FEPTU
Food & Environmental Proficiency Testing Unit
Operated by UK Health Security Agency

Summary of Results
Legionella Molecular Scheme

External Quality Assessment for Water Microbiology

Distribution Number: **LM16**
Sample Numbers: **LM16A & LM16B**

Distribution Date:	**17 February 2025**
Results due:	**28 March 2025**
Report Date:	**9 April 2025**
Samples prepared and quality control tested by:	**Divya George** **Afifa Halim** **Nafeesa Hussain** **Sabine Naujokat** **Zak Prior** **Jake Videlefsky**
Data analysed by:	**Joanna Donn** **Nita Patel**
Report compiled by:	**Joanna Donn** **Nita Patel**
Authorised by:	**Nita Patel**

UK Health Security Agency
Food and Environmental Proficiency Testing Unit (FEPTU)
61 Colindale Avenue
London
NW9 5EQ
Tel: +44 (0) 20 8327 7119
Email: foodeqa@ukhsa.gov.uk

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 12869:2019 - 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. Please note that currently FEPTU have deviated from testing three samples after dispatch, however additional checks are done to assure the samples are still stable during this period. FEPTU reference number is FPD21-4. This deviation will come to an end in May 2022.

FEPTU's quantification results were obtained using: Bio-Rad iQ-Check® 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.

Guidelines and general advice:
If you experience difficulties with any of the examinations, please refer to section 17.0 of the Scheme Guide: Scheme Guide - Food and Environmental Proficiency Testing Unit

Please contact FEPTU staff for advice and information:

Repeat samples	Carmen Gomes or Kermin Daruwalla	
Data analysis	Nita Patel	**Telephone: +44 (0)20 8327 7119**
Microbiological advice	Zak Prior or Nita Patel	**E-mail: foodeqa@ukhsa.gov.uk**
General comments and complaints	Zak Prior or Nita Patel	
Scheme Co-ordinator	Nita Patel	
Scheme Consultant	Charles Fuller	

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

UKAS PROFICIENCY TESTING
0006

Sample: LM16A

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^{-1}** (FEPTU median results from six data sets)
Legionella pneumophila serogroup 5 (wild strain) *Brevibacillus laterosporus* (wild strain) *Pseudomonas fluorescens* (NCTC 3756)	$2.4x10^2$ $3.9x10^4$ $8.2x10^4$	$5.60x10^4$ and $2.70x10^4$ – for total *Legionella* spp.

cfu = colony forming units, GU L^{-1} = genomic units per litre

Expected Results:

	Expected Result	**Your Result**	**Ct value**	**UKHSA score**	**Z-Score**
Identification: *Legionella pneumophila*	Detected				
Legionella spp.	Detected				
Quantification (GU L^{-1}): *Legionella pneumophila*	$6.73x10^3 – 4.79x10^5$ (3.83 – 5.68 $\log_{10}$)				
Total *Legionella* spp.	$1.13x10^4 – 2.27x10^5$ (4.05 – 5.36 $\log_{10}$)				

Performance information

	Reported result	**Total participants reporting**	**Number of participants reporting correctly**	**Percentage (%) of correct results**
Identification (*L. pneumophila* or spp.)	*L. pneumophila* – detected	28	27	96.4
	Legionella spp. - detected	28	27	96.4

