UK Health Security Agency

Summary of Results
Legionella Molecular Scheme

External Quality Assessment for Water Microbiology

Distribution Number: **LM11**
Sample Numbers: **LM11A & LM11B**

Distribution Date:	**1 August 2022**
Results due:	**9 September 2022**
Report Date:	**4 October 2022**
Samples prepared and quality control tested by:	**Divya George** **Cansev Katar** **Zak Prior** **Sidney Sandiford** **Jake Videlefsky**
Data analysed by:	**Joanna Donn** **Nita Patel**
Report compiled by:	**Joanna Donn** **Nita Patel**
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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 12869:2019 - 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
https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide

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
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Sample: LM11A

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 1 (ST2110) *Citrobacter braakii* *Brevundimonas vesicularis* *Staphylococcus saprophyticus*	$1.8x10^4$ $5.4x10^3$ $1.9x10^4$ $1.1x10^5$	$2.1x10^5$ $2.3x10^5$ – 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*	Detected				
Legionella spp.	Detected				
Quantification (GU L^{-1}): *Legionella pneumophila*	$3.12x10^4$ – $1.02x10^6$) (4.49 – 6.01 log_{10})				
Total *Legionella* spp.	$3.77x10^4$ – $1.60x10^6$) (4.58 – 6.20 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	31	31	100
	Legionella spp. - detected	32	32	100

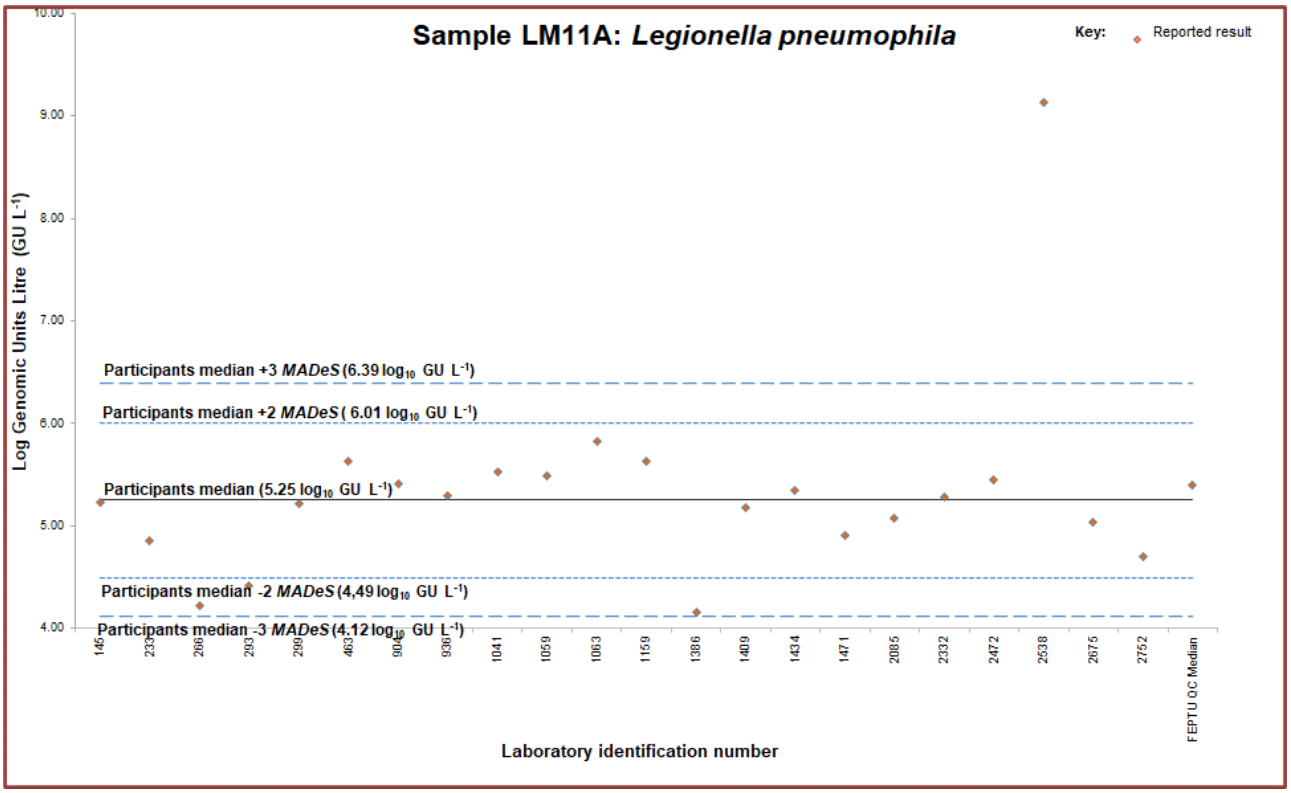
***Legionella pneumophila* quantification results**

