false positive for Other *Legionella* species.

Sample G112B contained *Legionella pneumophila* serogroup 2 to 15, *Aeromonas hydrophila* and *Pseudomonas lundensis*. Two participants (laboratory codes 5A and 9) obtained an outlier as their Z-score >3.0.

Sample G112C contained *Legionella bozemanii*, *Acinetobacter junii* and *Pseudomonas lundensis*. No laboratory reported an outlier result. One participant (laboratory code 9) reported a false positive for *Legionella pneumophila* serogroup 1, and a false negative for Other *Legionella* species.

Results of Serotyping (refer Appendix A: page A1)

The serotyping results for all laboratories were considered satisfactory, with the exception of two participants (laboratory codes 4 and 9). Laboratory code 4 reported a false negative result for Other *Legionella* with regard to sample G112C. Laboratory code 9 reported a false negative result for *Legionella pneumophila* serogroup 1 (sample G112A) and a false negative result for Other *Legionella* (G112A). Laboratory code 9 also reported a false positive result for *Legionella pneumophila* serogroup 1 (G112C) and a false negative result for Other *Legionella* species (G112C). With regards to the identification kit used, laboratory code 4 used a Liofilm Chemistry Diagnostic Latex kit, whilst laboratory 9 did not divulge what type of identification was used.

Most participating laboratories used the Microgen Legionella Latex Test or the Oxoid kit for identification.

Participants are reminded to check the labels on samples prior to testing.

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 do not appear to be examining sufficient representative colonies as described in AS/NZS 3896:2017 (reference [2]). The AS standard requires up to three colonies 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 G112A, G112B and G112C and is tabulated in Table A: Total *Legionella* Summary Statistics, and also in the summary statistics tables in Appendix A.

Measurement Uncertainty (MU)

Approximately 47.2% of participants in this round reported the measurement uncertainty (MU) associated with their results. This is an increase in comparison to Round 57 where 22.2% of participants reported their calculated MU.

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 3896:2017 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 G112A	MU	Sample G112B	MU	Sample G112C	MU	Method Code
1	340	2	310	2	300	2	2
2	500	300-840	500	240-1050	200	130-320	2
3	30	-	100	-	100	-	1
4	300	-	300	-	200	-	2
5A	2	2-2	5	4-6	26	21-32	2
5B							
6	120	0.2 log 10	190	0.2 log 10	140	0.2 log 10	1
7	80	±15	200	±39	60	±12	1
8	200	0.22	170	0.22	300	0.22	1
9	1200	-	1600	-	580	-	2

Method Code: 1 = AS/NZS 3896:2017
2 = Other

The above results are tabulated as reported on the *Results Sheet*.

Lab Code	<i>L. pneumophila</i> Serogroup 1					
	Sample G112A	MU	Sample G112B	MU	Sample G112C	MU
1	340	2	-	-	-	-
2	500	300-840	<10	-	<10	-
3	30	-	<10	-	<10	-
4	300	-	0	-	0	-
5A	-	-	-	-	-	-
5B	-	-	-	-	-	-
6	120	0.2 log 10	<10	0.2 log 10	<10	0.2 log 10
7	80	±15	<10	±2	<10	±2
8	200	0.22	<10	0.22	<10	0.22
9	0	-	0	-	580	-

The above results are tabulated as reported on the *Results Sheet*.

Lab Code	<i>L. pneumophila</i> other than Serogroup 1					
	Sample G112A	MU	Sample G112B	MU	Sample G112C	MU
1	-	-	310	2	-	-
2	<10	-	500	240-1050	<10	-
3	<10	-	100	-	<10	-
4	0	-	300	-	0	-
5A	-	-	L.pneumophila serogroup 2-14	-	-	-
5B	-	-	-	-	-	-
6	<10	0.2 log 10	190	0.2 log 10	<10	0.2 log 10
7	<10	±2	200	±39	<10	±2
8	<10	0.22	170	0.22	<10	0.22
9	0	-	1600	-	0	-

The above results are tabulated as reported on the *Results Sheet*.