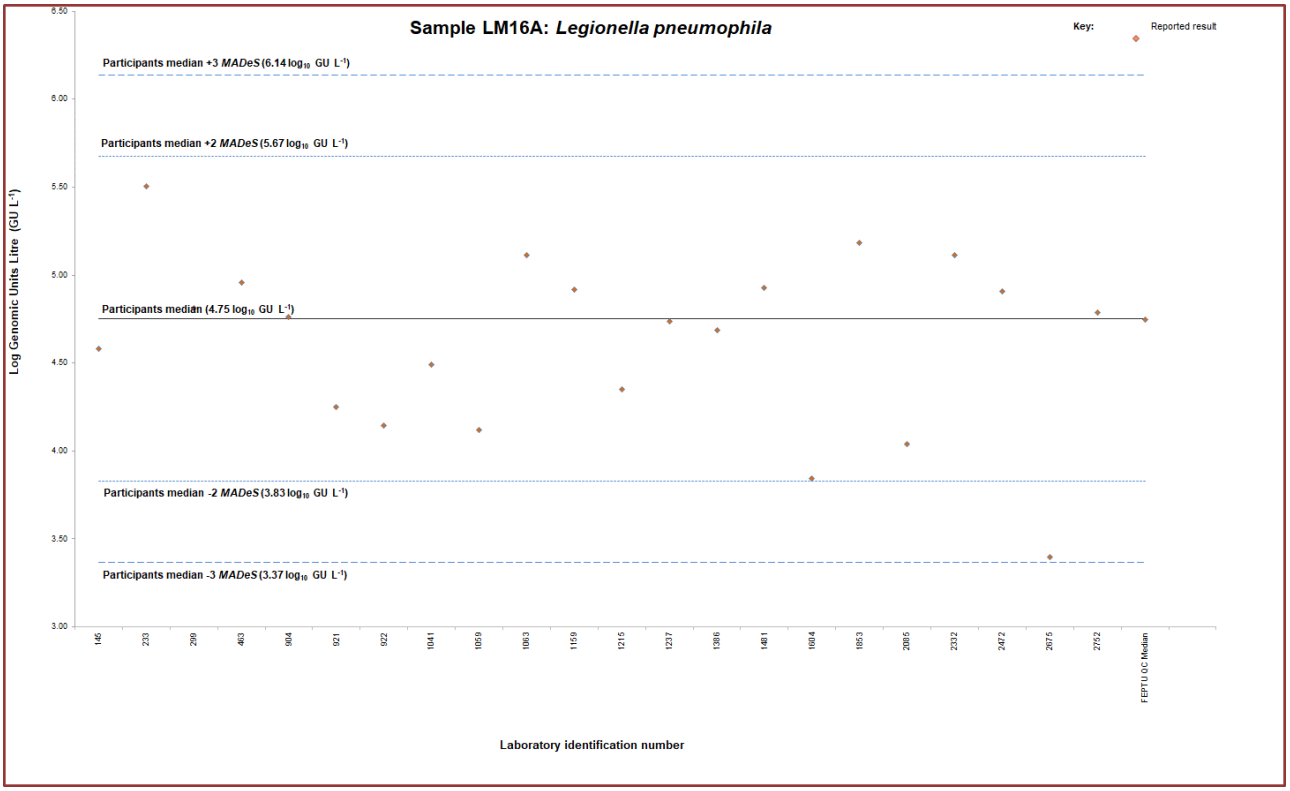
Legionella pneumophila quantification results

Total number of participants also quantifying for *Legionella pneumophila*	22

Assigned value (participants' median)	$5.63x10^4$ ($4.75 \log_{10}$ GU L^{-1})	
Standard deviation of participants results **	$0.46 \log_{10}$ GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= $\log_{10}$ GU L^{-1})	0.12	
Minimum and maximum genomic values	$2.51x10^3$ ($3.40 \log_{10}$ GU L^{-1})	$3.23x10^5$ ($5.51 \log_{10}$ GU L^{-1})
Number of outlying results	1 low	
FEPTU's median	$5.60x10^4$ ($4.75 \log_{10}$ GU L^{-1})	

The fixed standard deviation value (σ_{pt} value) used for calculation of the z-scores is 0.55 for all parameters