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

Assigned value (participants' median)	$1.79x10^5$ ($5.25 \log_{10}$ GU L^{-1})	
Standard deviation of participants results **	$0.38 \log_{10}$ GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= $\log_{10}$ GU L^{-1})	0.10	
Minimum and maximum genomic values	$1.45x10^4$ ($4.16 \log_{10}$ GU L^{-1})	$1.38x10^9$ ($9.14 \log_{10}$ GU L^{-1})
Number of outlying results	4 (3 low, 1 high)	
FEPTU's median	$2.06x10^5$ ($5.31 \log_{10}$ 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*)



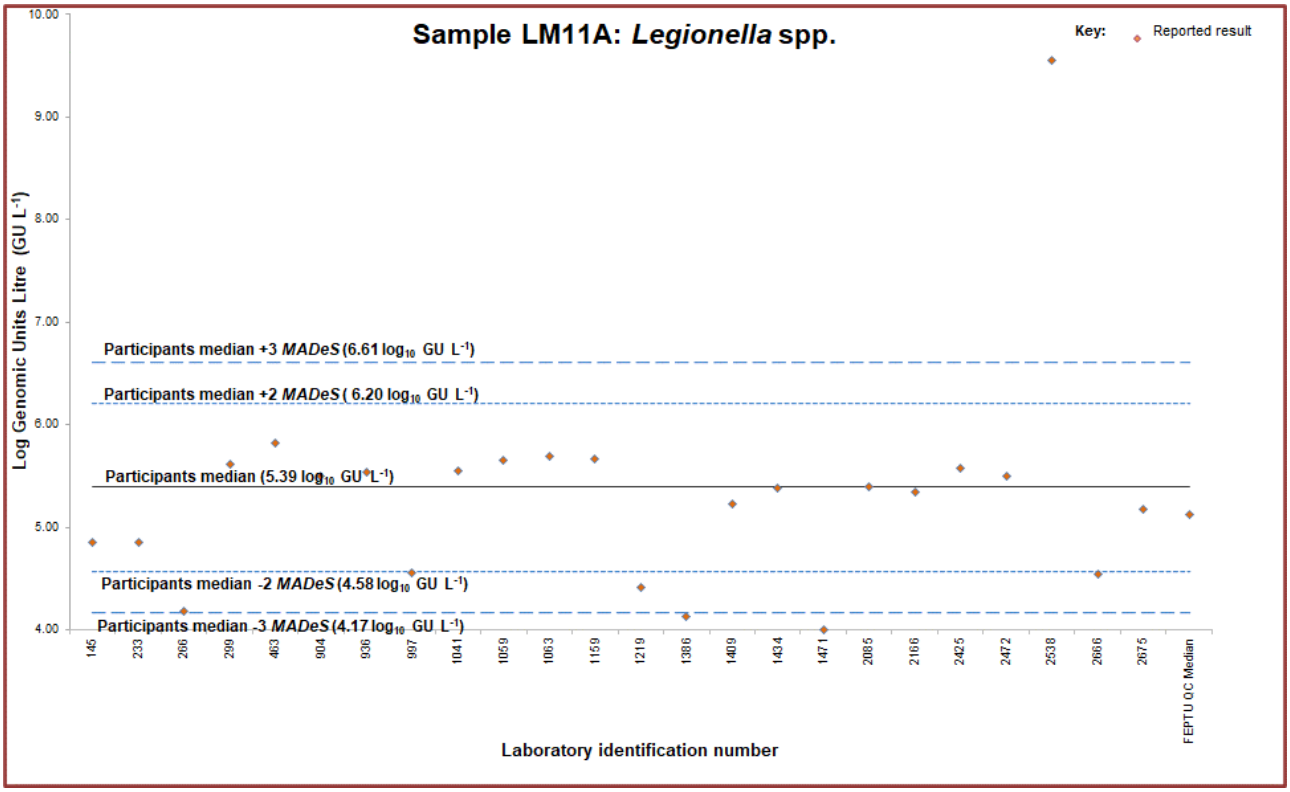
***Legionella* spp. quantification results**

