



## Summary of Results *Legionella* Molecular Scheme

### External Quality Assessment for Water Microbiology

**Distribution Number:** LM5  
**Sample Numbers:** LM5A & LM5B

Distribution Date:	February 2019
Results due:	19 April 2019
Report Date:	2 May 2019
Samples prepared and quality control tested by:	Angela Appea Richard Borrill Thomas Harper Margaret Njenga Zak Prior Lili Tsegaye
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Report compiled by:	Nita Patel Manchhari Rajkumar
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Lab No:

**Overview:**

*Legionella* spp. are the causative agent of legionellosis infections, varying in severity from a mild self-limiting febrile illness (Pontiac fever) to a potentially fatal atypical pneumonia (Legionnaires' disease). *Legionella* is recognised as a significant cause of sporadic and epidemic community-acquired and nosocomial-acquired pneumonia with many cases being associated with travel making it difficult to identify the source of infection.

Molecular methods are now being used in conjunction with traditional culture methods. However molecular methods should only be used as an alternative to traditional methods once you have validated the kit/s and understood the limitations for detection and quantification of the kit/s being used.

Participants are advised to refer to ISO/TS 12869:2012 - Water quality - Detection and quantification of *Legionella* spp. and/or *Legionella pneumophila* by concentration and genic amplification by quantitative polymerase chain reaction (qPCR) for more information on the method.

**FEPTU Quality Control:**

For homogeneity of the colony counts a minimum of 10 LENTICULE® discs, selected randomly from the batch, are examined for *Legionella* spp. The FEPTU results are determined using a method based on ISO 11731:1998: Water quality Detection and enumeration of *Legionella*.

To demonstrate homogeneity of the sample for genomic values, a minimum of 10 LENTICULE® discs, selected randomly from a batch, are tested.

To demonstrate stability of the sample for genomic values, a minimum of six LENTICULE discs, selected randomly from a batch, are examined throughout the distribution period.

FEPTU's quantification results were obtained using: Bio-Rad iQ-Cheq® screen *L. pneumophila*, Bio-Rad iQ-Check® screen *Legionella* spp. and Bio-Rad iQ-Check® *Legionella* quantification standards kits.

The intended results letters provide guidance for participants regarding the assigned values.

**Statistical analysis used for this scheme:**

Median absolute deviation from the median (MADeS) values has been used as there are less than 50 data sets. The use of MADeS values provides a statistically robust method for calculating the acceptable range using an analysis that requires calculation of the median difference from the median for every participant's result, which is then multiplied by a constant (1.4826) to obtain a robust estimate of the standard deviation (MADeS value).

The expected range has been calculated as the assigned quantification value  $\pm 2$  MADeS. The assigned quantification value used was the participant's median.

**Guidelines and general advice:**

If you experience difficulties with any of the examinations, please refer to section 17.0 of the Scheme Guide <https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide>

**Please contact FEPTU staff for advice and information:**

<b>Repeat samples</b>	Carmen Gomes or Kermin Daruwalla	<b>Tel: +44 (0)20 8327 7119</b>
<b>Data analysis</b>	Manchari Rajkumar and Nita Patel	<b>Fax: +44 (0)20 8200 8264</b>
<b>Microbiological advice</b>	Zak Prior or Nita Patel	<b>E-mail: foodeqa@phe.gov.uk</b>
<b>General comments and complaints</b>	Zak Prior or Nita Patel	
<b>Scheme Co-ordinator</b>	Nita Patel	
<b>Scheme Consultant</b>	Charles Fuller	

**Accreditation:** PHE Water EQA for *Legionella* Molecular Scheme is accredited to United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010.



A total of 39 participants were sent this distribution, of which 31 examined the samples, three did not and five did not return a result.

**Sample: LM5A**

**Sample type:** Simulated water

**Request:** (i) Examine for the presence of legionellae  
(ii) Quantify legionellae in samples

**Contents:**

	cfu/disc (FEPTU median results from six data sets)	GU L <sup>-1</sup> (FEPTU median results from six data sets)
<i>Legionella pneumophila</i> serogroup 1	4.4x10 <sup>2</sup>	1.8x10 <sup>3</sup>
<i>Aerococcus viridans</i>	ca. 4.0x10 <sup>2</sup>	
<i>Brevundimonas vesicularis</i>	ca. 1.4x10 <sup>4</sup>	

cfu = colony forming units, GU L<sup>-1</sup> = genomic units per litre

**Expected Results:**

	Expected Result	Your Result	PHE Score	Z-Score
Isolation of legionellae	Detected	Detected		
<u>Identification:</u> <i>Legionella pneumophila</i>	Detected	Detected		
<i>Legionella</i> spp.	Detected	Detected		
<u>Quantification (GU L<sup>-1</sup>):</u> <i>Legionella pneumophila</i>	1.4x10 <sup>3</sup> – 8.3x10 <sup>4</sup>	7300		
<i>Legionella</i> spp.	3.8x10 <sup>3</sup> – 3.8x10 <sup>4</sup>	12000		
Total score out of				

**Performance information**

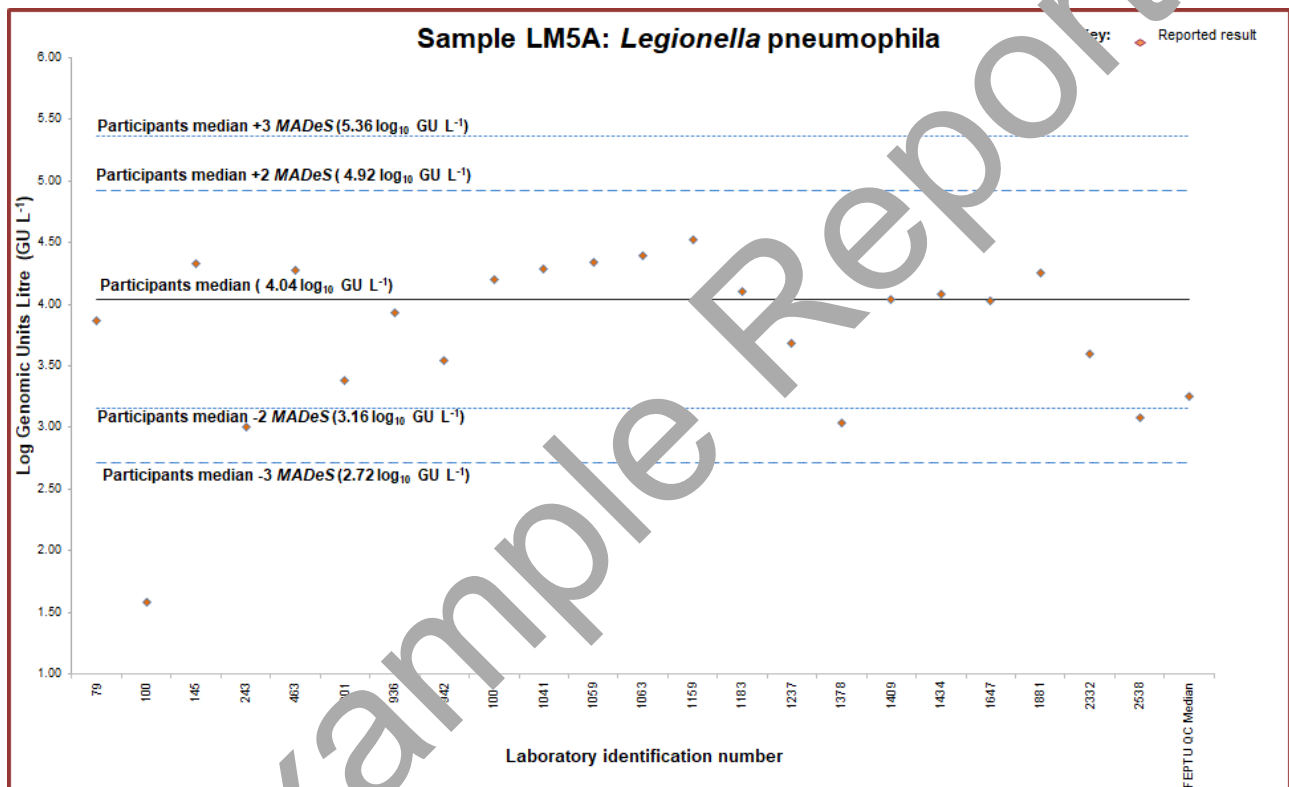
	Reported result	Total participants reporting	Participants reporting correctly	Percentage (%) of correct results
<b>Legionella detection</b>	Detected	30	29	97
<b>Identification (<i>L. pneumophila</i> or spp.)</b>	<i>L. pneumophila</i>	28	25	89
	<i>Legionella</i> spp.	22	18	82
	Participants reporting not examined	3		

