



Amended Report - Report No. 829B

Issued April 2014

***Legionella* Proficiency Testing**

Round 47

October 2013

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 forty-seventh round of an ongoing series of programs.

The program was conducted in August 2013 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 Advisor was Mr F Lagala of the Institute of Medical and Veterinary Science. This report was authorised by Ms W Fajloun, PTA Quality Coordinator.

2. FEATURES OF THE PROGRAM

- (a) Participants were provided with three lenticule discs, labelled G82A, G82B and G83A.
- (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 six laboratories received samples, comprising:
 - four Australian participants; and
 - two overseas participants, of which one was from Hong Kong and the other New Zealand.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, 2012* (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].

Note: Statistical analysis was not performed for sample G82B.

- (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 G82A contains *Legionella pneumophila* serogroup 1, *Brevibacillus laterosporus* and *Citrobacter braakii*.

Sample G82B contains *Brevundimonas vesicularis* and *Ralstonia pickettii*.

Sample G83A contains *Legionella pneumophila* serogroup 2-14, *Bacillus pumilus* and *Pseudomonas fluorescens*.

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. Tables listing all of the outlier results are on page 4.

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

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

Statistic	Sample G82A	Sample G82B	Sample G83A
No. of Results	6	1	6
Median	3.176	NA	3.021
Normalised IQR	0.200	NA	0.199
Robust CV	6.3%	NA	6.6%
Uncertainty (Median)	0.103	NA	0.102
<i>Approx. Median Levels (CFU/mL)</i>	<i>1500</i>	<i>100</i>	<i>1050</i>

Note: Statistical analysis was not performed for sample G82B.

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 G82A</u> Laboratory Code Number	<u>Sample G82B</u> Laboratory Code Number	<u>Sample G83A</u> Laboratory Code Number
<i>L. pneumophila</i> serogroup 1 FALSE NEGATIVE	-		
<i>L. pneumophila</i> serogroup 1 FALSE POSITIVE		-	-
<i>L. pneumophila</i> serogroup 2 - 14 FALSE NEGATIVE			-
<i>L. pneumophila</i> serogroup 2 - 14 FALSE POSITIVE	-	6	
Other <i>Legionella</i> species FALSE NEGATIVE			
Other <i>Legionella</i> species FALSE POSITIVE	-	-	-

TABLE C - STATISTICAL OUTLIERS - TOTAL *LEGIONELLA*

Total <i>Legionella</i>	Sample G82A	Sample G82B	Sample G83A
Robust Z-Score	-	-	6

6. PTA AND TECHNICAL ADVISOR'S COMMENTS

A brief summary of the results appear in Table A. Outlier 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 six laboratories which returned results, one laboratory (laboratory code 6) reported an outlier for the enumeration of *Legionella* in addition to a false positive for the detection of *Legionella*.

***Legionella* Enumeration Results**

(refer Appendix A: pages A1 to A7)

All *Legionella* results were obtained using the standard method AS/NZS 3896:2008 (method code 1).

Histograms are used to check for the normal distribution of data and upon inspection of the histograms it was found that the data were 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 G82A contained *Legionella pneumophila* serogroup 1, *Brevibacillus laterosporus* and *Citrobacter braakii*. All laboratories reported the presence of *Legionella* in the sample, and hence were considered satisfactory.

Sample G82B contained *Brevundimonas vesicularis* and *Ralstonia pickettii*. One laboratory (laboratory code 6) reported the presence of *Legionella*, which was therefore a false positive. The remainder of results reported were evaluated as being satisfactory.

Sample G83A contained *Legionella pneumophila* serogroup 2-14, *Bacillus pumilus* and *Pseudomonas fluorescens*. The majority of results were evaluated as being satisfactory, with the exception of one laboratory (laboratory code 6) which reported a result identified as an outlier.

Results of Serotyping

(refer Appendix A: pages A1 to A2)

The serotyping results for all laboratories were considered satisfactory, with the exception of laboratory code 6 which reported a false positive for *Legionella pneumophila* (serogroup 2-14) for sample G82B.

Number of Colonies

(refer Appendix A: pages A8 to 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 laboratories appear to be examining sufficient representative colonies as described in AS/NZS 3896:2008 (reference [2]).

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.

Reference materials (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 G82A and G83A and is tabulated in Table A: Total *Legionella* Summary Statistics, and also in the summary statistics tables in Appendix A.