Total number of participants also quantifying for *Legionella* spp.	25
Number of participants reporting a low censored value	1

Assigned value (participants' median)	$2.45x10^5$ (5.39 log_{10} GU L^{-1})	
Standard deviation of participants results **	0.41 log_{10} GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= log_{10} GU L^{-1})	0.10	
Minimum and maximum genomic values	$1.00x10^4$ (4.00 log_{10} GU L^{-1})	$3.63x10^9$ (9.56 log_{10} GU L^{-1})
Number of outlying results	7 (6 low, 1 high)	
FEPTU's median	$2.30x10^5$ (5.36 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: LM11B

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 1 (ST2454) *Citrobacter braakii* *Brevundimonas vesicularis*	$6.0x10^3$ $1.2x10^3$ $6.6x10^3$	$2.5x10^5$ $2.5x10^5$ – 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*	Detected				
Legionella spp.	Detected				
Quantification (GU L^{-1}): *Legionella pneumophila*	$3.89x10^4$ –$1.48x10^6$ (4.59 – 6.17 log_{10})				
Total *Legionella* spp.	$2.63x10^4$ – $3.55x10^6$ (4.42 – 6.55 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	30	29	97
	Legionella spp. - detected	32	31	97

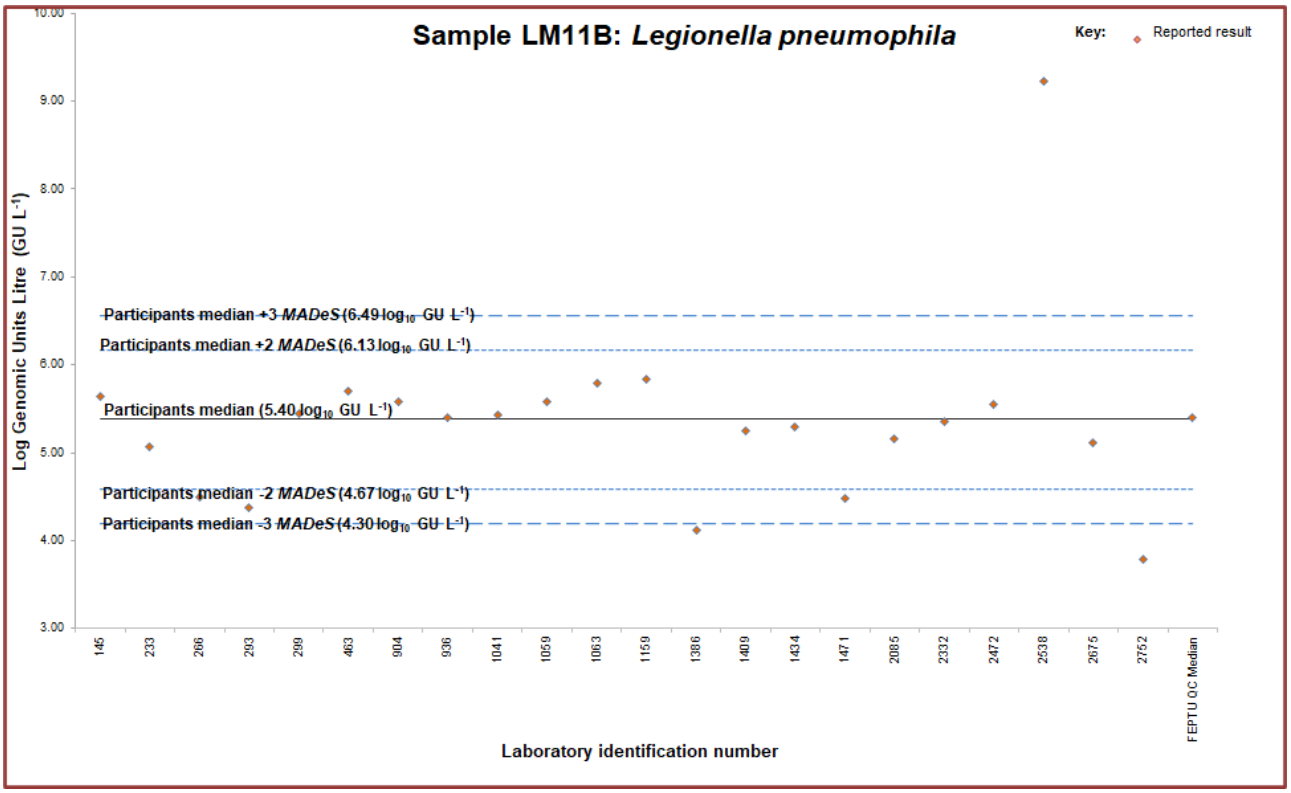
***Legionella pneumophila* quantification results**