**Legionella pneumophila** quantification results

<b>Total number of participants quantifying for Legionella pneumophila</b>	22	
<b>Assigned value (participants' median)</b>	1.1x10 <sup>4</sup> (4.04 log <sub>10</sub> GU L <sup>-1</sup> )	
<b>Standard deviation of participants results **</b>	0.44 log <sub>10</sub> GU L <sup>-1</sup>	
<b>Uncertainty of assigned value (U(X<sub>pt</sub>)= log<sub>10</sub> GU L<sup>-1</sup>)</b>	0.12	
<b>Minimum and maximum genomic values</b>	38 (1.58 log <sub>10</sub> GU L <sup>-1</sup> )	3.3x10 <sup>4</sup> (4.52 log <sub>10</sub> GU L <sup>-1</sup> )
<b>Number of outlying results</b>	4(4 low)	
<b>FEPTU's median</b>	1.8x10 <sup>3</sup> (3.26 log <sub>10</sub> GU L <sup>-1</sup> )	

The fixed standard deviation value (σ<sub>pt</sub> value) used for calculation of the z-scores is 0.35 for all parameters

\*\* Robust S\* based on median absolute deviation about the participants' median (MADe)

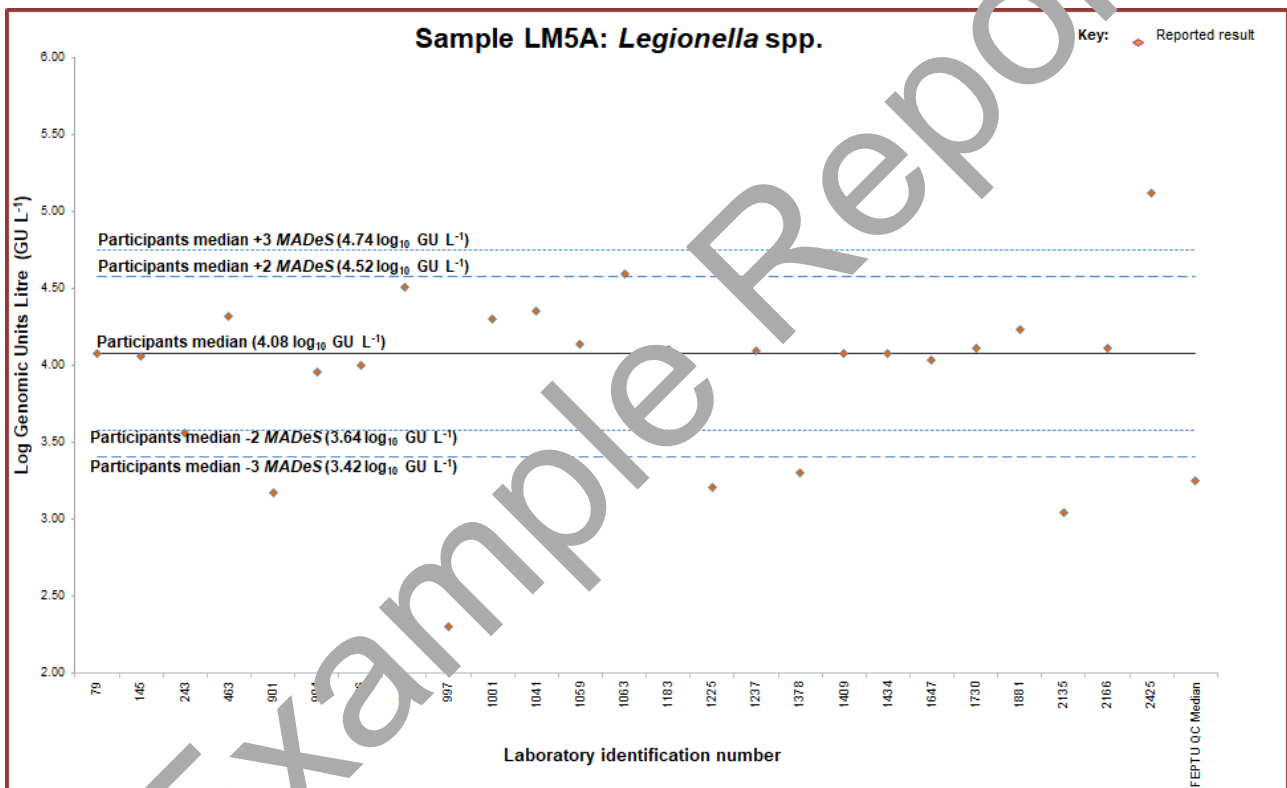


**Legionella spp. quantification results**

<b>Total number of participants quantifying for Legionella spp.</b>	27	
<b>Number of participants reporting a low censored value</b>	2	
<b>Assigned value (participants' median)</b>	1.2x10 <sup>4</sup> (4.08 log <sub>10</sub> GU L <sup>-1</sup> )	
<b>Standard deviation of participants results **</b>	0.22 log <sub>10</sub> GU L <sup>-1</sup>	
<b>Uncertainty of assigned value (U(X<sub>pt</sub>)= log<sub>10</sub> GU L<sup>-1</sup>)</b>	0.06	
<b>Minimum and maximum genomic values</b>	2.0x10 <sup>2</sup> (2.30 log <sub>10</sub> GU L <sup>-1</sup> )	1.3x10 <sup>5</sup> (5.11 log <sub>10</sub> GU L <sup>-1</sup> )
<b>Number of outlying results</b>	7 (5 low / 2 high)	
<b>FEPTU's median</b>	1.8x10 <sup>3</sup> (3.26 log <sub>10</sub> GU L <sup>-1</sup> )	

The fixed standard deviation value (σ<sub>pt</sub> value) used for calculation of the z-scores is 0.35 for all parameters