7. REFERENCES

- [1] *Guide to Proficiency Testing Australia (2012)*. [This document is located on the PTA website at www.pta.asn.au, under Programs/Documents.]
- [2] AS/NZS 3896:2008 "*Waters – Examination for Legionella spp. including Legionella pneumophila*".

APPENDIX A

Tables of Results and Z-scores, Summary Statistics 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.

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 and is analogous to the standard deviation.

(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.

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 G82A	MU	Sample G82B	MU	Sample G83A	MU	Method Code
1	2200	-	<10	-	1100	-	1
2	1060	-	0	-	550	-	1
3	1500	-	<10	-	700	-	1
4	2040	±394	<10	±2	1000	±193	1
5	1500	0.26 log 10	<10	0.26 log 10	1300	0.26 log 10	1
6	810	-	200	-	27000	-	1

Method Code: 1 = AS/NZS 3896:2008 (standard method)

A dash (-) means no result was submitted.

Lab Code	<i>L. pneumophila</i> Serogroup 1					
	Sample G82A	MU	Sample G82B	MU	Sample G83A	MU
1	2200	-	<10	-	<10	-
2	1060	-	-	-	-	-
3	1500	-	<10	-	<10	-
4	2040	±394	<10	±2	<10	±2
5	1500	0.26 log 10	<10	0.26 log 10	<10	0.26 log 10
6	810	-	0	-	0	-

A dash (-) means no result was submitted.

Lab Code	<i>L. pneumophila</i> other than Serogroup 1					
	Sample G82A	MU	Sample G82B	MU	Sample G83A	MU
1	<10	-	<10	-	1100	-
2	-	-	-	-	550	-
3	<10	-	<10	-	700	-
4	<10	±2	<10	±2	1000	±193
5	<10	0.26 log 10	<10	0.26 log 10	1300	0.26 log 10
6	0	-	200	-	27000	-

A dash (-) means no result was submitted.

Lab Code	Other <i>Legionella</i> species					
	Sample G82A	MU	Sample G82B	MU	Sample G83A	MU
1	<10	-	<10	-	<10	-
2	-	-	-	-	-	-
3	<10	-	<10	-	<10	-
4	<10	±2	<10	±2	<10	±2
5	<10	0.26 log 10	<10	0.26 log 10	<10	0.26 log 10
6	0	-	0	-	0	-

A dash (-) means no result was submitted.

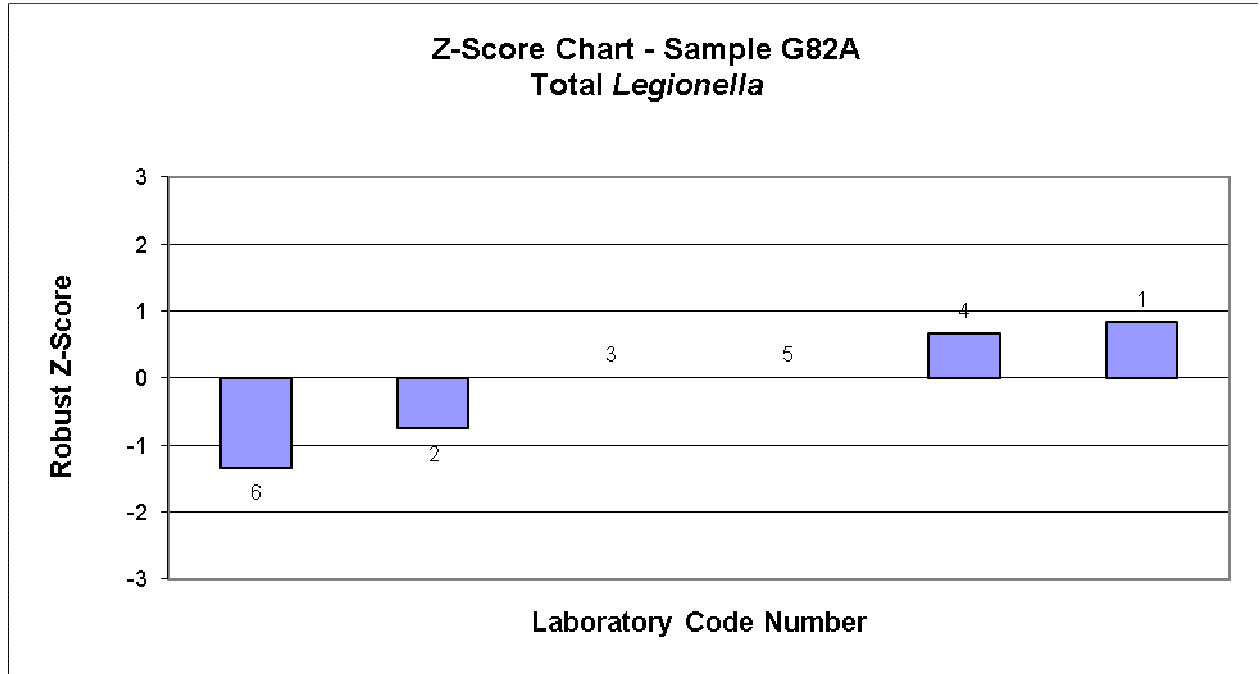
**TOTAL *LEGIONELLA* (Sample G82A)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G82A	Total <i>Legionella</i> [\log_{10} (CFU/mL)]	Robust z-score
1	2200	3.34	0.83
2	1060	3.03	-0.75
3	1500	3.18	0.00
4	2040	3.31	0.67
5	1500	3.18	0.00
6	810	2.91	-1.34