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



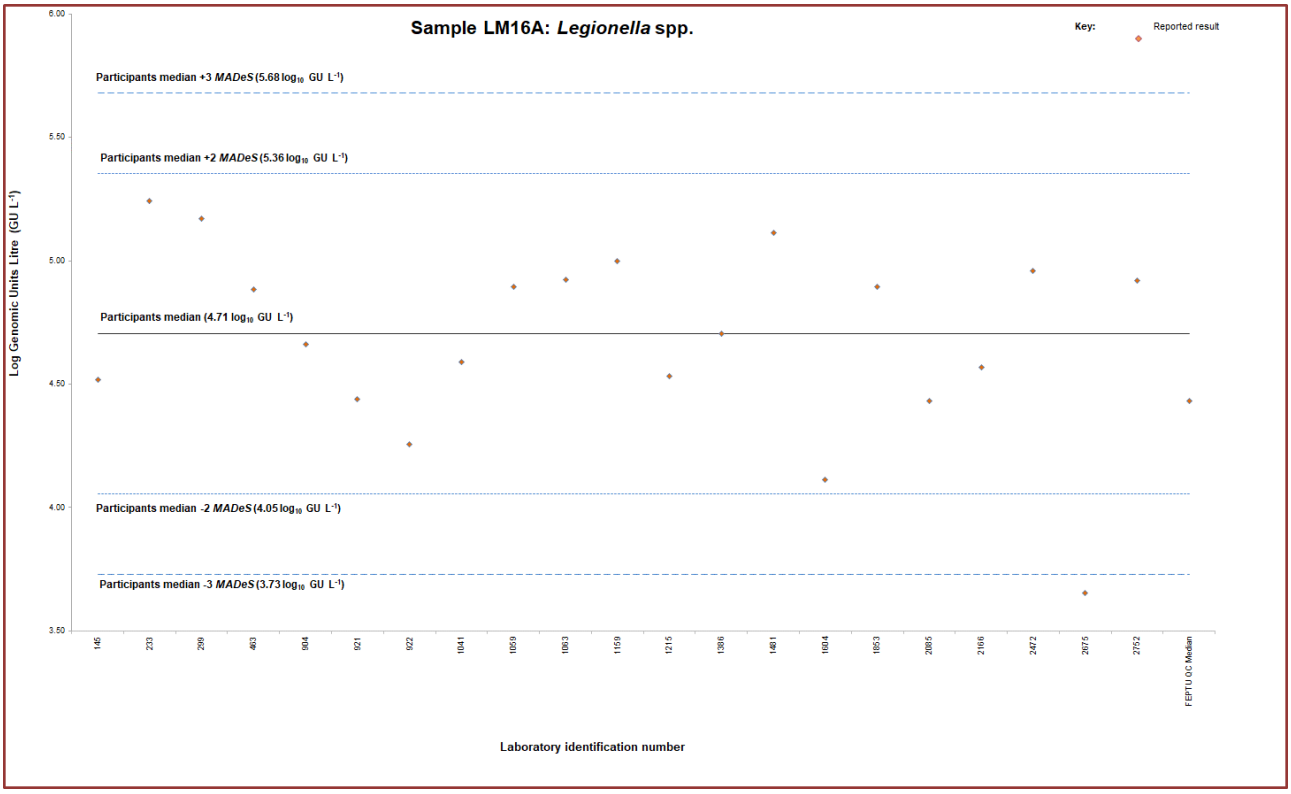
Legionella spp. quantification results

Total number of participants also quantifying for *Legionella* spp.	21

Assigned value (participants' median)	$5.07x10^4$ (4.71 log_{10} GU L^{-1})	
Standard deviation of participants results **	0.33 log_{10} GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= log_{10} GU L^{-1})	0.09	
Minimum and maximum genomic values	$4.45x10^3$ (3.65 log_{10} GU L^{-1})	$1.73x10^5$ (5.24 log_{10} GU L^{-1})
Number of outlying results	1 low	
FEPTU's median	$2.70x10^4$ (4.43 log_{10} GU L^{-1})	

The fixed standard deviation value (σ_{pt} value) used for calculation of the z-scores is 0.55 for all parameters

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



Sample: LM16B

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^{-1}** (FEPTU median results from six data sets)
Legionella longbeachae (wild strain) *Enterococcus faecalis* (wild strain) *Pseudomonas fluorescens* (NCTC 3756)	$4.1x10^3$ $1.1x10^3$ $6.7x10^3$	$2.70x10^5$ – for total *Legionella* spp.

cfu = colony forming units, GU L^{-1} = genomic units per litre

Expected Results:

	Expected Result	**Your Result**	**Ct value**	**UKHSA score**	**Z-Score**
Identification: *Legionella pneumophila*	Not detected				
Legionella spp.	Detected				
Quantification (GU L^{-1}): *Legionella pneumophila*	- -				
Total *Legionella* spp.	$2.98x10^5$ – $1.15x10^7$ (5.47 – 7.06 log_{10})				

