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External quality assessment schemes to support European surveillance of Legionnaires' disease in the EU/EEA countries, 2025

EQA organizer: European Reference Laboratory in Public Health on *Legionella* (EURL-PH-LEGI)

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Figures and tables

Abbreviations

AD	Allelic differences
BCYE	Buffered charcoal yeast extract
Cfu	Colony-forming units
cgMLST	Core genome multilocus sequence typing
COPD	Chronic obstructive pulmonary disease
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EQA	External Quality Assessment
ELDSNet	European Legionnaires' Disease Surveillance Network
EU	European Union
EURL	European Union Reference Laboratory
EURL-PH-LEGI	European Union Reference Laboratory for Public Health on Legionella
GVPC	Glycine–vancomycin–polymyxin B–cycloheximide
HCL	Hospices Civils de Lyon
IATA	International Air Transport Association
IQLS	Integrated Quality Laboratory System
ISO	International Organization for Standardization
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
mL	Millilitre
mompS	Major outer membrane protein S gene
NFP	National Focal Point
OCP	Operational Focal Point
PCR	Polymerase chain reaction
QC	Quality control
SBT	Sequence-based typing
Sg	Serogroup
SNP	Single nucleotide polymorphism
SPU	Sputum (specimen code)
ST	Sequence type
TALD	Travel-Associated Legionnaires' Disease
UN3373	United Nations category for Biological Substance, Category B
URI	Urine (specimen code)
WGS	Whole genome sequencing

Executive summary

This report describes the External Quality Assessment (EQA) schemes established by the EURL-PH-LEGI for participation by laboratories of the European Legionnaires' disease Surveillance network. It includes methods for the detection by PCR, isolation by culture, and identification of *Legionella* species, as well as cluster analyses using bioinformatic tools. The EQA schemes provided an outbreak scenario with packages of whole genome sequencing data, a DNA control, clinical specimens, and environmental samples for the participating laboratories to process, according to their technical capacity and protocols.

The purpose of the 2025 exercise was to continue monitoring the accuracy of *Legionella* testing and results reported by individual participating laboratories from 26 participating EU/EEA countries. It also includes information on the methods/kits in use as reported by the participating laboratories in Annex 1.

For the bioinformatic scheme, whole genome sequencing data from 15 isolates were sent on 2 June 2025 through a secure server to 18 participants, and 16 answered. Participants were invited to analyze the quality of the sequences provided and, if quality was satisfactory, to perform species identification, sequence-based typing, and cluster analysis by tool of choice (e.g., cgMLST or SNP). This scheme represented an outbreak associated with a hotel. The outbreak strain *Legionella pneumophila* serogroup (sg) 3, sequence type (ST) 93, was isolated from clinical specimens and environmental samples. Overall concordance with the intended results was very good (93.7%) for the identification of the species. Concordance was good (89.3%) for reporting the sequence type, with a lower concordance for STs with two copies of the *momps* gene. Concordance was excellent (94.8%) for the identification of the outbreak and the source.

For the clinical and environmental schemes, samples were shipped on 22-23 September 2025. Each package contained 10 specimens representing clinical material and/or 5 water samples, as well as a DNA control sample. Urine specimens and strains of *Legionella* were provided by the French national reference center, and they were fully characterized using conventional and molecular methods. Laboratories only needed to examine the samples they would routinely test or process and report whether any contained a *Legionella* spp., and then provide identification, serogroup (Sg), and sequence type (ST), where relevant. This scheme represented an outbreak associated with a campground. The outbreak strain *L. pneumophila* serogroup 1, ST23, was isolated from both the clinical specimens and environmental samples.