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

Assigned value (participants' median)	$2.40x10^5$ ($5.38 \log_{10}$ GU L^{-1})	
Standard deviation of participants results **	$0.39 \log_{10}$ GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= $\log_{10}$ GU L^{-1})	0.10	
Minimum and maximum genomic values	$6.17x10^3$ ($3.79 \log_{10}$ GU L^{-1})	$1.70x10^9$ ($9.23 \log_{10}$ GU L^{-1})
Number of outlying results	6 (5 low, 1 high)	
FEPTU's median	$2.50x10^5$ ($5.40 \log_{10}$ 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*)



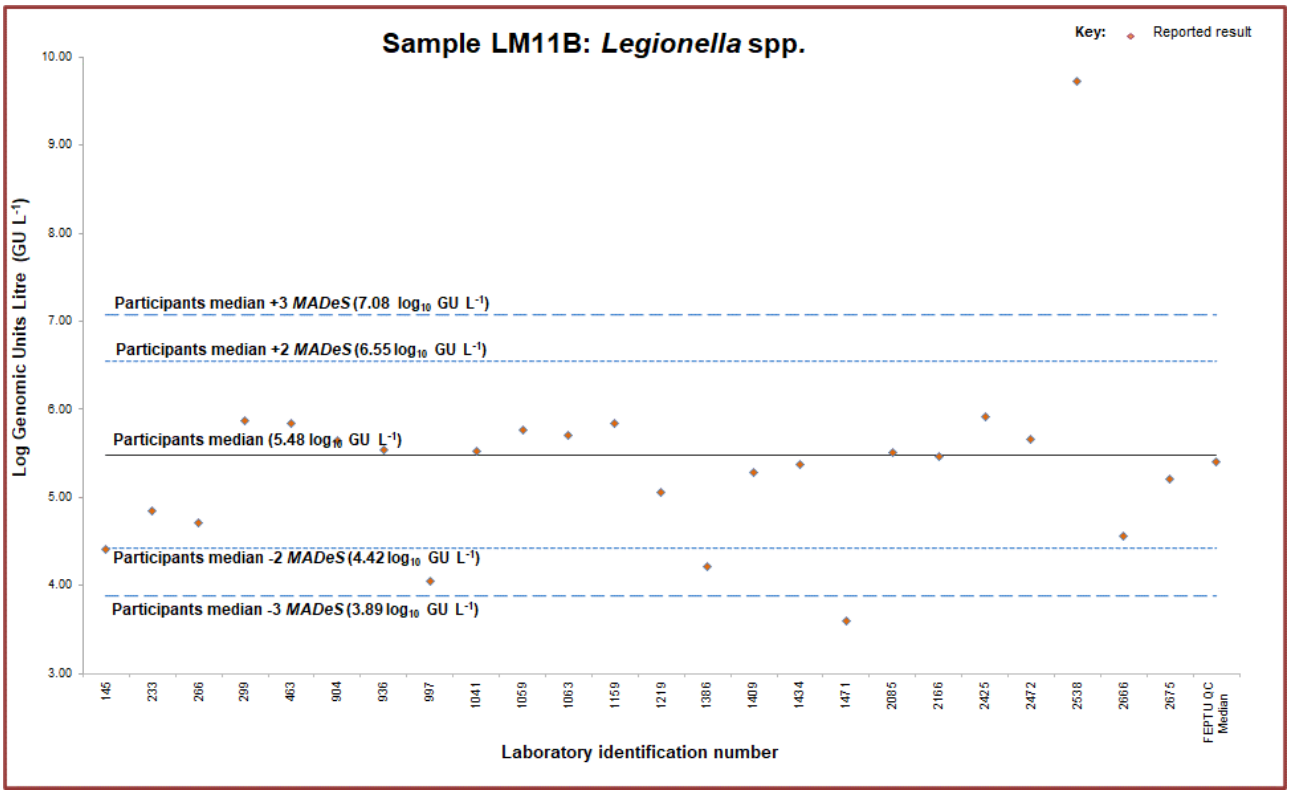
Legionella spp. quantification results

Total number of participants also quantifying for *Legionella* spp.	25
Number of laboratories reporting a low censored value	1