\*\* Robust S\* based on median absolute deviation about the participants' median (MADe)



**Sample: LM5B**

**Sample type:** Simulated water

**Request:** (i) Examine for the presence of legionellae  
(ii) Quantify legionellae in samples

**Contents:**

	<b>cfu/disc</b> (FEPTU median results from six data sets)	<b>GU L<sup>-1</sup></b> (FEPTU median results from six data sets)
<i>Legionella pneumophila</i> serogroup 3	4.2x10 <sup>2</sup>	1.7x10 <sup>4</sup>
<i>Legionella jamestowniensis</i>	2.7x10 <sup>3</sup>	1.6x10 <sup>4</sup>

cfu = colony forming units, GU L<sup>-1</sup> = genomic units per litre

**Expected Results:**

	<b>Expected Result</b>	<b>Your Result</b>	<b>PHE Score</b>	<b>Z-Score</b>
Isolation of legionellae	Detected	Detected		
<u>Identification:</u> <i>Legionella pneumophila</i>	Detected	Detected		
<i>Legionella</i> spp.	Detected	Detected		
<u>Quantification (GU L<sup>-1</sup>):</u> <i>Legionella pneumophila</i>	2.7x10 <sup>4</sup> – 3.7x10 <sup>5</sup>	4500		
<i>Legionella</i> spp.	4.7x10 <sup>4</sup> – 7.4x10 <sup>5</sup>	180000		
Total score out of				

**Performance information**

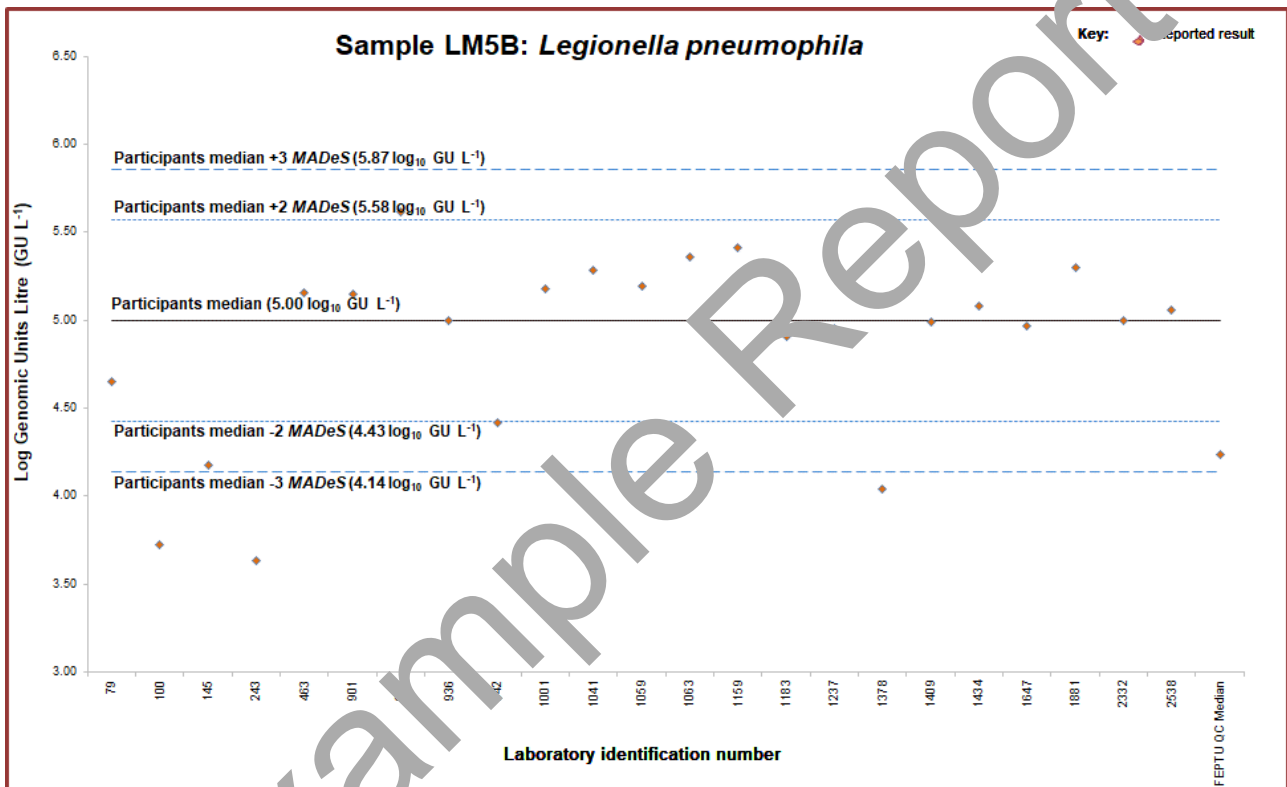
	<b>Reported result</b>	<b>Total participants reporting</b>	<b>Participants reporting correctly</b>	<b>Percentage (%) of correct results</b>
<b><i>Legionella</i> detection</b>	Detected	30	30	100
<b>Identification (<i>L. pneumophila</i> or spp.)</b>	<i>L. pneumophila</i>	28	27	96
	<i>Legionella</i> spp.	21	18	86
	Participants reporting not examined	3		

**Legionella pneumophila quantification results**

<b>Total number of participants quantifying for Legionella pneumophila</b>	23	
<b>Assigned value (participants' median)</b>	1.0x10 <sup>5</sup> (5.00 log <sub>10</sub> GU L <sup>-1</sup> )	
<b>Standard deviation of participants results **</b>	0.29 log <sub>10</sub> GU L <sup>-1</sup>	
<b>Uncertainty of assigned value (U(X<sub>pt</sub>)= log<sub>10</sub> GU L<sup>-1</sup>)</b>	0.07	
<b>Minimum and maximum genomic values</b>	4.3x10 <sup>3</sup> (3.65 log <sub>10</sub> GU L <sup>-1</sup> )	4.1x10 <sup>5</sup> (5.61 log <sub>10</sub> GU L <sup>-1</sup> )
<b>Number of outlying results</b>	6 (5 low / 1 high)	
<b>FEPTU's median</b>	1.7x10 <sup>4</sup> (4.24 log <sub>10</sub> GU L <sup>-1</sup> )	

The fixed standard deviation value (σ<sub>pt</sub> value) used for calculation of the z-scores is 0.35 for all parameters

\*\* Robust S\* based on median absolute deviation about the participants' median (MADe)



**Legionella spp. quantification results**

<b>Total number of participants also enumerating for <i>Legionella</i> spp.</b>	27
<b>Number of participants reporting a low censored value</b>	2

<b>Assigned value (participants' median)</b>	1.9x10 <sup>4</sup> (5.28 log <sub>10</sub> GU L <sup>-1</sup> )	
<b>Standard deviation of participants results **</b>	0.30 log <sub>10</sub> GU L <sup>-1</sup>	
<b>Uncertainty of assigned value (U(X<sub>pt</sub>)= log<sub>10</sub> GU L<sup>-1</sup>)</b>	0.07	
<b>Minimum and maximum genomic values</b>	2.5x10 <sup>3</sup> (3.4 log <sub>10</sub> GU L <sup>-1</sup> )	1.1x10 <sup>6</sup> (6.04 log <sub>10</sub> GU L <sup>-1</sup> )
<b>Number of outlying results</b>	6 (5 low / 1 high)	
<b>FEPTU's median</b>	1.6x10 <sup>4</sup> (4.21 log <sub>10</sub> GU L <sup>-1</sup> )	