Lab Code	Other <i>Legionella</i> species					
	Sample G112A	MU	Sample G112B	MU	Sample G112C	MU
1	-	-	-	-	300	2
2	<10	-	<10	-	200	130-320
3	<10	-	<10	-	100	-
4	0	-	0	-	200	-
5A	-	-	-	-	-	-
5B	-	-	-	-	-	-
6	<10	0.2 log 10	<10	0.2 log 10	140	0.2 log 10
7	<10	±2	<10	±2	60	±12
8	<10	0.22	<10	0.22	300	0.22
9	1200	-	0	-	0	-

The above results are tabulated as reported on the *Results Sheet*.

**TOTAL LEGIONELLA (Sample G112A)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

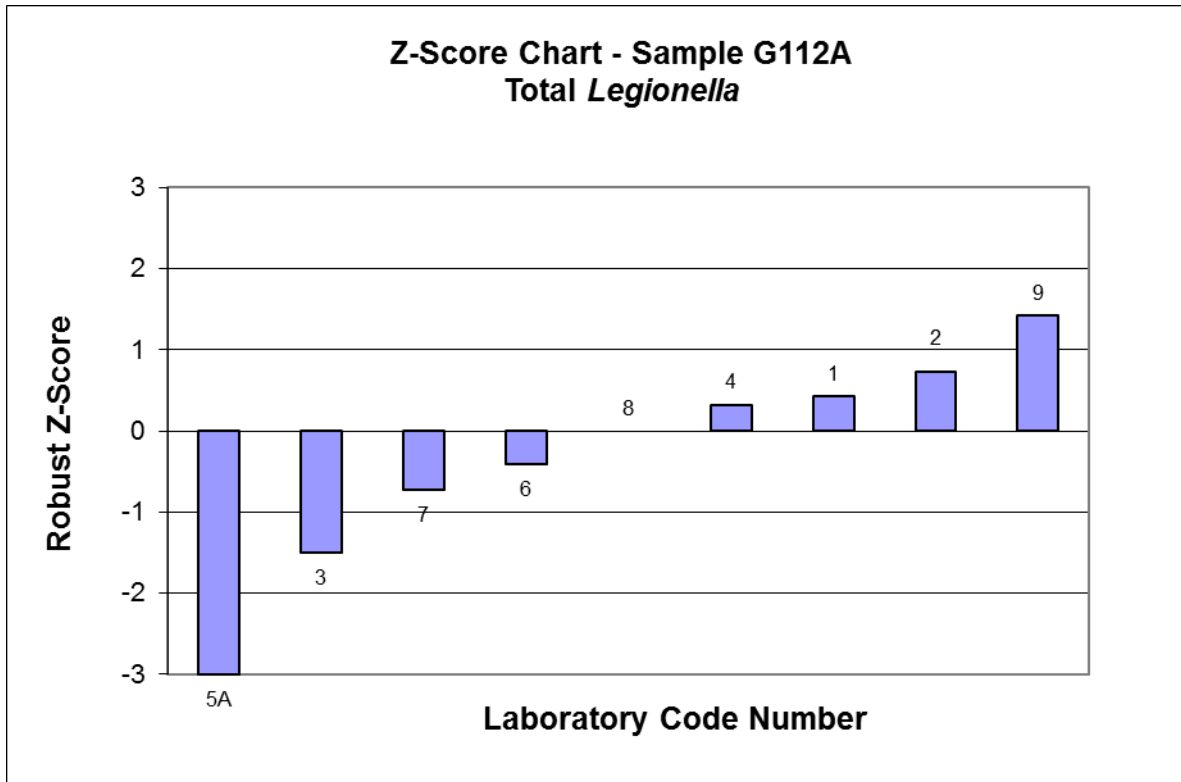
Lab Code	Sample G112A	Total <i>Legionella</i> [log(CFU/mL)]	Robust z-score	
1	340	2.53	0.42	
2	500	2.70	0.72	
3	30	1.48	-1.50	
4	300	2.48	0.32	
5A	2	0.30	-3.64	§
5B				
6	120	2.08	-0.40	
7	80	1.90	-0.72	
8	200	2.30	0.00	
9	1200	3.08	1.42	

§ Denotes an outlier result

SUMMARY STATISTICS

No. of Results	9
Median	2.301
Normalised IQR	0.549
Robust CV	23.87%
Minimum	0.30
Maximum	3.08
Range	2.78
Uncertainty (Median)	0.229

**TOTAL *LEGIONELLA* (Sample G112A)
ORDERED ROBUST Z-SCORE CHART**



Please note laboratory code 5B did not submit results.

