



Report No. 804

***Legionella* Proficiency Testing**

Round 46

May 2013

Acknowledgments

PTA gratefully acknowledges the technical advice provided for this program by Mr F Lagala of the Institute of Medical and Veterinary Science.

Also thank you to Ms N Patel from the Food and Environmental Proficiency Testing Unit (FEPTU) of the Health Protection Agency (HPA) - UK who supplied the samples and Ms S Giannoulidis of Global Proficiency Pty Ltd who distributed the samples.

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PO Box 7507 SILVERWATER NSW 2128, Australia

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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-sixth round of an ongoing series of programs.

The program was conducted in February 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 G78A, G79A and G79B.
- (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:
 - seven Australian participants; and
 - two overseas participants from Hong Kong.All laboratories returned results for inclusion in the report with one laboratory submitting an extra set of results for inclusion in the final report, i.e. a total of ten sets of results.
- (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. Please note that a laboratory reported more than one set of results and, therefore, their code number (with letter) could appear several times in the same data set.

- (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: No statistical analysis was performed for sample G78A.

- (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 the Health Protection Agency (HPA) - UK in the form of lenticule discs.

Sample G78A contains *Brevundimonas vesicularis* and *Enterobacter cloacae*.

Sample G79A contains *Legionella pneumophila*, *Brevibacillus laterosporus* and *Citrobacter* spp.

Sample G79B contains *Legionella bozemanii*.

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 G79A	Sample G79B
No. of Results	10	10
Median	2.519	2.998
Normalised IQR	0.138	0.244
Robust CV	5.5%	8.2%
Uncertainty (Median)	0.055	0.097
<i>Approx. Median Levels (CFU/mL)</i>	330	1000

Note: No statistical analysis was performed for sample G78A.

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 G78A</u> Laboratory Code Number	<u>Sample G79A</u> Laboratory Code Number	<u>Sample G79B</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	-	-	-
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 G78A	Sample G79A	Sample G79B
Robust Z-Score	-	-	-

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 nine laboratories which returned results, zero laboratories reported an outlier for the enumeration of *Legionella* and zero laboratories reported outliers for the detection of *Legionella*.

***Legionella* Enumeration Results**

(refer Appendix A: pages A1 to A6)

Nine of the *Legionella* results were obtained using the standard method AS/NZS 3896:2008 (method code 1) and one set of results was obtained using an ISO method (ISO 11731-2:2004).

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 G78A contained *Brevundimonas vesicularis* and *Enterobacter cloacae*. No laboratory reported the presence of *Legionella* in the sample, and hence was considered as satisfactory.

Regarding sample G79A, two laboratories (laboratory codes 7B and 9) reported results resulting in z-scores ≥ 2.0 . The remainder of results reported were evaluated as being satisfactory.

All results reported for sample G79B were evaluated as being satisfactory.

Results of Serotyping

(refer Appendix A: pages A1 to A2)

The serotyping results for all laboratories were considered satisfactory.

Number of Colonies

(refer Appendix A: pages A7 to A8)

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 HPA-UK 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 G79A and G79B 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 G78A	MU	Sample G79A	MU	Sample G79B	MU	Method Code
1	-	-	219	-	1900	-	1
2	<10	±0.26	430	±0.26	900	±0.26	1
4	<10	-	330	±220 - 490	700	±450 - 1100	1
5	<10	-	340	-	1400	-	1
6	<10	±2	270	±52	560	±108	1
7A	0	-	330	±1.37	1800	±1.5	1
7B	0	-	160	±1.32	570	±1.41	2
8	<10	-	380	-	1400	-	1
9	<10	-	700	-	800	-	1
10	<10	-	250	-	1100	-	1

Method Code: 1 = AS/NZS 3896:2008 (standard method)
2 = Other method (ISO 11731-2:2004)

A dash (-) means no result was submitted.

Lab Code	<i>L. pneumophila</i> Serogroup 1					
	Sample G78A	MU	Sample G79A	MU	Sample G79B	MU
1	-	-	219	-	-	-
2	<10	±0.26	430	±0.26	<10	±0.26
4	<10	-	330	±220 - 490	<10	-
5	<10	-	340	-	<10	-
6	<10	±2	270	±52	<10	±2
7A	0	-	330	±1.37	0	-
7B	0	-	160	±1.32	0	-
8	<10	-	380	-	<10	-
9	<10	-	700	-	<10	-
10	<10	-	250	-	<10	-

A dash (-) means no result was submitted.