For the clinical scheme, 25 participants were sent the sample package, and all returned at least one result. Overall concordance was very good (93.4%) for urinary antigen testing. Concordance with the intended results was good (83%) for the PCR results on sputum samples. All the participants were able to correctly identify *L. pneumophila* in sputum samples. However, the concordance was much lower in the case of co-infection, with only 55.6% of participants identifying both species. Concordance was excellent (100%) for the identification of *L. pneumophila* serogroup 1 even in the absence of strains. However, concordance reduced dramatically when a non-Sg 1 strain was included. In the presence of a strain, 80% the participants reported a sequence type, and concordance was very good (92%).

For the environmental scheme, 23 participants were sent the sample package, and 20 returned a result. Only 35% of participants reported results for PCR, but all reported results for isolation, identification, and serogroup at least once. Up to 17 participants reported an enumeration count, and 9 reported a sequence type at least once. The overall isolation performance for culture was good (77%), and concordance for *Legionella* species identification was very good (92%).

The **DNA control** was included in the package for 30 participants; only 17 participants performed PCR for at least one target, with 76.5% participants performing *Legionella* spp. and *L. pneumophila* PCR, and only 35.3% performing *L. pneumophila* serogroup 1-specific PCR. As expected, intra-laboratory variation was low across the three targets (71% of participants reporting a variation under 0.23 Ct).

Normal flora relevant to the specimen or sample type was included to simulate a real sample, but also to challenge the laboratories' processing techniques and to confirm the performance of the selective agar used. Participants with suboptimal performance were advised to contact the EQA organizer for support in investigating and identifying the root cause.

The overall conclusion of this 2025 EQA exercise is that on average laboratories reached 87% of the full scores for the results they submitted across all schemes and all samples. The overall results of this EQA, within the limits of its design, provide assurance that EU/EEA laboratories can undertake effective public health investigations for *L. pneumophila*. Laboratories need to continue to take part in available EQA programs to maintain up-to-date data on performance and confirm their proficiency. In addition, the EQA organizer will improve their processes to reduce inter-laboratory variation and to clarify the guidelines for the testing of control material.

1 Introduction

Background

Legionnaires' disease is a serious, and potentially fatal, infection caused by Gram-negative bacteria of the genus *Legionella*. These organisms are ubiquitous in natural freshwater environments and soil and may colonize man-made water systems. More than 60 *Legionella* species have been described, over 20 of which are known to cause human disease. *Legionella pneumophila* is the species most frequently detected in both environmental samples and clinical cases. This species is classified into at least 16 serogroups based on surface antigenic properties, with serogroup (Sg) 1 being responsible for the majority of outbreaks.

Routine laboratory capacity to distinguish *L. pneumophila* from other *Legionella* species, as well as to differentiate Sg 1 from other *L. pneumophila* serogroups, is therefore essential. In outbreak situations, higher-resolution typing methods are required to accurately identify clusters and trace sources of contamination.

In Europe, the majority of reported cases (approximately 72.6% in 2024, according to ECDC) are community-acquired and sporadic. Surveillance of Legionnaires' disease at EU level is conducted through the European Legionnaires' Disease Surveillance Network (ELDSNet), with ECDC responsible for coordinating the collection and analysis of annual surveillance data across the EU/EEA. Although Legionnaires' disease is legally notifiable in all EU/EEA countries, the true burden is likely underestimated. Under-reporting may result from clinical underdiagnosis, particularly when empirical antibiotic treatment is initiated without specific testing for *Legionella*, as well as from incomplete notification by healthcare professionals. In addition, differences in case ascertainment and laboratory practices may contribute to the variability in notification rates observed between countries.

The European Reference Laboratory in public health on *Legionella* (EURL-PH-LEGI) is responsible to implement Regulation (EU) 2024/892 of the European Parliament and of the Council of 23 November 2022 on serious cross-border threats to health, in particular relating to *Legionella*. Its core mandate is to promote best practices and provide technical support to EU/EEA Member States in diagnostics, testing, and typing, thereby ensuring data quality, comparability, and strengthened laboratory capacity. Within this framework, the EURL-PH-LEGI is responsible for organizing External Quality Assessment (EQA) schemes to support and enhance the surveillance of Legionnaires' disease across the EU/EEA.