The fixed standard deviation value (σ<sub>pt</sub> value) used for calculation of the z-scores is 0.35 for all parameters

\*\* Robust S\* based on median absolute deviation about the participants' median (MADe)

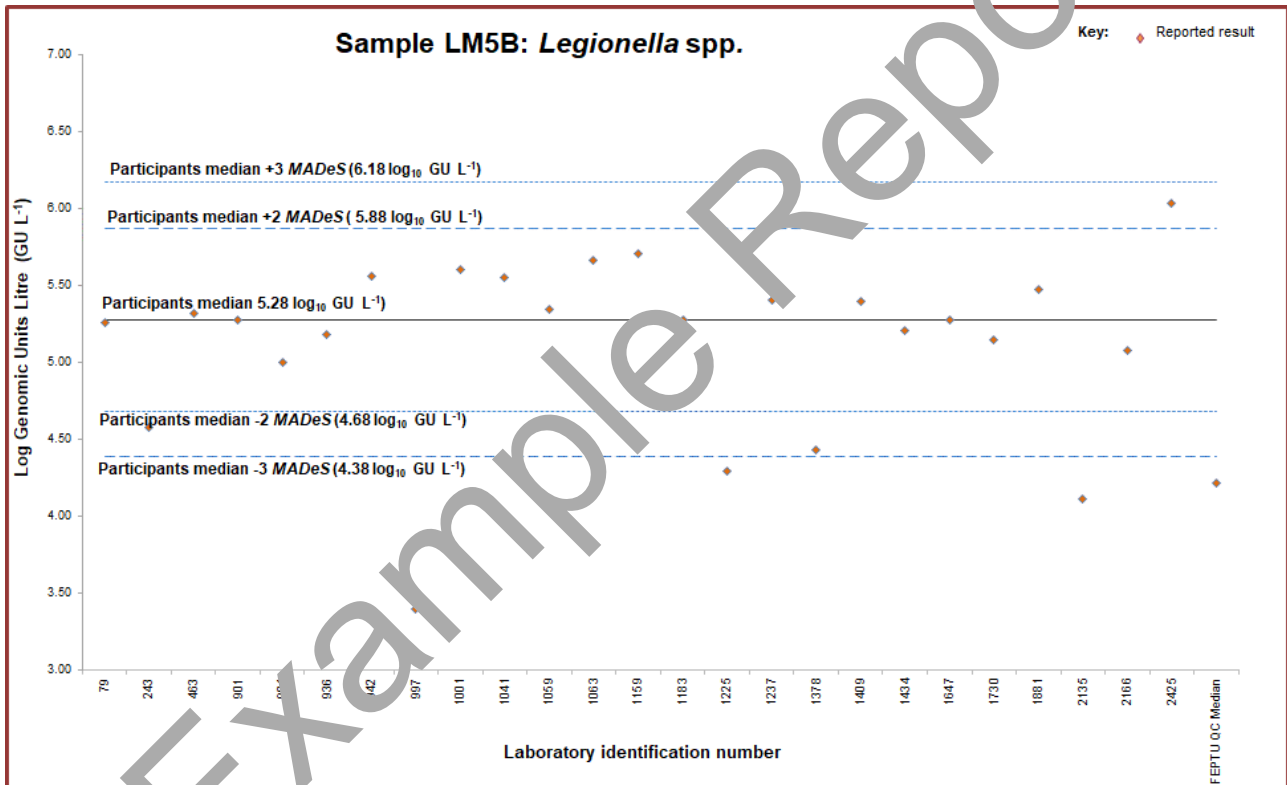




Table 1: Summary of participant's results for LM5 (incorrect results are shown in red and outlying quantification results in green).

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction Assay Platform used
	Detected Detected	Detected / Detected Detected / Detected	7300 45000	32.9 30.4	12000 180000	31.6 28	Pall Corporation GeneDisc® system Pall Corporation GeneDisc® Plate – Legionella DUO Pall Corporation GeneDisc® Cyclor
	Detected Detected	Detected / Not detected Detected / Not detected	38 5300	32.8 28.2	<10 10		Roche Diagnostics MagNA Pure LC DNA Isolation Kit III Roche Diagnostics LightCycler® 2.0 Instrument
	Detected Detected	Detected / Detected Detected / Not detected	21280 15003	21.13 33.18	11488 <166	34.82 -	Bio-Rad InstaGene™ Matrix In-house Applied Biosystems® 7500 Fast Real-Time PCR System
	Detected Detected	Detected / Detected Detected / Detected	1019 4326	31.9 29.7	3642 37610	26.8 23.2	Bio-Rad Aquadient™ Bacterial DNA Extraction and Purification Primerdesign genesig® Kit for <i>Legionella pneumophila</i> Applied Biosystems® StepOnePlus™ Real-Time PCR
	Not examined						

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction Assay Platform used
	Detected Detected	Detected / Detected Detected / Detected	18838 143250	29 26	20613 208750	28 24	bioMérieux NucliSENS® miniMag®  Roche Diagnostics LightCycler® 2.0 Instrument
Non-return of results							
	Detected Detected	Detected / Detected Detected / Detected	2400 141700	31.9 29.1	1500 188300	30.4 26.1	PALL Corporation Extraction Pack Environment 03 and GeneDisc® Ultra-Lyser  Applied Biosystems® 7500 Fast Real-Time PCR System
	Detected Detected	Not detected / Detected Detected / Detected	- 410000	28	9000 100000	34 30	Qiagen DNeasy UltraClean 96 Microbial Kit  Roche Diagnostics LightCycler® 96 System
Non-return of results							
	Detected Detected	Detected / Detected Detected / Detected	8500 100000	32.67 29.01	10000 150000	32.32 28.3	Roche Diagnostics MagNA Pure LC DNA Isolation Kit III  Applied Biosystems® 7300 Fast Real-Time PCR System