Assigned value (participants' median)	3.02×10^5 ($5.48 \log_{10}$ GU L^{-1})	
Standard deviation of participants results **	$0.53 \log_{10}$ GU L^{-1}	
Uncertainty of assigned value ($U(X_{pt})$= $\log_{10}$ GU L^{-1})	0.14	
Minimum and maximum genomic values	3.98×10^3 ($3.60 \log_{10}$ GU L^{-1})	5.37×10^9 ($9.73 \log_{10}$ GU L^{-1})
Number of outlying results	4 (3 low, 1 high)	
FEPTU's median	2.50×10^5 ($5.40 \log_{10}$ 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*)



Scheme specific comment for LM11A and LM11B

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.

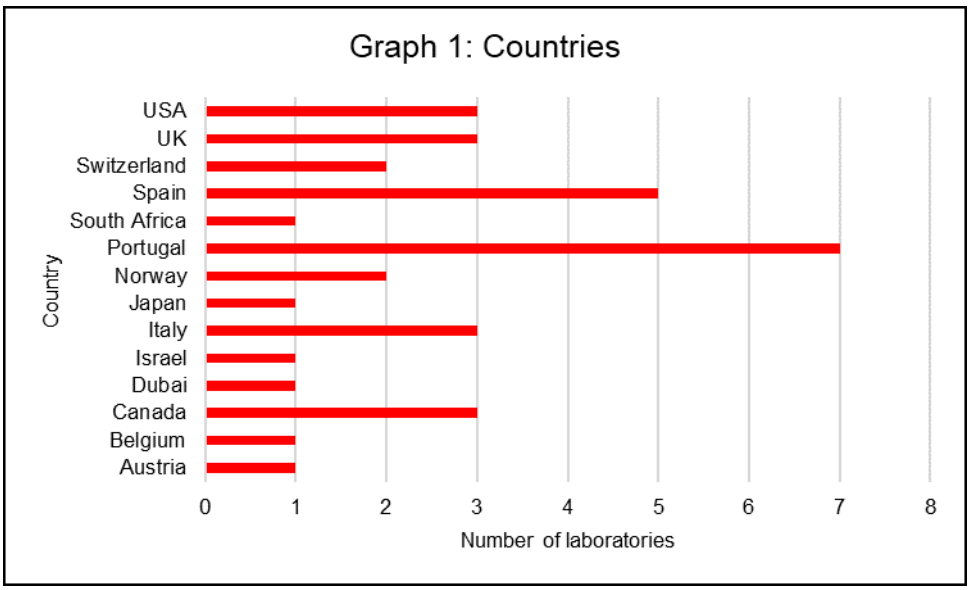
Statistical evaluation
Participants are advised that for a robust statistical evaluation at least 20 reported results are required for a parameter. When statistical calculation is based on 10 – 19 result, they should be interpreted with caution as they may be overly influenced by outlying results. This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

Z-scores
Following a review of all the participants' data and the standard deviation calculated from all the previous distributions (LM1 – LM6), the organisers have amended the fixed standard deviation value (σ_{pt} value) used to calculate the z-scores to 0.55 instead of 0.35. This is due to the wide variability of results that is observed with this examination.