As specified in the Guide for EU-level external quality assessments (EQAs) for public health microbiology laboratories, the objective of implementing EQAs for EU-level public health microbiology laboratories is to assess the laboratories' proficiency in order to strengthen and maintain the high quality and comparability of public health laboratory data, ensure robust capacity for the detection and characterization of pathogens, and identify needs for capacity and capability building related to pathogens of public relevance.

Within this framework, the present EQA evaluates the competency of participating laboratories in analyzing whole genome sequencing data, clinical specimens, and environmental samples for the detection of *Legionella* spp. and the identification of clusters. It provides an independent mechanism to assess and improve laboratory performance, support accreditation and quality systems, and ultimately strengthen public health and clinical responses through reliable, high-quality testing.

External quality assessment, 2025

The primary purpose of this EQA program year was to determine the accuracy of *Legionella* testing and results reported by individual participating laboratories This report presents an analysis of the results.

The secondary objectives of the 2025 EQA program were:

- to understand the current baseline level of testing undertaken in laboratories in response to routine outbreak scenarios, for both clinical and environmental samples.
- to assess if there were any general performance concerns for specific issues relating to the different species, concentrations, and normal flora included
- to provide data to design EURL-PH-LEGI wet lab training, webinars, and country visits to improve laboratory performance.

The objectives of this EQA program align closely with broader EU/EEA public health priorities by supporting enhanced laboratory diagnostic capabilities and data accuracy for Legionnaires' disease surveillance. By ensuring laboratories can accurately identify and characterize *Legionella* spp., this initiative contributes to improved outbreak detection, more effective epidemiological investigations, and timely public health interventions. These efforts directly support the EU's commitment to reducing health inequalities, safeguarding public health, and strengthening cross-border collaboration to address infectious disease threats.

2 Study design and methods

Organization of the External Quality Assessment schemes

The EQA schemes were organized by the National Reference Centre for Legionella in the Hospices Civils de Lyon (HCL, France), coordinator of the EURL-PH-LEGI in collaboration with ECDC. The HCL is not ISO/IEC 17043:2013 accredited; however, all requirements of the standard were followed in the delivery of these schemes. Sample design and format for each scheme were agreed in advance with ECDC and the EURL-PH-LEGI consortium.

Overall, 29 EU/EEA countries were contacted to nominate up to two laboratories (to cover clinical and/or environmental samples) for their participation in the EQA program 2025. Nominations were made through ECDC NFPs for Legionnaires' disease and OCP for Microbiology – Legionellosis. Participation is free for nominated participants. Nominated laboratories received an email to confirm their consent to participate in the different schemes of the EQA, and a list of participating laboratories was established. If a laboratory did not meet the reporting deadline, they were not considered for any statistical analyses, and they did not receive a certificate of participation.

The first scheme was launched on 2 June 2025 based on whole genome sequence data of 15 Illumina short read sequences (in fastq format). For clinical and environmental schemes, the packages of samples were sent to participating laboratories on 22 and 23 September 2025. The distribution comprised a total of 16 simulated samples: 1 DNA control, 5 urine, 5 simulated sputum, and 5 water samples. Sample design and format were agreed in advance with ECDC and the EURL-PH-LEGI consortium.

HCL undertook testing of the samples in accordance with published methods, to replicate – where possible – the testing methods that would be used by the participants. Detection, identification, enumeration, confirmation, and further characterization tests (serogrouping and sequence-based typing) were also undertaken.

In total, 26 of the 29 participating countries tested WGS data, clinical specimens, and/or environmental samples (Table 1). Three countries (Iceland, Ireland and Luxembourg) did not register for participation in the EQA.