SUMMARY STATISTICS

Statistic	Sample G82A
No. of results	6
Median	3.176
Normalised IQR	0.200
Robust CV	6.3%
Minimum	2.91
Maximum	3.34
Range	0.43
Uncertainty (Median)	0.103

**TOTAL *LEGIONELLA* (Sample G82A)
ORDERED ROBUST Z-SCORE CHART**



**TOTAL *LEGIONELLA* (Sample G82B)
TRANSFORMED RESULTS (\log_{10} CFU/mL)**

Lab Code	Sample G82B	Total <i>Legionella</i> [\log_{10} (CFU/mL)]
1	<10	NA
2	0	NA
3	<10	NA
4	<10	NA
5	<10	NA
6	200	2.30

Note: 1. Statistical analysis was not performed for sample G82B.
2. Laboratory code 6 reported a false positive result.

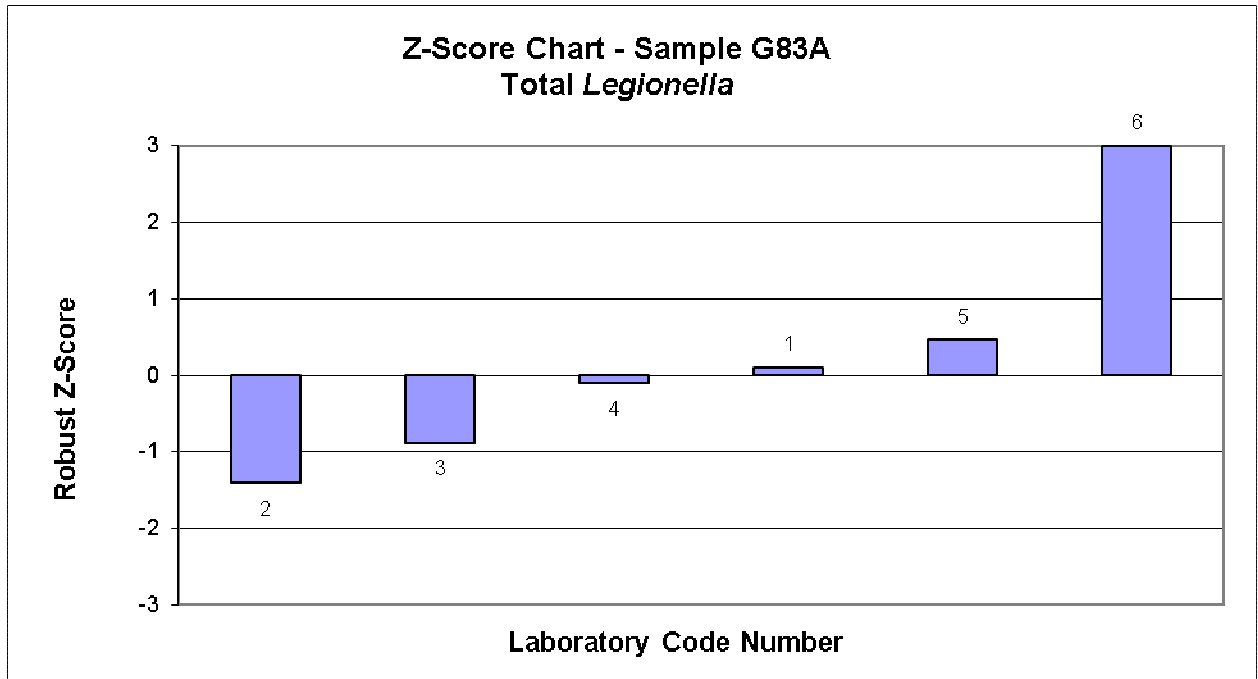
**TOTAL *LEGIONELLA* (Sample G83A)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G83A	Total <i>Legionella</i> [\log_{10} (CFU/mL)]	Robust z-score
1	1100	3.04	0.10
2	550	2.74	-1.41
3	700	2.85	-0.88
4	1000	3.00	-0.10
5	1300	3.11	0.47
6	27000	4.43	7.08 §