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i>		<i>Legionella</i> spp.		Extraction Assay Platform used
			Genomic units per Litre LM5A LM5B	Ct value LM5A LM5B	Genomic units per Litre LM5A LM5B	Ct value LM5A LM5B	
	Detected Detected	Detected / - Detected / -	3500 26000	32 29	32000 360000	33 30	Qiagen DNeasy UltraClean microbial kit  Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System
	Detected Detected		Not examined Not examined		210 2500	37.9 35.03	Macherey-Nagel NucleoSpin® gDNA Clean-up  TaKaRa Bio Inc. Cycleave® PCR <i>Legionella</i> (16S rRNA) Detection Kit  TaKaRa Bio Inc. Thermal Cycler Dice™ Real Time System
	Detected Detected	Detected / <b>Not detected</b> Detected / <b>Not detected</b>	16000 150000	29,24 26,12	20000 400000	29 25.8	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification  Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit  Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / Detected Detected / Detected	13500 94000	30,95 / 30,75 27,54 / 27,47	22700 358000	31,34 / 31,27 27,61 / 28,00	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification  Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit  Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / - Detected / -	21800 156000	29,82 / 29,76 27,11 / 27,13	13600 220000	31,02 / 31,05 27,56 / 27,59	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification  Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit  Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction Assay Platform used
	Detected Detected	Detected / Detected Detected / Detected	25000 230000	27.08 23.8	39000 460000	4.53 19.95	DIATHEVA DNApure Water Isolation Kit DIATHEVA <i>Legionella</i> spp. and <i>Legionella pneumophila</i> quantitative Qiagen Rotor-Gene Q
	Detected Detected	Detected / <b>Not detected</b> Detected / Detected	33000 260000	29.04 25.97	10000 510000	- 25.77	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L.</i> <i>pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / - Detected / -	12600 80200		12700 190000		Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L.</i> <i>pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	<b>Not detected</b> / Detected <b>Not detected</b> / Detected			1600 19800		Primerdesign genesig® Easy DNA/RNA Extraction Kit Primerdesign genesig® Kit for <i>Legionella</i> spp. Primerdesign genesig® q16
	Detected Detected	Detected / Detected Detected / Detected	4820 90500	31 26	12448 252800	30 25	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>L. pneumophila</i> Real-Time PCR Quantification Kit Agilent Technologies Mx3005P qPCR System

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i>		<i>Legionella</i> spp.		Extraction Assay Platform used	
			Genomic units per Litre LM5A LM5B	Ct value LM5A LM5B	Genomic units per Litre LM5A LM5B	Ct value LM5A LM5B		
			Non-return of results					
			Non-return of results					
	Detected Detected	Detected / Detected Detected / Detected	1100 11000	32,53 29,46	2000 27000	32,12 28,68	Qiagen DNeasy PowerWater kit Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	
	Detected Detected	Detected / - Detected / -	11000 98000	295,86 266,82	12000 250000	297,309 257,342	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. Real-Time PCR Quantification Kit Applied Biosystems® StepOnePlus™ Real-Time PCR System	
	Detected Detected	Detected / - Detected / -	12000 20000	30,51 27,61	12000 160000	31,23 27,82	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	
	Detected Detected	Detected / Detected Detected / Detected	10700 93500		10900 189000		Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction Assay Platform used
		Detected / Detected Detected / Detected		30,41 / 30,28 28,10 / 27,85	13000 140000	30,05 / 29,55 26,12 / 26,3	Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / Detected Detected / Detected	18000 200000		17 000 30 000		Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / Detected Detected / Detected					Bio-Rad InstaGene™ Matrix Applied Biosystems® 7500 Fast Real-Time PCR System
	Non-return of results						
	Not examined						
	Detected Detected				1100 13000	32.74 / 33 29.97 / 29.25	wizbiosolutions WizPrep™ gDNA Mini Kit (Cell/Tissue) Promega Microbial Legiofast® Species Agilent Technologies Mx3005P qPCR System

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction Assay Platform used
	Detected Detected	- / Detected - / Detected			13000 120000	34 32	Bio-Rad Aquadient™ Bacterial DNA Extraction and Purification Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. Real-Time PCR Quantification Kit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected Detected	Detected / <b>Not detected</b> Detected / -	3900 100000	33.1 28.1	Not examined		PALL Corporation Extraction Pack Environment 01 Pall Corporation GeneDisc® Plate - <i>Legionella pneumophila</i> Pall Corporation GeneDisc® Cyclor
	Detected Detected	Detected / Detected Detected / Detected			132000 1090000	31,020 27,671	Bio-Rad Chelex® 100 Applied Biosystems® QuantStudio™ 5 Real-Time PCR
	<b>Not detected</b> Detected	<b>Not detected</b> / - Detected / -					Roche Diagnostic MagNa Pure Compact Nucleic Isolation kit I TIB MolBiol LightMix® kit for <i>Legionella</i> (16S RNA) Roche Diagnostics LightCycler® 96 System
	Not examined						

Lab	Results LM5A LM5B	Identification <i>L. pneumophila</i> / <i>Legionella</i> spp. LM5A LM5B	<i>L. pneumophila</i> Genomic units per Litre LM5A LM5B	<i>L. pneumophila</i> Ct value LM5A LM5B	<i>Legionella</i> spp. Genomic units per Litre LM5A LM5B	<i>Legionella</i> spp. Ct value LM5A LM5B	Extraction
							Assay Platform used
	Detected Detected	Detected / - Detected / -	1202 114840	38 32			In house
							BIOTECON microproof® Legionella Quantification Lyokit
							Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System

Example Report



## **Scheme specific comment for LM5A and LM5B**

There are number of incorrect result or outlying quantification results reported, a further analysis did not highlight an issue with any specific method/kit used.

Participants reporting an incorrect detection result or an outlying genomic result are encouraged to investigate the reason for this by requesting a repeat sample from FEPTU.

## **Legionella spp.**

Participants are reminded that the detection of *Legionella* spp. is also an important factor in determining the effectiveness of control measures in an artificial water system. *Legionella* spp. other than *L. pneumophila*, have also been implicated in causing infection, particularly in nosocomial cases. However the Organisers are aware that national guidance documents may only refer to *L. pneumophila* and not necessarily include the requirement of testing for other species of *Legionella*.

## **General comments on methods and results**

There was a large variation in the methods used to examine the samples (please refer to questionnaire section of this report for further information).

Participants should know the limits of detection and quantification for the method they are using and it is useful to record this when reporting their results. This would include knowing the impact such as the volume of sample processed, DNA extraction method, reagent ratios and PCR reaction conditions etc. would have on results obtained.

## **Scoring**

The samples in this distribution have been scored using the below PHE scoring criteria.

### **Presence/absence results**

Participants' correct results for detection are allocated scores up to a maximum of two points as follows:

Fully correct result for the intended result	2
False positive / false negative result	0

### **Quantification results**

The expected range for each quantification result reported is calculated using the median absolute deviation from the median (*MADeS*) values which are determined from the median result reported by participants' and take into account the following criteria:

- (1) median  $\pm 2$  *MADeS*\*
- (2) median  $\pm 3$  *MADeS*\*
- (3) median  $\pm 0.5$  log<sub>10</sub> units

If the ranges in (1) and/or (2) are less than the value of the median  $\pm 0.5$  log<sub>10</sub> units then the expected range is extended as described in (3).