Table 1. EU/EEA countries participating in the bioinformatic, clinical and environmental EQA, 2025

Country	Participated in bioinformatic scheme 1	Participated in clinical scheme 2	Participated in environmental scheme 3	Number of schemes	Number of participating laboratories per country
Austria	Yes	Yes	Yes	3	1
Belgium	Yes	Yes	No	3	1
Bulgaria	No	Yes	Yes	2	1
Croatia	No	Yes	Yes	2	1
Cyprus	No	Yes	Yes	2	2
Czechia	No	Yes	Yes	2	1
Denmark	No	Yes	Yes	2	1
Estonia	Yes	Yes	Yes	3	1
Finland	Yes	No	No	1	1
France	Yes	Yes	Yes	3	1
Germany	Yes	Yes	Yes	3	1
Greece	Yes	Yes	Yes	3	1
Hungary	Yes	Yes	Yes	3	1
Italy	Yes	Yes	Yes	3	1
Latvia	Yes	Yes	Yes	3	2
Lithuania	Yes	Yes	No	2	1
Malta	No	Yes	Yes	2	2
Netherlands	Yes	Yes	Yes	3	1
Norway	Yes	Yes	Yes	3	2
Poland	No	Yes	Yes	2	2
Portugal	Yes	Yes	Yes	3	1

Romania	Yes	Yes	Yes	3	1
Slovakia	No	Yes	No	1	1
Slovenia	No	Yes	No	1	1
Spain	No	Yes	No	1	1
Sweden	Yes	Yes	Yes	3	2
Total	**16**	**25**	**20**	**-**	**32**

The packages were dispatched in approved UN3373 containers that included dispatch letters and an EQA protocol. This protocol contained information on the sample details, instructions on how to process, the safety data information, and instructions on how to enter the results online. Further information was also sent to all participants electronically.

A dedicated web interface was available for laboratories to enter and submit their results (hosted by EURL-PH-LEGI subcontractor IQLS). Detailed instructions were included in a user manual on how to access the secure website using the username and password provided. The deadline for the final submission of results was stated in the protocol. Laboratories were allowed six weeks (42 days) from the date of dispatch to examine the samples and return all their results. The length of time allowed for this exercise was determined according to the length of time required to isolate the *Legionella* spp. on culture media (minimum 10 days) and undertake the relevant confirmatory testing, which includes the time a reference laboratory may require to provide a result for specialist tests, such as sequence-based typing.

Six weeks after the dispatch date, the web-based platform was closed to results submission. Individual reports providing a detailed evaluation of participant results were made available in August 2025 for the bioinformatics scheme and in December 2025 for the clinical and environmental schemes. Although participants were advised to investigate the root causes of any deviations, the EURL-PH-LEGI did not yet receive any requests for technical support. Certificates of participation were sent electronically to the laboratories on 15 December 2025. A hard copy of the certificate was available by request.

From 15 December 2025 to 15 January 2026, EURL-PH-LEGI conducted a short online satisfaction survey to obtain feedback on the 2025 EQA exercise and to enable the laboratories to suggest improvements for future EQAs organized by EURL-PH-LEGI.

Exercise scenario and sample design

The strains selected for the exercise were chosen in consultation with EURL-PH-LEGI experts in clinical and environmental microbiology and in collaboration with ECDC experts.

Strains of *Legionella* were provided by HCL as fully characterized isolates. Commensal/background flora was taken from a bank of organisms held by the EQA organizers, and these strains were fully characterized using conventional methods. Homogeneity and stability results were assessed to ensure suitability for use and that the defined criteria were met. Samples were authorized for inclusion in the distribution if they were homogeneous and their contents matched those obtained from HCL for identification, serogroup, and sequence type. Samples were quality-controlled by the EQA management team of the Hospices Civils de Lyon and processed in the same ways they would have been by the participating laboratories at week 1, 3, 5, and 8 after distribution of the packages.

All packages were dispatched at -20°C, in accordance with the latest International Air Transport Association regulations, using an approved airfreight company.