Participation

Total samples sent	**44**
Not examined	**2**
Non return of results	**8**

A total of 14 countries participated in this distribution (graph 1). The majority of which were in Europe (n=34)

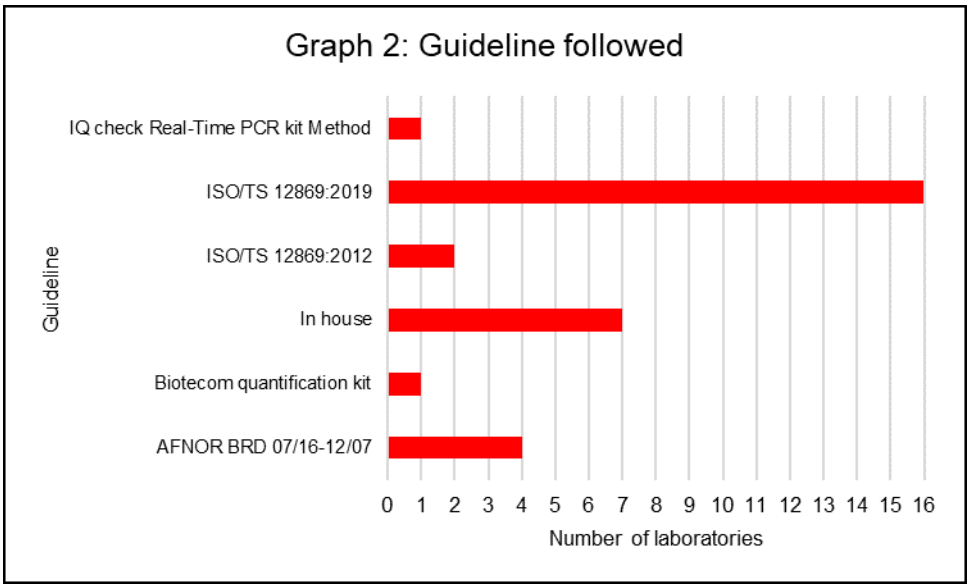


Questionnaire results:

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 31 responses received, the majority used ISO/TS 12869:2019 (graph 2).



2. Filtration of samples

- Of the 33 responses received, 32 laboratories routinely filter 1 litre of a water sample and one laboratory filtered 100mL. In addition one laboratory commented that they also concentrate the sample by centrifugation.

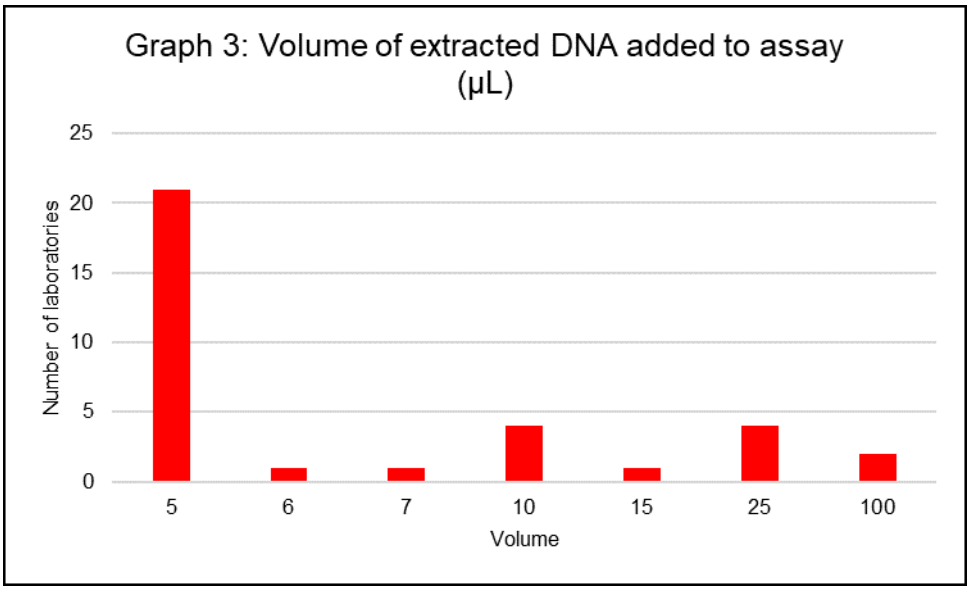
3. Details of the DNA extraction method used

- There was a variation of DNA extraction kits used by participants as shown in the table below (n=34).