	Score
Expected range within the range according to criteria (1)	2
Outlying results (1) within the range of criteria (2) but not within criteria (1)	1
Outlying results (2) outside the range of criteria (2)	0

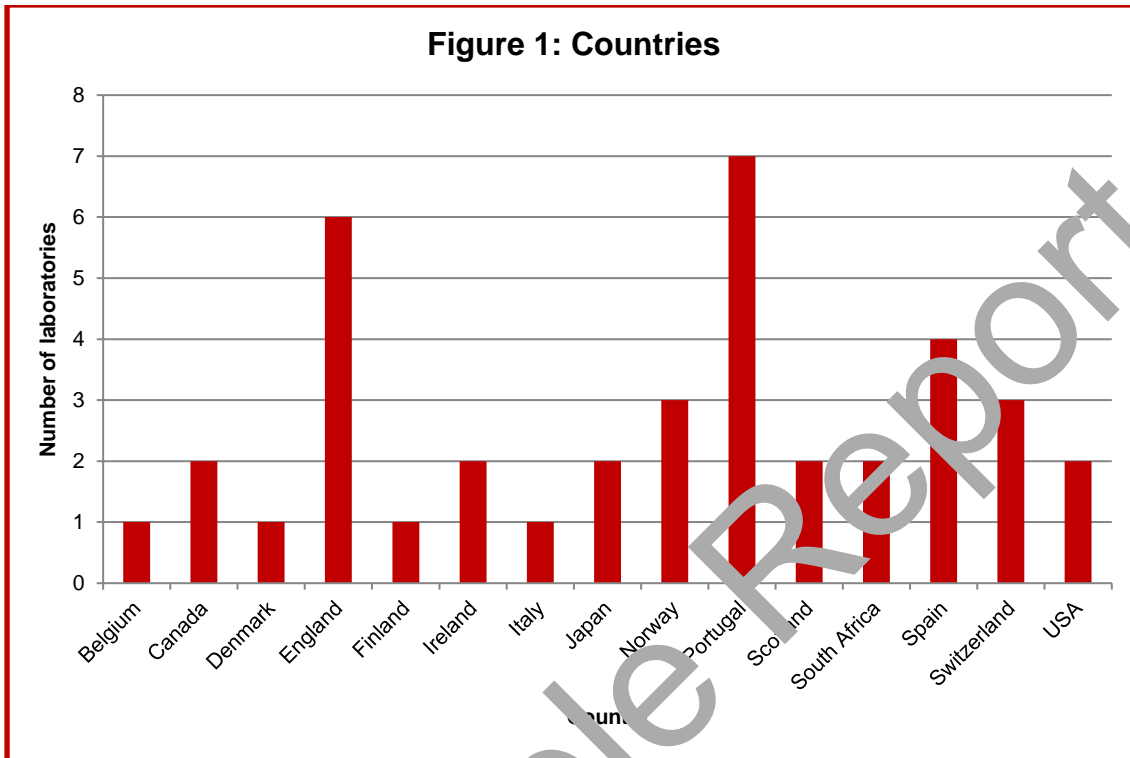
### **Non-return of results**

Participants who do not return a result by the specified date are allocated a PHE score of zero for all tests.

**Participation**

Total samples sent	39
Not examined	3
Not returned	5

A total of 15 countries participated in this distribution (figure 1). The majority of which were in Europe.



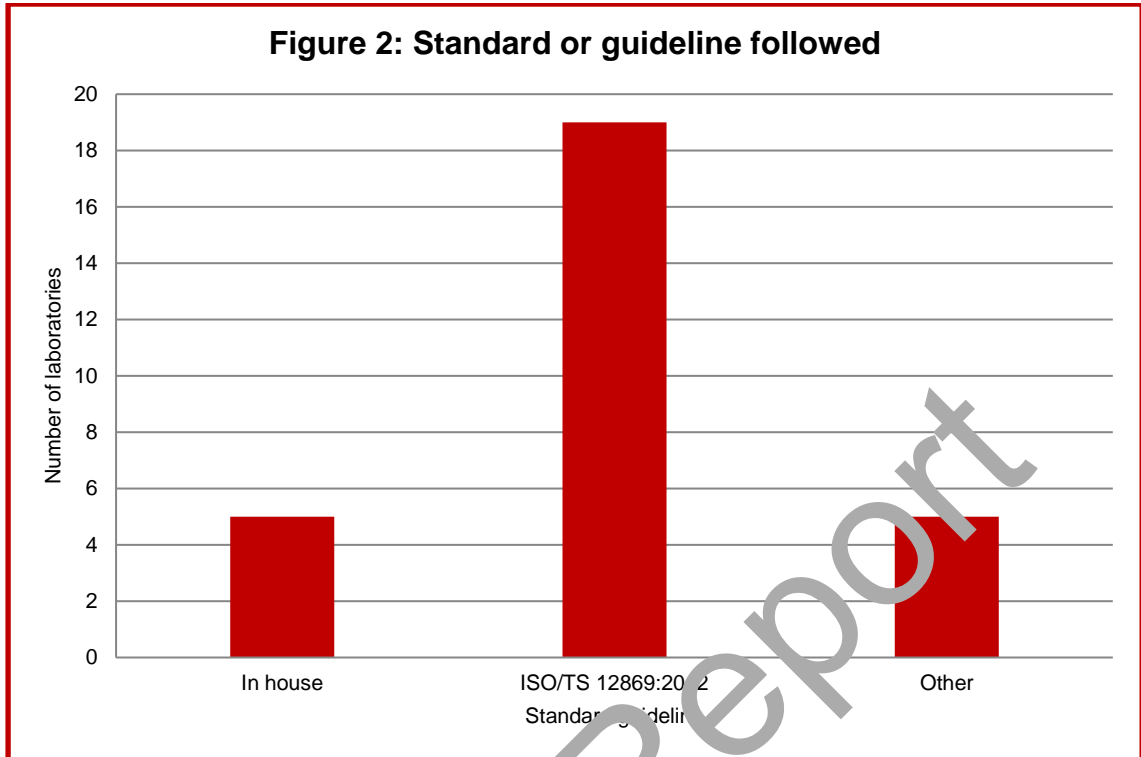
**Questionnaire results:**

Please note that not all participants provided the relevant information. FEPTU are aware that processes are different and therefore have not attempted to categorise the information into specific groups such as automation versus manual etc.

The data shown below is for information only. It does not evaluate or associate the data with a failure with PT to a method/process used nor does it attempt to compare performance of the various molecular kits/processes with each other.

**1. Standard and or guideline used for the sample examination**

- Of the 29 responses received, the majority used ISO/TS 12869:2012 (figure 2).



**2. Filtration of samples**

- Most participants routinely filter 1 litre of a water sample. Filtration volume ranged from 100 mL to 1 litre, two laboratories concentrated the sample by centrifugation and one performed an enrichment step.