**TOTAL *LEGIONELLA* (Sample G112B)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

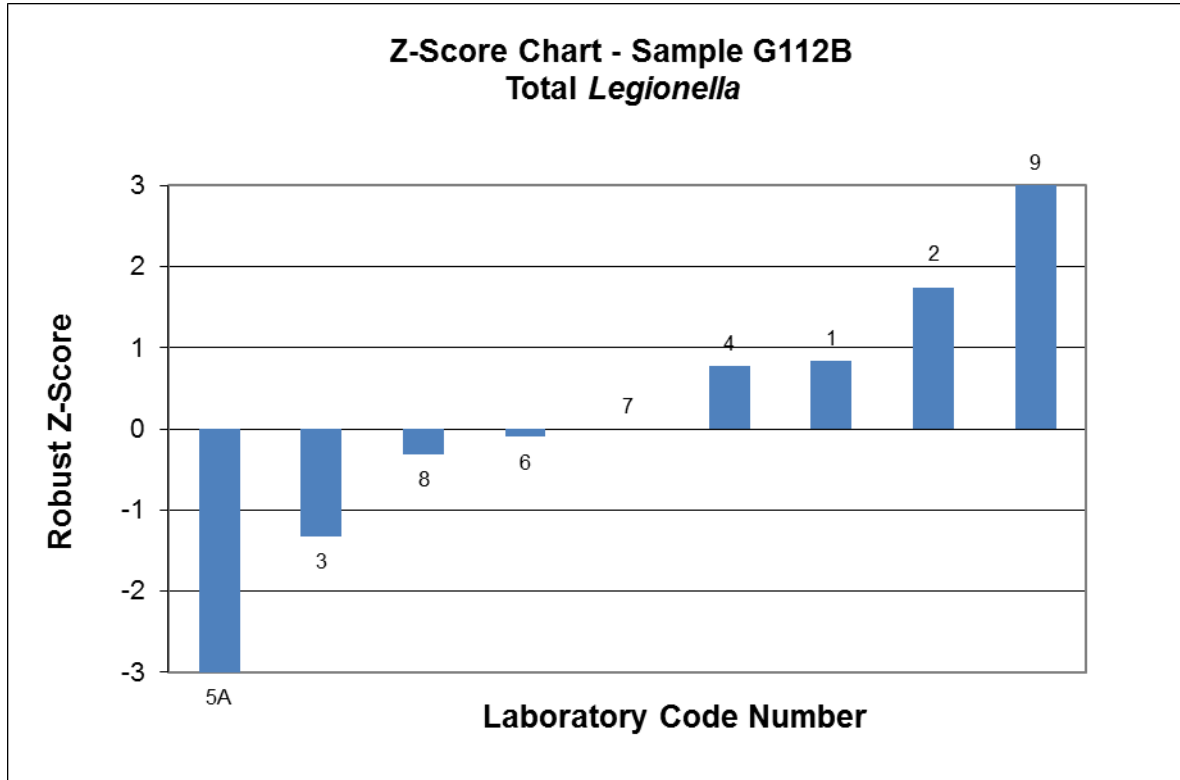
Lab Code	Sample G112B	Total <i>Legionella</i> [$\log(\text{CFU/mL})$]	Robust z-score	
1	310	2.49	0.83	
2	500	2.70	1.74	
3	100	2.00	-1.32	
4	300	2.48	0.77	
5A	5	0.70	-7.02	§
5B				
6	190	2.28	-0.10	
7	200	2.30	0.00	
8	170	2.23	-0.31	
9	1600	3.20	3.96	§

§ Denotes an outlier result

SUMMARY STATISTICS

No. of Results	9
Median	2.301
Normalised IQR	0.228
Robust CV	9.9%
Minimum	0.70
Maximum	3.20
Range	2.51
Uncertainty (Median)	0.095

**TOTAL *LEGIONELLA* (Sample G112B)
ORDERED ROBUST Z-SCORE CHART**



Please note laboratory code 5B did not submit results.