Performance information

	Reported result	**Total participants reporting**	**Number of participants reporting correctly**	**Percentage (%) of correct results**
Identification (*L. pneumophila* or spp.)	*L. pneumophila* – not detected	28	27	96.4
	Legionella spp. - detected	25	25	100

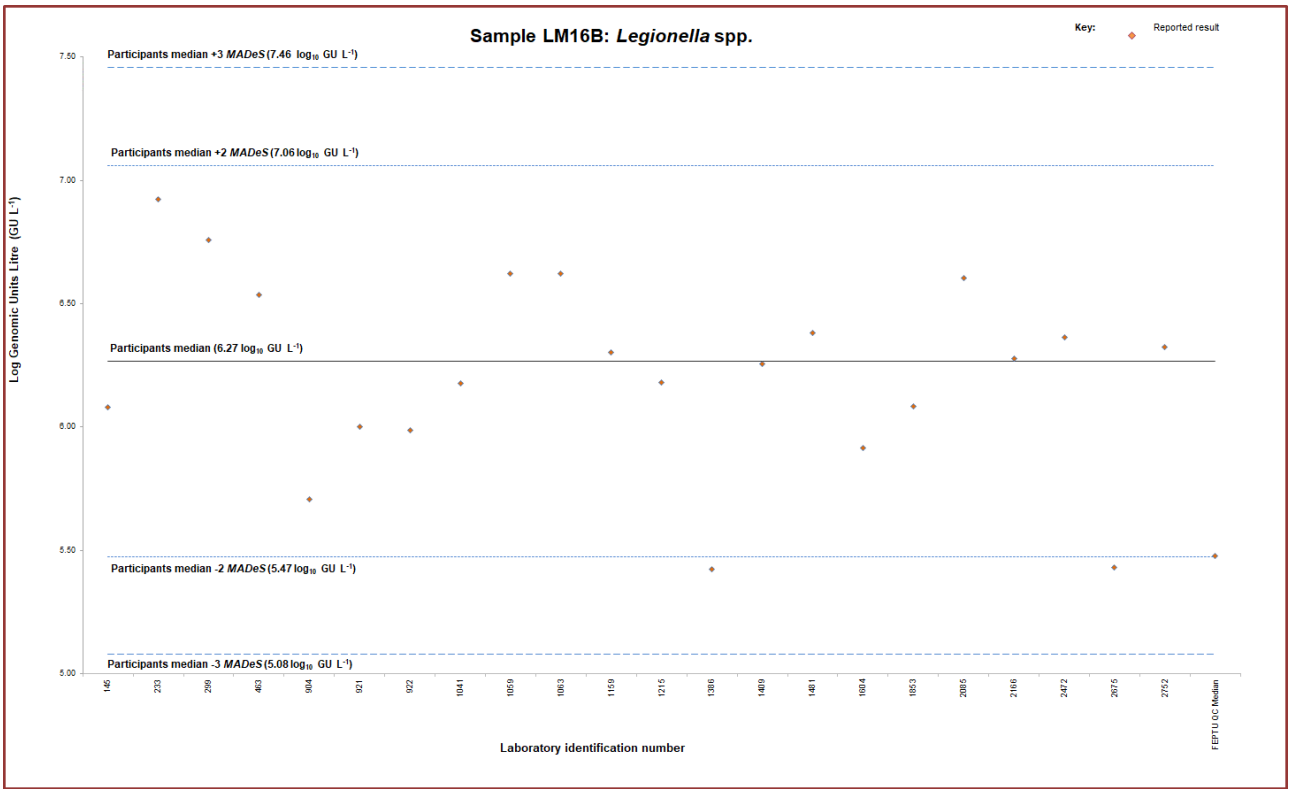
Legionella spp. quantification results

Total number of participants also quantifying for *Legionella* spp.	22

Assigned value (participants' median)	1.89×10^6 (6.28 $\log_{10}$ GU L^{-1})	
Standard deviation of participants results **	0.40 $\log_{10}$ GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= $\log_{10}$ GU L^{-1})	0.11	
Minimum and maximum genomic values	2.65×10^5 (5.42 $\log_{10}$ GU L^{-1})	8.40×10^6 (6.92 $\log_{10}$ GU L^{-1})
Number of outlying results	2 low	
FEPTU's median	3.01×10^5 (5.48 $\log_{10}$ GU L^{-1})	