Assay	Number of users
bioMérieux NucliSENS® miniMag®	2
Bio-Rad Aquadien™ Bacterial DNA Extraction and Purification	16
Bio-Rad InstaGeneTM Matrix	1
Bioside qualyfast™ *Legionella* PCR and DNA Extraction Kits	1
BIOTECON foodproof® StarPrep Two Kit	3
Chelex	1
DIATHEVA DNApure Water Isolation Kit	1
In-house method	1
Macherey-Nagel NucleoSpin® gDNA Clean-up	1
Maxwell® RSC PureFood GMO and Authentication Kit	1
PALL Corporation Extraction Pack Environment 01	1
PrepSEQ Rapid Spin	1
Qiagen DNeasy PowerSoil Pro Kit	2
Roche Diagnostic MagNa Pure Compact Isolation kit I	1
Roche Diagnostic MagNa Pure Compact Isolation kit I	1

4. Volume of extracted DNA used in your assay

- Of the 34 responses received, the volume of extracted DNA added to the assay is shown in graph 3.



5. Type of PCR used

- 31/34 (91%) used a real-time PCR
- 1/34 (3%) used a conventional RT-PCR
- 2/34 (6%) used a ddPCR™

6. The commercial assays used are shown in the table below from 29 participants. Some laboratories used more than one assay.

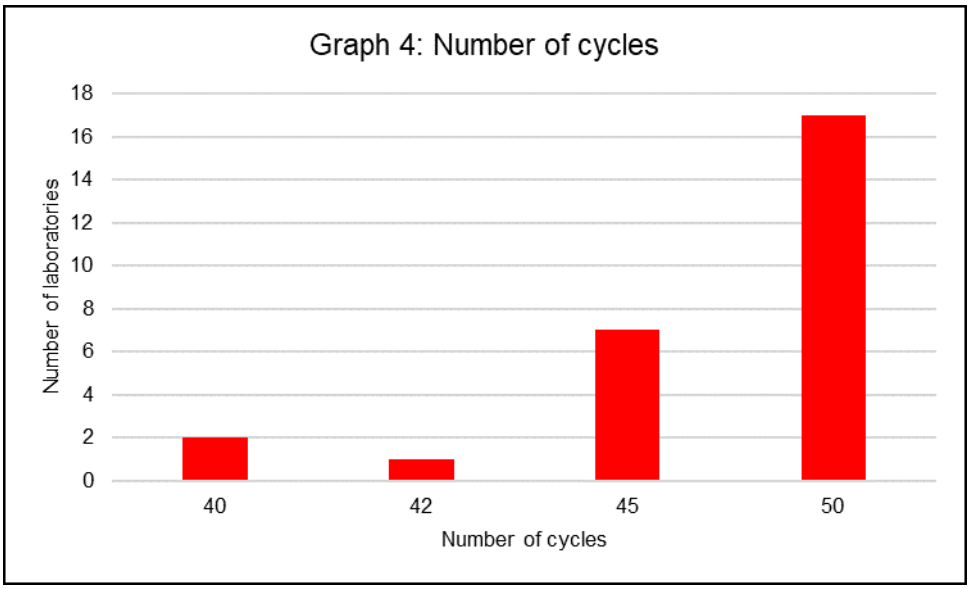
Assay	Number of users
Bio-Rad iQ-Check®	1
Bio-Rad iQ-Check® *Legionella* Real-Time PCR Kit	1
Bio-Rad iQ-Check® Quanti *Legionella* spp. Real-Time PCR Quantification Kit	6
Bio-Rad iQ-Check® Quanti *L. pneumophila* Real-Time PCR Detection Kit	5
Bio-Rad iQ-Check® Screen *L. pneumophila* Real-Time PCR Detection Kit	4
Bio-Rad iQ-Check® Screen *Legionella* spp. Real-Time PCR Detection Kit	2
Bioside qualyfast™ *Legionella* Kit	1
BIOTECON microproof® *Legionella* Quantification Lyokit	3
Diatheva DI-Check	1
Diatheva DI-Check *Legionella pneumophila*	1
Diatheva DI-Check *Legionella* spp.	1
GPS kit	1
ielab *L. pneumophila* detection and quantification kit	1
In-house	4
Roche LightCycler® 480 Probes Master	1
TIB MolBiol LightMix® kit for *Legionella* (16S RNA)	1
TaKaRa Bio Inc. Cycleave® PCR *Legionella* (16S rRNA) Detection Kit	1