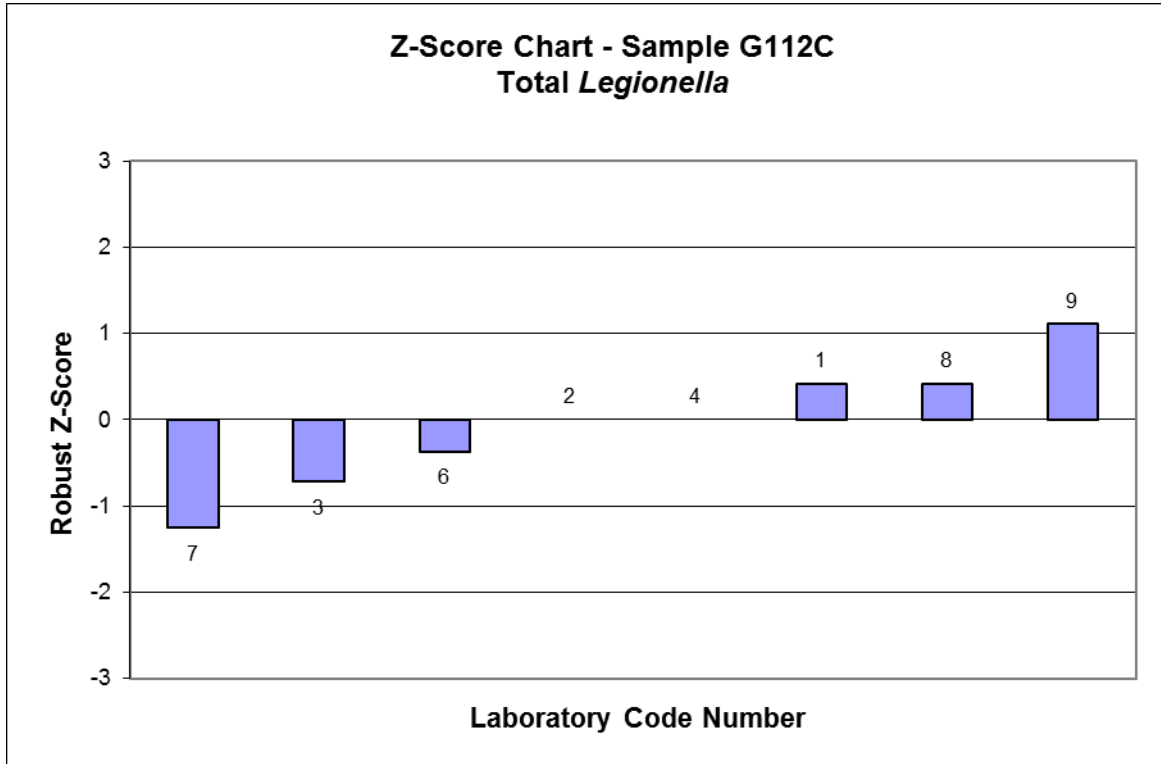
**TOTAL LEGIONELLA (Sample G112C)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G112C	Total <i>Legionella</i> [log(CFU/mL)]	Robust z-score
1	300	2.48	0.42
2	200	2.30	0.00
3	100	2.00	-0.72
4	200	2.30	0.00
5A	26	1.41	-2.12
5B			
6	140	2.15	-0.37
7	60	1.78	-1.25
8	300	2.48	0.42
9	580	2.76	1.11

SUMMARY STATISTICS

No. of Results	9
Median	2.301
Normalised IQR	0.417
Robust CV	18.1%
Minimum	1.41
Maximum	2.76
Range	1.35
Uncertainty (Median)	0.174

**TOTAL *LEGIONELLA* (Sample G112C)
ORDERED ROBUST Z-SCORE CHART**



Please note laboratory code 5B did not submit results.