The fixed standard deviation value (σ_{pt} value) used for calculation of the z-scores is 0.55 for all parameters

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



Your method/kit details

Guideline / Standard	
Extraction platform	
Commercial kit	
Amplification platform	

Scheme specific comment for LM16A and LM16B

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.*

Scoring

The samples in this distribution have been scored using the below UKHSA 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 (*MADe*) values which are determined from the median result reported by participants' and take into account the following criteria:

(1) median ± 2 *MADeS**
(2) median ± 3 *MADeS**
(3) median ± 0.5 $\log_{10}$ units

If the ranges in (1) and/or (2) are less than the value of the median ± 0.5 $\log_{10}$ 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 UKHSA score of zero for all tests.

Statistical evaluation
Participants are advised that for a robust statistical evaluation at least 20 reported results are required for a parameter. When statistical calculation is based on 10 – 19 result, they should be interpreted with caution as they may be overly influenced by outlying results. This is the reason why the standard deviation of the enumeration results reported can be wide.

New website

We are pleased to announce the launch of our new website: https://www.feptu.org.uk/. Please refer to this website to obtain the latest information for your proficiency testing.

Information of importance

To understand more about the proficiency testing schemes, please use the following links for information on:

1. Report format explained: Annotated report

2. Performance rating: Performance-over-time and Scheme guide (section 16.0)

3. Scoring and statistics used: Scoring information and stats

4. Homogeneity and stability: Scheme guide (section 9.0)

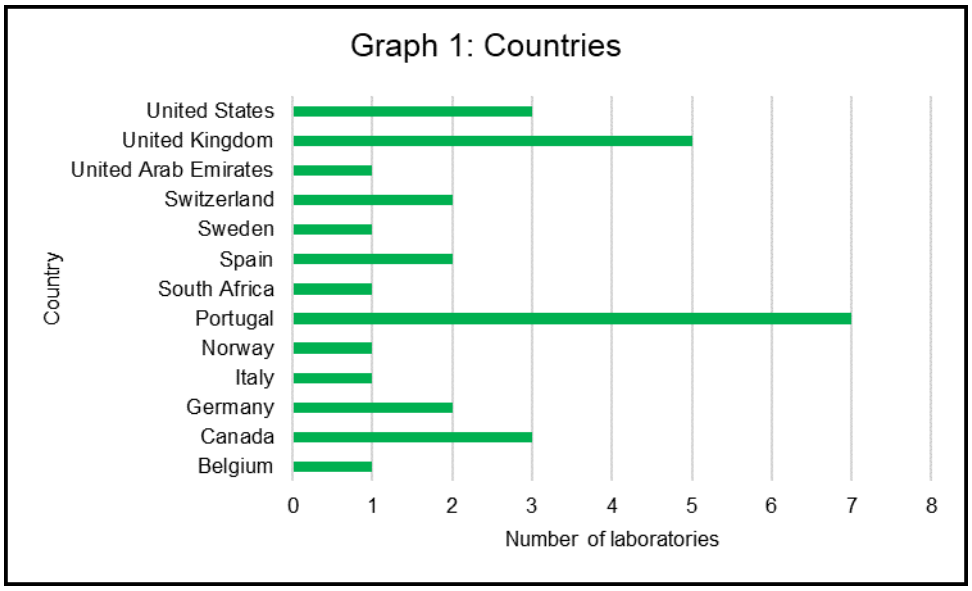
5. Complaints and appeal process: Scheme guide (section 20.0 and 21.0)

For further information about the operation of the service including confidentiality and terms of participation, please refer to the Scheme Guide: Scheme guide

Participation

Total samples sent	**33**
Not examined	**2**
Non return of results	**2**

A total of 13 countries participated in this distribution (graph 1). The majority of which were in Europe (n=30)