7. The Amplification platforms used are shown in the table below from 25 participants.

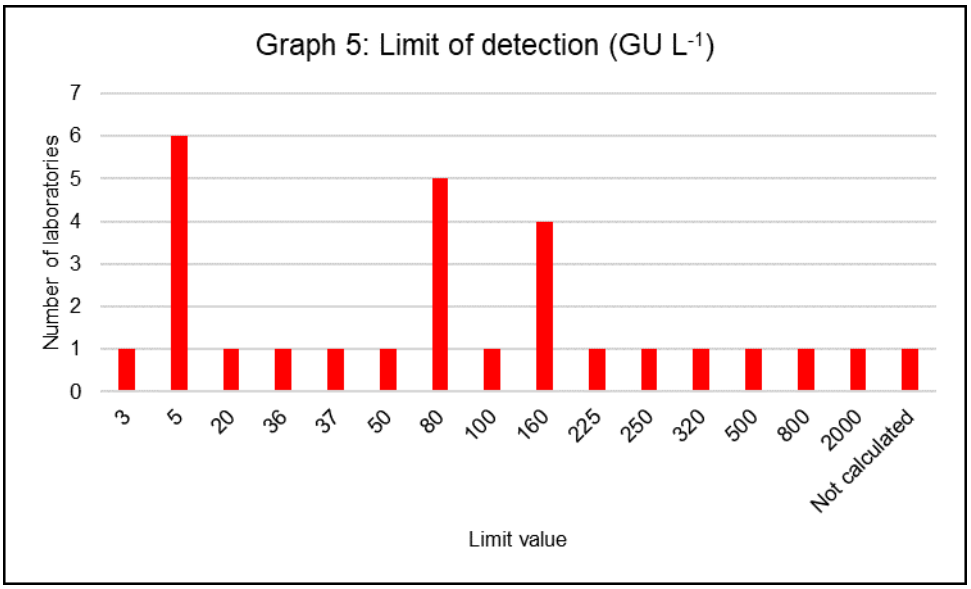
Platforms	Number of users
Applied Biosystems® Quantstudio™ 5 Real-Time PCR System	3
Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System	1
Applied Biosystems® 7500 Fast Real-Time PCR System	4
Applied Biosystems™ StepOnePlus™ Real-Time PCR System	1
Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	11
Bio-Rad T100™ Thermal Cycler	1
Qiagen Rotor-Gene Q	1
Roche Diagnostics LightCycler® 2.0 Instrument	1
Roche Diagnostics LightCycler® 96 System	2

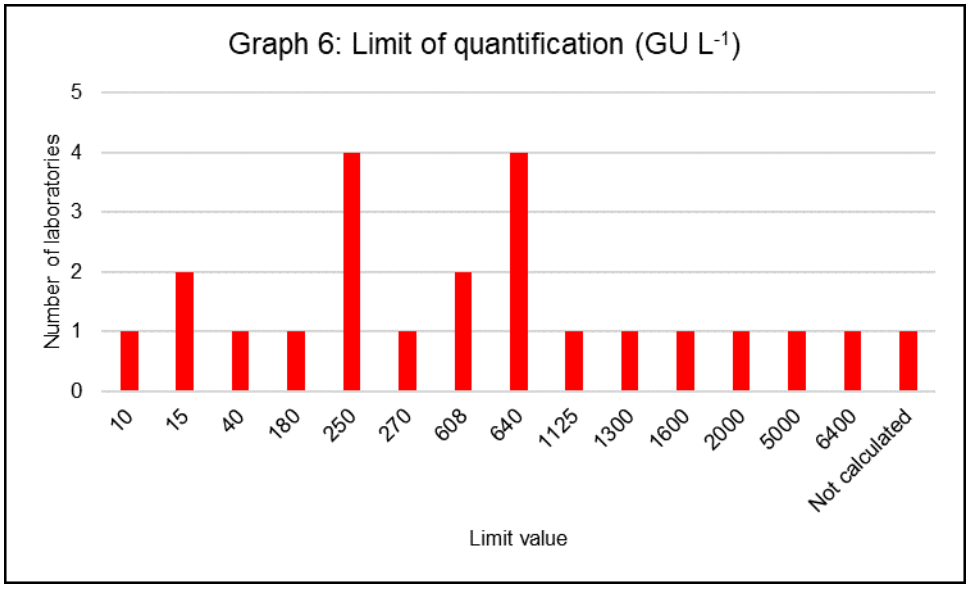
8. Cycling

27 of the participants used between x 40-50 cycles (graph 4).



9. Limit of detection (LOD) is shown in graph 5 from 28 participants and limit of quantification (LOQ) in graph 6 from 23 participants.





End of report.