NUMBER OF COLONIES CONFIRMED

Lab Code	Plate Type	Direct inoculation 0.1mL					
		G112A		G112B		G112C	
		pick	conf	pick	conf	pick	conf
1	MWY	20	Leg serogroup 1	12	Leg serogroup 2-15	20	Leg. Species
1	BMPA	1	Leg serogroup 1	0	-	3	Leg. Species
2	MWY	4/27	4/27	1/18	1/18	1/8	1/8
2	BMPA	1/5	1/5	1/5	1/5	1/2	1/2
3	MWY	9	3	4	2	4	0
3	BMPA	6	0	3	3	4	0
4	BCYE	5	3	5	3	5	5
4	MWY	5	3	5	5	5	5
5A	-	-	-	-	-	-	-
5A	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
6	BMPA	3	2	3	3	3	3
6	MWY	3	2	3	3	3	3
7	MWY	4	4	5	5	4	4
7	BMPA	6	6	6	6	6	6
8	MWY	6	6	5	5	4	4
8	BMP	6	6	6	6	2	2
9	BYCE	3-4 colonies	yes	3-4 colonies	yes	3-4 colonies	yes
9	BCYE without L-cysteine	No growth	-	No growth	-	No growth	-

NUMBER OF COLONIES CONFIRMED

Lab Code	Plate Type	Heat treated 0.1mL					
		G112A		G112B		G112C	
		pick	conf	pick	conf	pick	conf
1	MWY	1	Legionella serogroup 1	11	Legionella serogroup 2-15	8	Legionella species
1	MWY	1	Legionella serogroup 1	5	Legionella serogroup 2-15	0	-
2	MWY	2/13	2/13	1/17	1/17	1/17	1/8
2	MWY	1/2	1/2	1/2	1/2	1/2	0
3	MWY	0	0	0	0	3	1
3	BMPA	0	0	0	0	3	1
4	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5A	-	-	-	-	-	-	-
5A	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
6	BMPA	1	1	1	1	1	1
6	MWY	0	0	2	2	1	1
7	MWY	8	8	1	1	5	5
7	BMPA	1	1	2	2	4	4
8	MWY	1	0	1	1	0	0
8	BMP	4	4	1	1	0	0
9	BCYE	No growth	-	No growth	-	No growth	-
9	BCYE without L-cysteine	No growth	-	No growth	-	No growth	-

NUMBER OF COLONIES CONFIRMED

Lab Code	Plate Type	Acid treatment					
		G112A		G112B		G112C	
		pick	conf	pick	conf	pick	conf
1	BMPA	0	-	0	-	1	Legionella species
1	BMPA	-	-	-	-	-	-
2	BMPA	0	0	1/2	1/2	1/2	0
2	-	-	-	-	-	-	-
3	MWY	0	0	0	0	0	0
3	BMPA	0	0	0	0	1	0
4	BCYE	3	3	4	3	5	5
4	MWY	2	1	0	0	1	1
5A	-	2	L.pneumop hila serogroup 1	5	L.pneumop hila serogroup 2-14	22	Legionella spp
5A	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
6	BMPA	3	1	0	0	1	0
6	MWY	3	3	1	1	3	1
7	MWY	0	0	0	0	0	0
7	BMPA	0	0	2	2	0	0
8	MWY	1	1	1	1	2	2
8	BMP	2	2	1	1	0	0
9	BCYE	No growth	-	No growth	-	No growth	-
9	BCYE without L- cysteire	No growth	-	No growth	-	No growth	-

NUMBER OF COLONIES CONFIRMED

Lab Code	Plate Type	Other plate/s or dilutions					
		G112A		G112B		G112C	
		pick	conf	pick	conf	pick	conf
1	NA	NA	NA	NA	NA	NA	NA
1	NA	NA	NA	NA	NA	NA	NA
2	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	BCYE	5	3	5	4	5	2
4	-	-	-	-	-	-	-
5A	-	-	-	-	-	-	-
5A	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
5B	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	Positive Control	3-4 colonies	Yes	3-4 colonies	Yes	3-4 colonies	Yes
9	-	-	-	-	-	-	-

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

Legionella Proficiency Testing Program**Round 58****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.

C2

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 5th April 2019** 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

LAB CODE:

RESULTS FOR ROUND 58

Samples received at (time) on / / (date) Temp on arrival °C

RESULTS (cfu/mL)	Sample G112A		Sample G112B		Sample G112C	
	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 3896:2017

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

Legionella identification kit and use by date:

NO. OF COLONIES...		Sample G112A		Sample G112B		Sample G112C	
0.1mL inoculation	Plate Type	..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 plate							
Direct plate							
Heat treated plate							
Heat treated plate							
Acid treated plate							
Acid treated plate							
Other plate/s or dilutions							

Signature: _____

Date: _____

Please return results **NO LATER THAN FRIDAY 5th April 2019** 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

- End of Report -