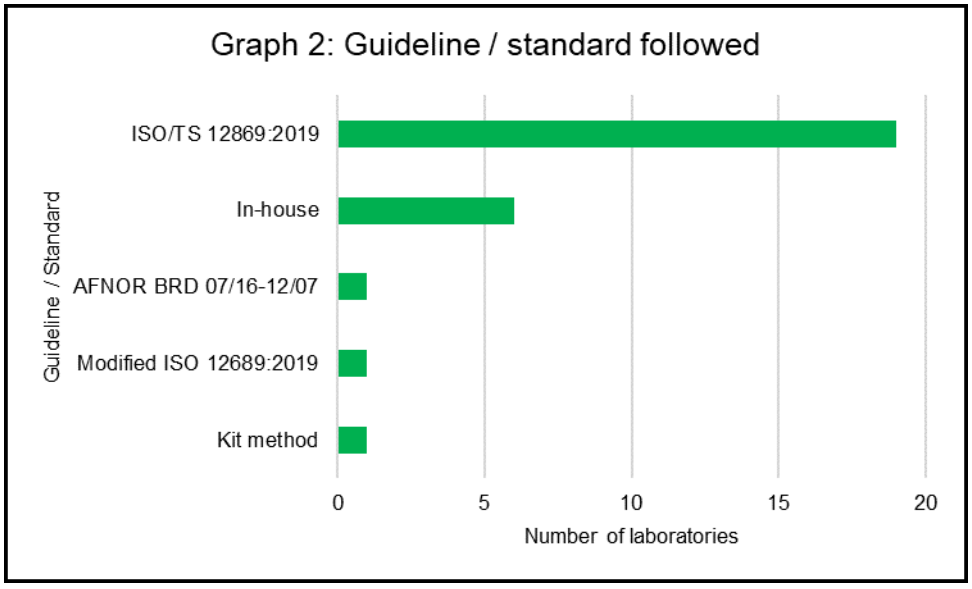


Questionnaire results:

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 28 responses received, the majority 19/28 (68%) used ISO/TS 12869:2019 (graph 2).



2. Filtration of samples

- Of the 28 responses received, 25 laboratories routinely filter 1 litre of a water sample, two laboratories filter 100mL and one laboratory filters 500mLs. 0.2 µm and 0.45 µm are the common membrane filter size used by the laboratories.

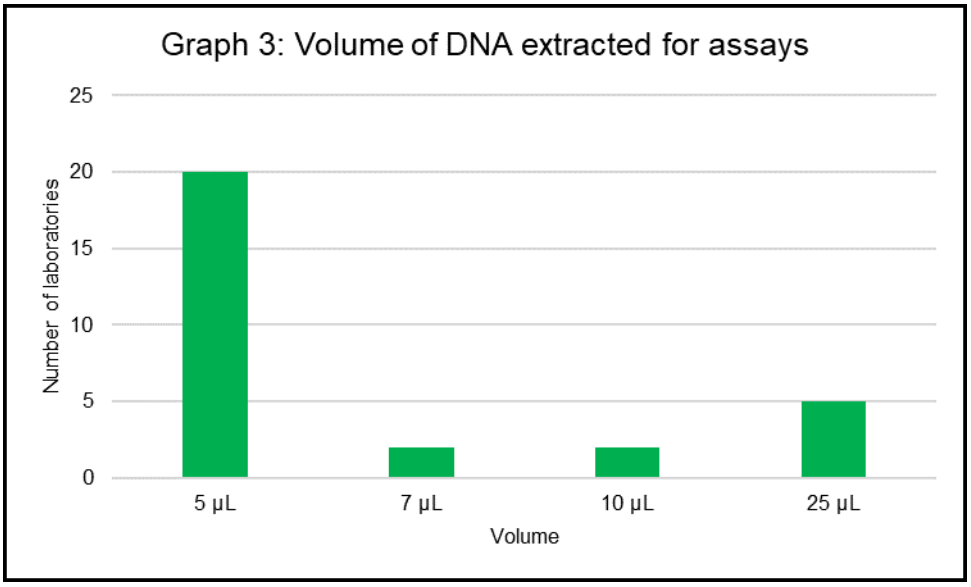
3. Details of the DNA extraction method used

- There was a variation of DNA extraction kits used by participants as shown in the table below (n=27). Some laboratories used multiple methods.