Lab Code	<i>L. pneumophila</i> other than Serogroup 1					
	Sample G78A	MU	Sample G79A	MU	Sample G79B	MU
1	-	-	-	-	-	-
2	<10	±0.26	<10	±0.26	<10	±0.26
4	<10	-	<10	-	<10	-
5	<10	-	<10	-	<10	-
6	<10	±2	<10	±2	<10	±2
7A	0	-	0	-	0	-
7B	0	-	0	-	0	-
8	<10	-	<10	-	<10	-
9	<10	-	<10	-	<10	-
10	<10	-	<10	-	<10	-

A dash (-) means no result was submitted.

Lab Code	Other <i>Legionella</i> species					
	Sample G78A	MU	Sample G79A	MU	Sample G79B	MU
1	-	-	-	-	1900	-
2	<10	±0.26	<10	±0.26	900	±0.26
4	<10	-	<10	-	700	±450 - 1100
5	<10	-	<10	-	1400	-
6	<10	±2	<10	±2	560	±108
7A	0	-	0	-	1800	±1.5
7B	0	-	0	-	570	±1.41
8	<10	-	<10	-	1400	-
9	<10	-	<10	-	800	-
10	<10	-	<10	-	1100	-

A dash (-) means no result was submitted.

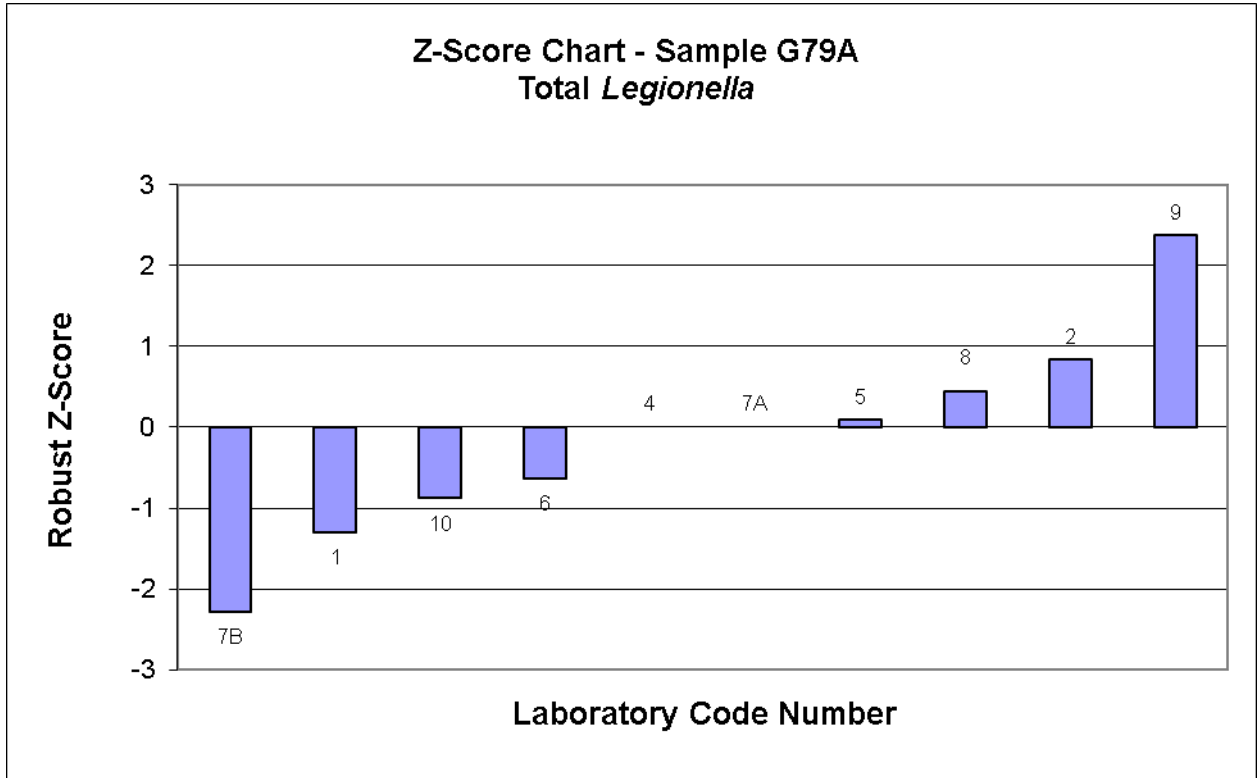
**TOTAL *LEGIONELLA* (Sample G79A)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G79A	Total <i>Legionella</i> [\log_{10} (CFU/mL)]	Robust z-score
1	219	2.34	-1.29
2	430	2.63	0.83
4	330	2.52	0.00
5	340	2.53	0.09
6	270	2.43	-0.63
7A	330	2.52	0.00
7B	160	2.20	-2.28
8	380	2.58	0.44
9	700	2.85	2.37
10	250	2.40	-0.88