**3. Details of the DNA extraction method used**

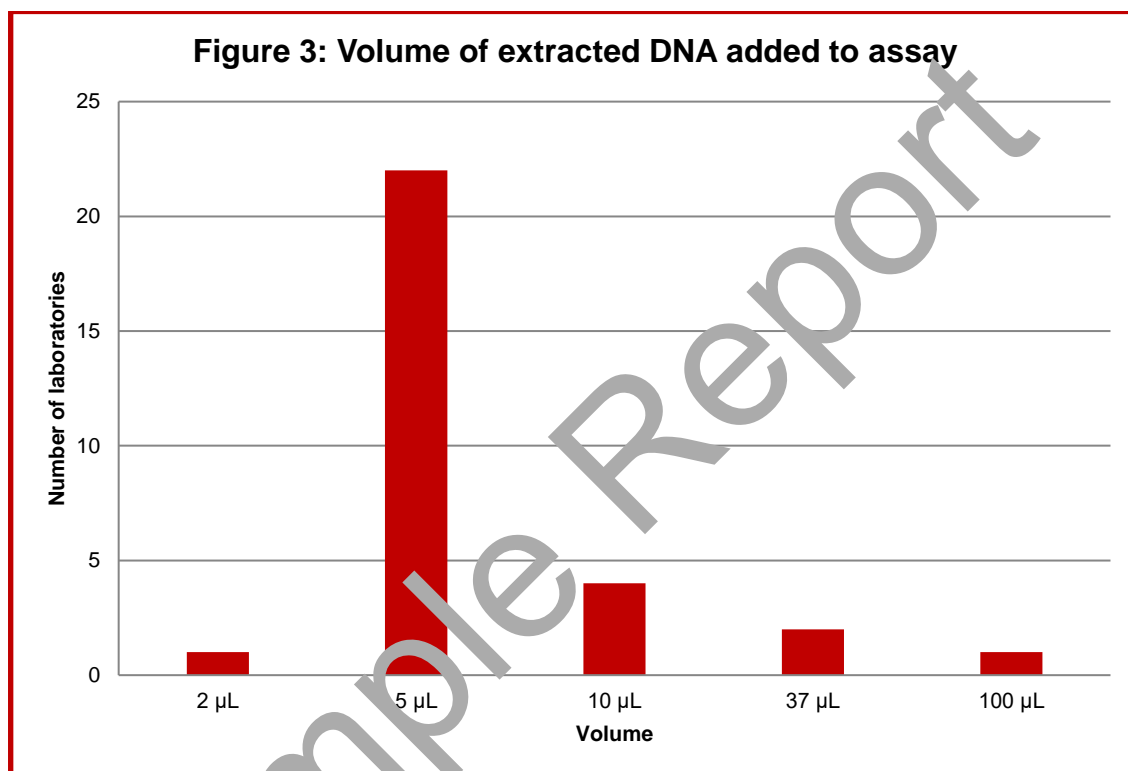
- There was a variation of DNA extraction kits used by participants as shown in the table below.

Assay	Number of users	% of users
bioMerieux NucliSENS easyMag®	1	3
Diatech DNApure Water Isolation kit (MBK0080)	1	3
Bio-Rad Aquaspin™ Bacterial DNA Extraction and Purification	13	42
Bio-Rad Chelex	1	3
Bio-Rad InstaGene™ Matrix	2	6
In house	1	3
Macherey-Nagel NucleoSpin® gDNA Clean-up	1	3
PALL Corporation Extraction Pack Environment 01	1	3
PALL Corporation Extraction Pack Environment 03 and GeneDisc® Ultra-Lyser	1	3
PALL Corporation GeneDisc® Systems	1	3
Primerdesign genesig Easy DNA/RNA Extraction Kit	1	3

Qiagen DNeasy PowerSoil Kit	1	3
Qiagen DNeasy PowerWater kit	1	3
Qiagen DNeasy UltraClean 96 Microbial Kit	1	3
Roche Diagnostic MagNa Pure Compact Isolation kit I	1	3
Roche Diagnostics MagNA Pure LC DNA Isolation Kit III	2	6

#### 4. Volume of extracted DNA used in your assay

- Of the 30 responses received, the volume of extracted DNA added to the assay is shown in figure 3.



#### 5. Type of PCR used

- 30/31 (97%) used a real-time PCR
- 1/31 (3%) used a conventional PCR

#### 6. The commercial assays used are shown in the table below from 23 participants.

Assay	Number of users	% of users
Primerdesign genesig® Kit for <i>Legionella</i> spp. kit	1	4
Bio-Rad iQ-Check® <i>Legionella</i> Real-Time PCR Kit	4	17
Bio-Rad iQ-Check® Quanti <i>L. pneumophila</i> Real-Time PCR Quantification Kit	1	4
Bio-Rad iQ-Check® Quanti <i>Legionella</i> spp. and <i>L. pneumophila</i> Real-Time PCR Quantification Kit	8	35
BIOTECON microproof® <i>Legionella</i> Quantification Lyokit	1	4
Diatheva <i>Legionella pneumophila</i> quantitative kit & <i>Legionella</i> spp. quantitative kit	1	4
In house	1	4
Microbial Legiofast® SPECIES	1	4

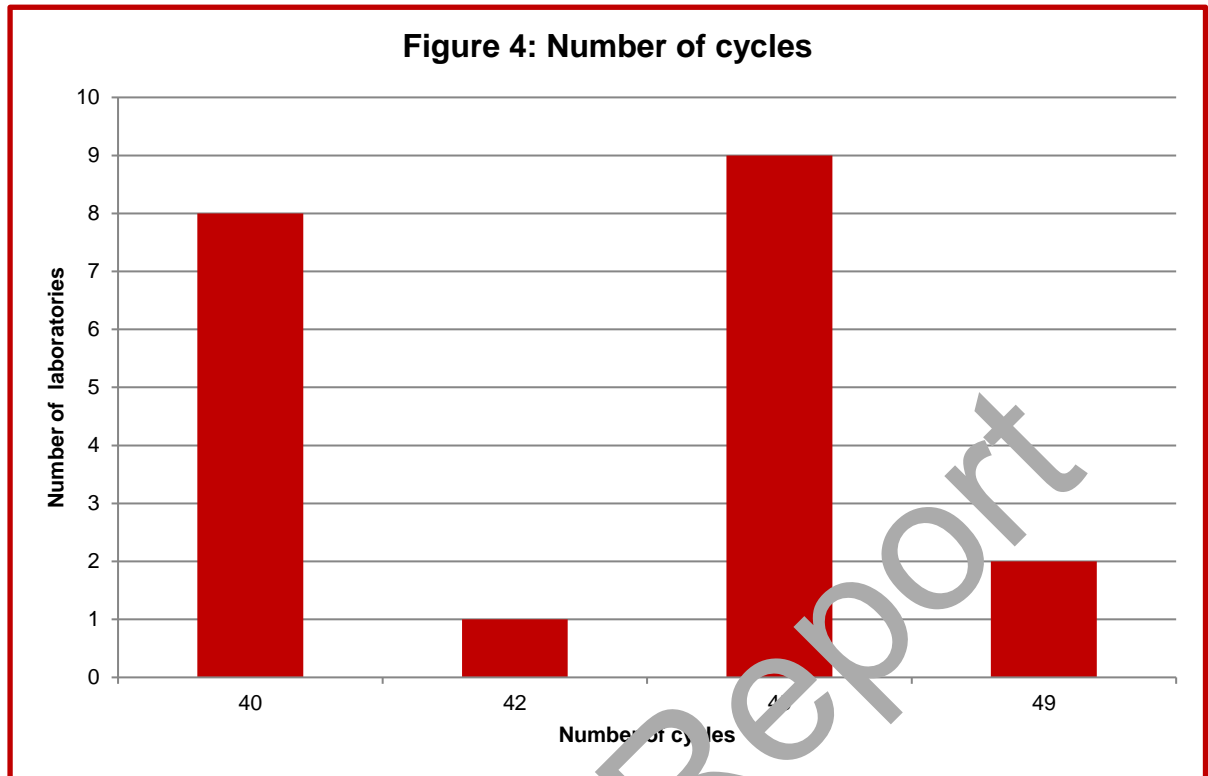
Pall Corporation GeneDisc® Plate - <i>Legionella</i> DUO	1	4
Pall Corporation GeneDisc® Plate - <i>Legionella pneumophila</i>	1	4
Primerdesign genesig® Kit for <i>Legionella pneumophila</i>	1	4
TaKaRa Bio Inc. Cycleave® PCR Legionella (16S rRNA) Detection Kit	1	4
TIB MolBiol LightMix® kit for <i>Legionella</i> (16S RNA)	1	4