The individual laboratory reports detailed a laboratory's results for each of the requested examinations and the microbiological contents for each sample. This included the identification of the *Legionella* spp., serogroup, sequence type, and PCR results, where applicable. The report also provided an overall performance result (i.e., the percentage of results that were concordant or partial) for each examination based on a pooled total of all the laboratories' reported results.

Scheme 1: Outbreak investigation using bioinformatics methods

Samples were quality controlled by the EQA organisers. Sequence quality was assessed using QUAST and FastQC. Species identification was performed using Kraken and sequence-based typing was performed using Chewbacca and el_gato. Cluster analysis based on cgMLST was realized with two schemes: i) the 50 genes scheme used in routine investigation and ii) the 1521 genes scheme. SNP analysis was performed using snippy. All isolates were also sequenced with ONT technology to confirm the results. The outbreak strain *L. pneumophila* serogroup 3, ST93, was

isolated from clinical specimens and environmental samples. This ST is reported sporadically in France and has the allelic profile: 3; 10; 1; 28; 14; 9; 13. Other strains included in this scheme were:

- *L. pneumophila* **Sg 1, ST259** – considered a subspecies of *L. pneumophila raphaeli* [1]. ST259 is among the most frequent sporadic ST in France, and it has been responsible for multiple outbreaks in the past. This ST has the allelic profile: 21; 27; 28; 2; 15; 29; 6.
- *L. pneumophila* **Sg 1, ST1** – one of the major ST in Europe and represented 12% of cases in France and 10% of cases in Spain. It has been responsible for several outbreaks in France and Europe. This ST has the allelic profile: 1; 4; 3; 1; 1; 1; 1.
- *L. pneumophila* **Sg 1, ST188** - rare in Europe, both in clinical and environmental isolates. It has been associated with antimicrobial resistance previously in France and Belgium [2]. This ST has the allelic profile: 2; 6; 17; 6; 13; 3; 11; 188.
- *L. pneumophila* **Sg 1, ST23** – isolated from clinical and environmental samples. This ST is endemic in France and has the allelic profile: 2; 3; 9; 10; 2; 1; 6.
- *L. anisa* – accounts for rare clinical cases. *L. anisa* colonizes water distribution systems and is frequently found with L. pneumophila; therefore, it could be a good indicator for increased risk of nosocomial infection [3].
- *L. bozemanii* – a distinguishing characteristic of this species is the ability of colonies to exhibit blue-white autofluorescence when viewed under ultraviolet light. *L. bozemanii* is within the top five most common *Legionella* species isolated from clinical samples and water distribution systems.
- *L. longbeachae* – a species first isolated from the respiratory tract of patients with pneumonia in 1982 [4]. *L. longbeachae* infections are associated with exposure to soil, compost, and potting mixes. Human infections are common in Australia and New Zealand and increasing in the North of Europe [5].

Each participant received 15 short reads (Illumina) sequences (fastq format) (Table 2). Participating laboratories were asked to perform organism identification, sequence typing, cluster analysis, and sequence quality control. Participants' results were analyzed and considered 'concordant' if the reported categorization agreed with the EQA organizer's interpretation.

Scheme 2: Outbreak scenario using diagnostic clinical methods

Samples were prepared and quality-controlled by the EQA organizer. The scheme contained four paired (sputum/urine) simulated specimens and two single specimens (sputum and urine) taken from six patients with suspected symptoms indicating Legionnaires' disease. The paired specimens were designed to mimic an outbreak.

The outbreak strain of *L. pneumophila* Sg 1, ST23, used for this exercise, was isolated from clinical and environmental samples. This ST is endemic in France and has the allelic profile: 2; 3; 9; 10; 2; 1; 6. Other strains included in this scheme were:

- *L. pneumophila* **Sg3, ST710** – a rarely reported ST in France observed in environmental isolates. The strain was present in low quantity, explaining the lack of growth for most participants. This ST has the allelic profile: 12; 29; 2; 5; 50; 20; 15.
- *L. bozemanii*.
- *L. longbeachae*.