SUMMARY STATISTICS

Statistic	Sample G83A
No. of results	6
Median	3.021
Normalised IQR	0.199
Robust CV	6.6%
Minimum	2.74
Maximum	4.43
Range	1.69
Uncertainty (Median)	0.102

**TOTAL *LEGIONELLA* (Sample G83A)
ORDERED ROBUST Z-SCORE CHART**



NUMBER OF COLONIES CONFIRMED

Lab Code	Direct inoculation 0.1 mL						Direct inoculation 0.01 mL					
	G82A		G82B		G83A		G82A		G82B		G83A	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	2	2	2	0	4	1	1	0	2	0	1	1
2	3	3	0	-	3	3	0	0	0	-	0	0
3	3	3	0	-	6	6	0	-	0	-	6	6
4	3	3	3	0	3	0	6	3	3	0	3	3
5	6	6	6	0	9	6	1	0	6	0	6	6
6	2	2	3	3	-	-	1	1	2	2	-	-

NUMBER OF COLONIES CONFIRMED

Lab Code	Heat treated 0.1 mL						Heat treated 0.01 mL					
	G82A		G82B		G83A		G82A		G82B		G83A	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	2	2	1	0	1	1	1	1	0	0	0	0
2	3	3	0	-	3	3	0	0	0	-	0	0
3	5	5	0	-	1	1	1	1	0	-	0	-
4	3	3	0	0	3	0	0	0	0	0	3	0
5	6	6	0	0	3	3	1	0	0	0	0	0
6	-	-	-	-	-	-	-	-	-	-	-	-

NUMBER OF COLONIES CONFIRMED

Lab Code	Acid treatment						Other plate/s or dilutions					
	G82A		G82B		G83A		G82A		G82B		G83A	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	1	1	1	0	3	1	2	2	1	0	3	1
2	0	0	0	-	0	0	-	-	-	-	-	-
3	0	-	0	-	1	1	-	-	-	-	-	-
4	3	0	0	0	3	3	-	-	-	-	-	-
5	0	0	0	0	4	4	-	-	-	-	-	-
6	2	2	-	-	5	5	-	-	-	-	-	-

The tables above and on the previous page show the quantified results only, as requested in the *Instructions to Participants*.

A dash (-) means no result was reported.

APPENDIX B

Homogeneity and Stability Testing

Homogeneity and Stability Testing

The FEPTU of PHE - UK's EQA 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 reference materials and are therefore considered 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 47 (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 **13th SEPTEMBER 2013** to:

Yvette Christie
Proficiency Testing Australia
PO Box 7507, Silverwater NSW 2128 Australia
Phone: +61 2 9739 8397,
Fax: +61 2 9743 6664
Email: yvette.christie@pta.asn.au

Proficiency Testing Australia

Legionella Proficiency Testing Program – Round 47

Results Sheet

Lab Code

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

RESULTS (CFU/mL)	<u>Sample G82A</u>		<u>Sample G82B</u>		<u>Sample G83A</u>	
	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 G82A</u>		<u>Sample G82B</u>		<u>Sample G83A</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 13 SEPTEMBER 2013** to:

Yvette Christie, Proficiency Testing Australia
 PO Box 7507, Silverwater NSW 2128 Australia
 Phone: +61 2 9739 8397, Fax: +61 2 9743 6664
 Email: yvette.christie@pta.asn.au

- End of Report -