SUMMARY STATISTICS

Statistic	Sample G79A
No. of results	10
Median	2.519
Normalised IQR	0.138
Robust CV	5.5%
Minimum	2.20
Maximum	2.85
Range	0.64
Uncertainty (Median)	0.055

**TOTAL *LEGIONELLA* (Sample G79A)
ORDERED ROBUST Z-SCORE CHART**



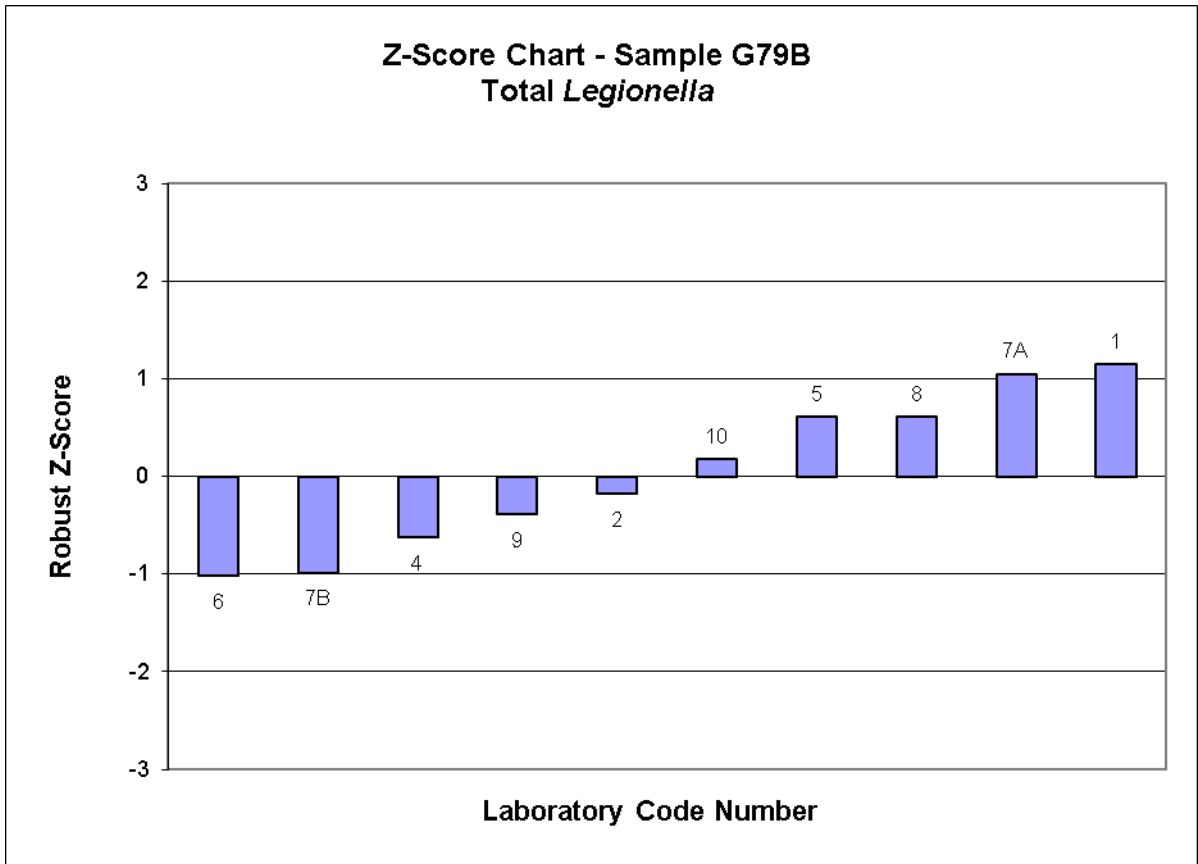
**TOTAL *LEGIONELLA* (Sample G79B)
TRANSFORMED RESULTS (\log_{10} CFU/mL) AND Z-SCORES**