**7. The Amplification platforms used are shown in the table below from 31 participants.**

<b>Platforms</b>	<b>Number of users</b>	<b>% of users</b>
Agilent Technologies Stratagene Mx3005P qPCR System	2	6
Applied Biosystems® StepOnePlus™ Real-Time PCR	2	6
Applied Biosystems® QuantStudio™ 5 Real-Time PCR	1	3
Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System	1	3
Applied Biosystems® 7300 Fast Real-Time PCR System	4	13
Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	12	39
Pall Corporation GeneDisc® Cyclor	2	6
Primerdesign genesig® q16	1	3
Qiagen Rotor-Gene Q	1	3
Roche Diagnostics LightCycler® 2.0 Instrument	2	6
Roche Diagnostics LightCycler® 96 System	2	6
TaKaRa Bio Inc. Thermal Cycler Dice™ Real-Time System	1	3

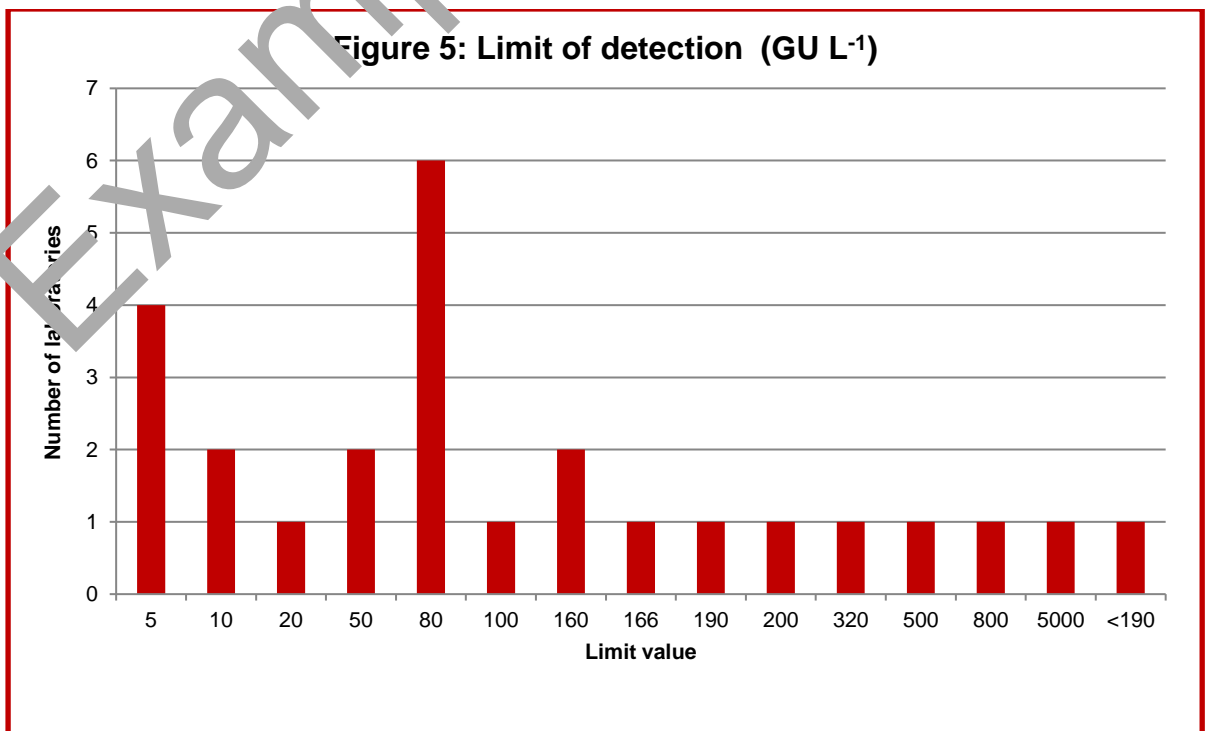
## 8. Cycling

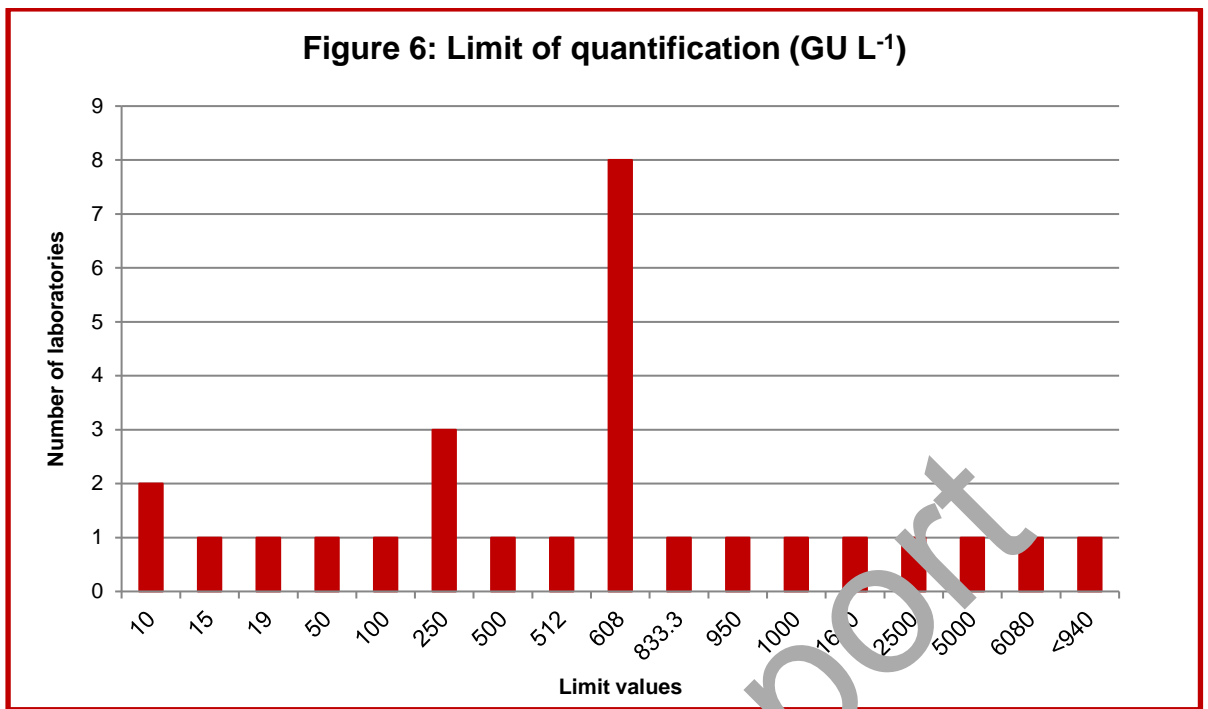
24 of the participants used between x 40-50 cycles (figure 4).



## 9. Limit of detection (LOD) is shown in figure 5 from 26 participants and limit of quantification (LOQ) in figure 6 from 27 participants.

- There was a large variation in limit values reported. Participants need to refer to ISO/TS 12869:2012 which prescribes acceptable limits of detection and quantification.





End of report.

Example Report