Assay	Number of users
bioMérieux NucliSENS® miniMag®	1
Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification	12
Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification – short protocol	3
Bio-Rad InstaGene™ Matrix	1
BIOTECON foodproof® StarPrep Two Kit	4
DIATHEVA DNApure Water Isolation Kit	1
In-house	2
ThermoFisher Scientific MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit	1
Promega, Maxwell® RSC PureFood GMO and Authentication Kit	1
Qiagen DNeasy PowerSoil Pro Kit	1
Qiagen DNeasy PowerWater kit	1
TANBead Nucleic acid extraction kits	1

4. Volume of extracted DNA used in your assay

- Of the 29 responses received, the volume of extracted DNA added to the assay is shown in graph 3.



5. **Type of PCR used**
    - 26/28 (93%) used a real-time PCR
    - 2/28 (7%) used a ddPCR™

6. **The commercial assays used are shown in the table below from 26 participants. Some laboratories used more than one assay.**

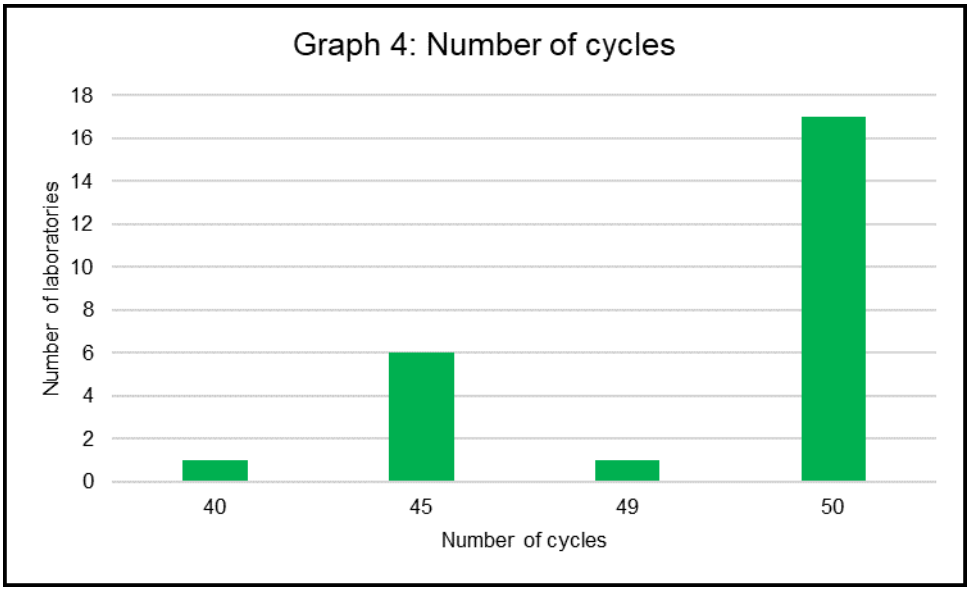
Assay	**Number of users**
Bio-Rad iQ-Check® Quanti *Legionella* spp. Real-Time PCR Quantification Kit	9
Bio-Rad iQ-Check® Quanti *Legionella pneumophila* Real-Time PCR Quantification Kit	10
Bio-Rad iQ-Check® Screen *L. pneumophila* Real-Time PCR Detection Kit	2
Bio-Rad iQ-Check® Screen *Legionella* spp. Real-Time PCR Detection Kit	3
BIOTECON microproof® *Legionella* Quantification Lyokit	5
Diatheva DI-Check	1
Diatheva DI-Check *Legionella pneumophila*	1
Diatheva DI-Check *Legionella* spp.	1
In-house	2
Integrated DNA Technologies PrimeTime™ qPCR Primer Assays	1
Promega GoTaq® Legionella qPCR and Viability qPCR Kits	1
TIB MolBiol LightMix® Modular *Legionella pneumophila*	1

7. **The Amplification platforms used are shown in the table below from 26 participants.**