Lab Code	Sample G79B	Total <i>Legionella</i> [\log_{10} (CFU/mL)]	Robust z-score
1	1900	3.28	1.15
2	900	2.95	-0.18
4	700	2.85	-0.62
5	1400	3.15	0.61
6	560	2.75	-1.02
7A	1800	3.26	1.05
7B	570	2.76	-0.99
8	1400	3.15	0.61
9	800	2.90	-0.39
10	1100	3.04	0.18

SUMMARY STATISTICS

Statistic	Sample G79B
No. of results	10
Median	2.998
Normalised IQR	0.244
Robust CV	8.2%
Minimum	2.75
Maximum	3.28
Range	0.53
Uncertainty (Median)	0.097

**TOTAL *LEGIONELLA* (Sample G79B)
ORDERED ROBUST Z-SCORE CHART**



NUMBER OF COLONIES CONFIRMED

Lab Code	Direct inoculation 0.1 mL						Direct inoculation 0.01 mL					
	G78A		G79A		G79B		G78A		G79A		G79B	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	-	-	3	3	0	0	-	-	0	0	3	3
2	4	0	4	4	4	4	1	0	6	4	2	2
4	3	0	3	3	3	3	-	-	3	0	2	2
5	3	0	3	3	3	3	1	0	1	1	3	3
6	2	0	2	2	2	2	-	-	-	-	1	1
7A	3	0	3	3	3	3	2	0	3	3	3	3
7B	0	0	3	3	3	3	-	-	-	-	-	-
8	1	0	2	2	2	2	0	0	0	0	2	2
9	3	0	3	3	3	3	2	0	3	3	3	3
10	2	0	6	6	3	3	1	0	0	0	3	3

NUMBER OF COLONIES CONFIRMED

Lab Code	Heat treated 0.1 mL						Heat treated 0.01 mL					
	G78A		G79A		G79B		G78A		G79A		G79B	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	-	-	0	0	0	0	-	-	0	0	0	0
2	2	0	2	2	2	2	0	0	1	1	0	0
4	2	0	3	3	2	2	1	0	-	-	-	-
5	2	0	-	-	2	2	-	-	-	-	1	1
6	-	-	2	2	1	1	-	-	1	1	-	-
7A	1	0	3	3	3	3	0	0	0	0	0	0
7B	-	-	-	-	-	-	-	-	-	-	-	-
8	0	0	2	2	2	2	0	0	0	0	0	0
9	2	0	3	3	0	-	0	-	0	-	1	1
10	0	-	3	3	1	1	0	-	0	0	0	0

NUMBER OF COLONIES CONFIRMED

Lab Code	Acid treatment						Other plate/s or dilutions					
	G78A		G79A		G79B		G78A		G79A		G79B	
	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf	pick	conf
1	-	-	0	0	3	3	-	-	-	-	-	-
2	0	0	4	1	3	3	-	-	-	-	-	-
4	-	-	2	0	2	2	-	-	-	-	-	-
5	-	-	-	-	1	1	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7A	0	0	3	3	3	3	-	-	-	-	-	-
7B	-	-	-	-	-	-	-	-	-	-	-	-
8	0	0	0	0	2	2	0	0	0	0	1	1
9	0	-	3	3	3	3	-	-	-	-	-	-
10	0	-	1	1	3	3	0	-	0	0	0	0

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 HPA - 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 46 (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 **8th March 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 46

Results Sheet

Lab Code

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

RESULTS (CFU/mL)	<u>Sample G78A</u>		<u>Sample G79A</u>		<u>Sample G79B</u>	
	Result	MU	Result	MU	Result	MU
Total <i>Legionella</i>						
<i>L. pneumophila</i> Serogroup 1						
<i>L. pneumophila</i> Other than 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 Sample G78A Sample G79A Sample G79B

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 8 MARCH 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 -