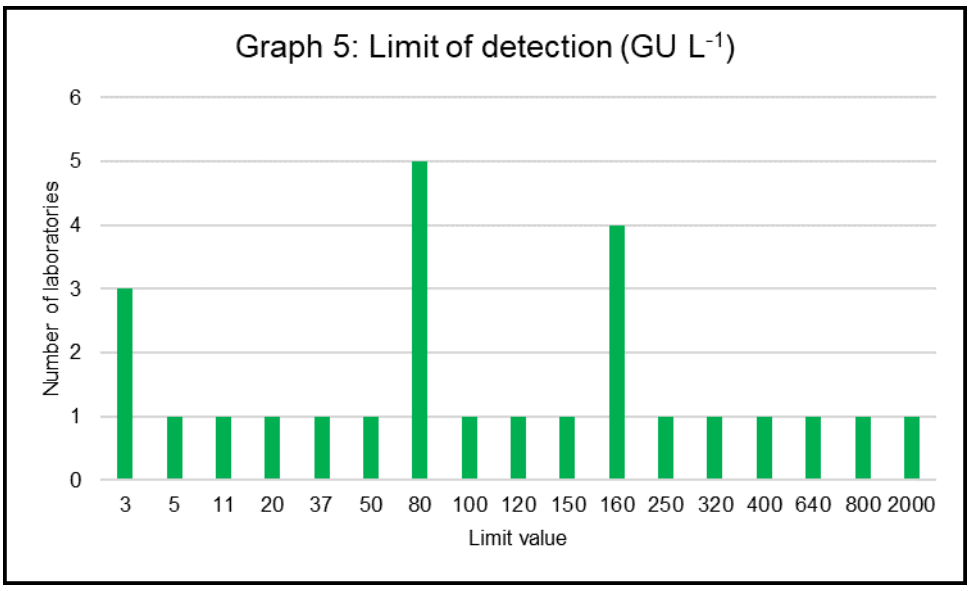
Platforms	**Number of users**
Agilent Technologies Stratagene Mx3005P qPCR System	1
Aligent Technologies AriaMx Real-time PCR System	1
Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System	2
Applied Biosystems® Quantstudio™ 5 Real-Time PCR System	3
Applied Biosystems® 7500 Fast Real-Time PCR System	1
Applied Biosystems™ StepOnePlus™ Real-Time PCR System	1
Bio-Rad CFX Opus Deepwell Real-Time PCR System	1
Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	9
Bio-Rad T100™ Thermal Cycler	1
Qiagen Rotor-Gene Q MDx	1
Roche Diagnostics LightCycler® 96 System	3
Roche Diagnostics LightCycler® 480 System	1
Techne The Prime Pro 48	1

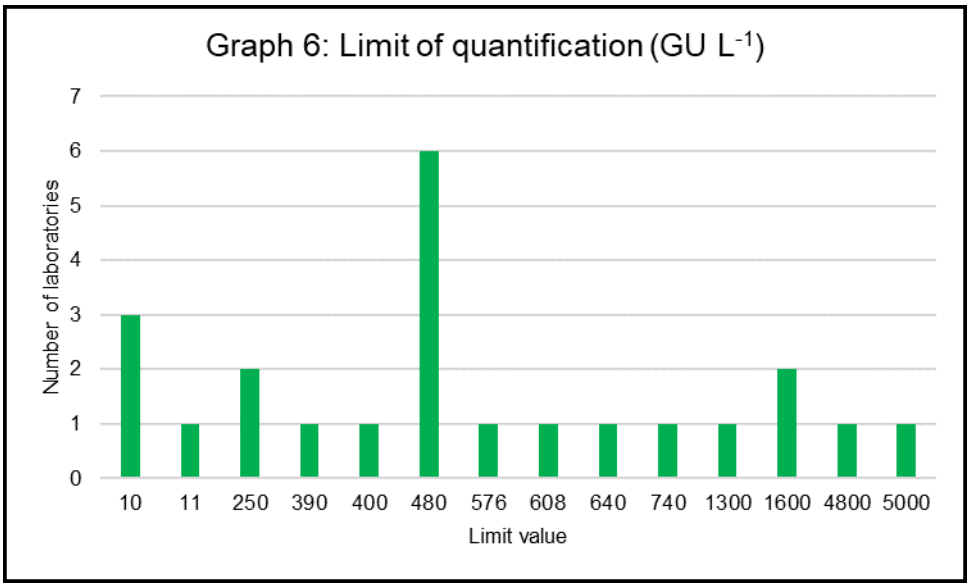
8. Cycling

25 of the participants used between x 45 - 50 cycles (graph 4).



9. Limit of detection (LOD) is shown in graph 5 from 26 participants and limit of quantification (LOQ) in graph 6 from 23 participants.





End of report.