Each participating laboratory received 5 urines samples and 5 simulated sputum (Table 3). Real urine samples were provided and participants were asked to perform urinary antigen testing. Simulated sputa were prepared in a liquid and viscous format to mimic a real sputum and were spiked with various concentrations of the pathogen *Legionella* spp. and other species. To simulate authentic clinical material, the vials also contained a strain of commensal flora commonly isolated from lower respiratory tract infections. Participating laboratories were asked to perform DNA detection, organism identification, and sequence typing (simulated sputum) and cluster analysis using the tool of their choice. Participants' results were analyzed and considered 'concordant' if the reported categorization agreed with the EQA organizer's interpretation.

The yield of the pathogen ranged between $10-10^2$ colony-forming units (cfu) per mL. The yield of the commensal flora ranged between 10^2-10^3 cfu/mL, but participants were not asked to report on the background flora included.

The instructions provided to participants addressed the following:

- how to inoculate the appropriate media with the appropriate incubation conditions to isolate any potential pathogens; and
- how to report results.

The simulated sputum specimens were examined using the national documents for the identification of *Legionella* spp. and for the investigation of bronchoalveolar lavage, sputum, and associated specimens, in accordance with the requirements for clinical laboratories accredited to ISO 15189:2012 and ISO 15189:2022 (Medical laboratories – Requirements for quality and competence).

Scheme 3: Outbreak scenario using water samples for Legionella testing

Samples were prepared and quality-controlled by the EQA organizers. Five simulated water samples were supplied to represent an outbreak investigation. These included samples provided from:

- cold water from the aero-refrigeration tower;
- hot water from the departure points of the distribution network – Camping B.
- hot water from the bathroom shower – Patient 4's home.
- water from a sink in the staff break room, which Patient 2 frequently used.
- Cold water from the shower located next to the swimming pool in a residential apartment complex.

The outbreak strain of L. pneumophila Sg 1, ST23, used for this exercise, was isolated from clinical and environmental samples. This ST is endemic in France and has the allelic profile: 2; 3; 9; 10; 2; 1; 6. Other strains included in this scheme were:

- *L. pneumophila* **Sg1, ST143** - a rarely reported ST in France observed in environmental isolates. This ST has the allelic profile: 4; 17; 11; 23; 5; 12; 19.
- *L. pneumophila* **Sg11** – observed in environmental isolates, ST was not evaluated because it was new.
- *L. anisa*.
- *L. longbeachae.*

Participating laboratories were asked to perform PCR, organism identification, serogrouping, and sequence typing. Simulated water samples were prepared as lenticuled pellets, which were shown to preserve organisms over a long period of time (Table 4). The samples that were positive for *Legionella* spp. contained bacteria at varying levels between 10–10^4 cfu/L. Background organisms relevant to the sample type were included to simulate a real sample, but also to challenge the laboratories' processing techniques, such as acid/heat treatment, and to confirm the performance of the selective agar used. Participants were not asked to report on the background flora included. Participants' results were analysed and considered 'concordant' if the reported categorisation agreed with the EQA organizer's interpretation.

The instructions provided to participants addressed the following:

- How to reconstitute the lenticuled pellets; and
- how to report results.

Samples were tested in the HCL laboratory according to the international method ISO 11731:2017 (Water quality – Enumeration of Legionella) for water, sludge, and swab samples. This is in accordance with water laboratories being accredited to ISO/IEC 17025:2010 (General requirements for the competence of testing and calibration laboratories).

Scheme 2 and 3: DNA control

An additional DNA control was supplied to evaluate PCR methods and the intra-laboratory variation. Participating laboratories were asked to perform PCR for Legionella spp., *L. pneumophila,* and *L. pneumophila* Sg1. Participants' results were analyzed and considered 'concordant' if the reported intra-laboratory variation was below the threshold of 0.23 Ct as published here [6].

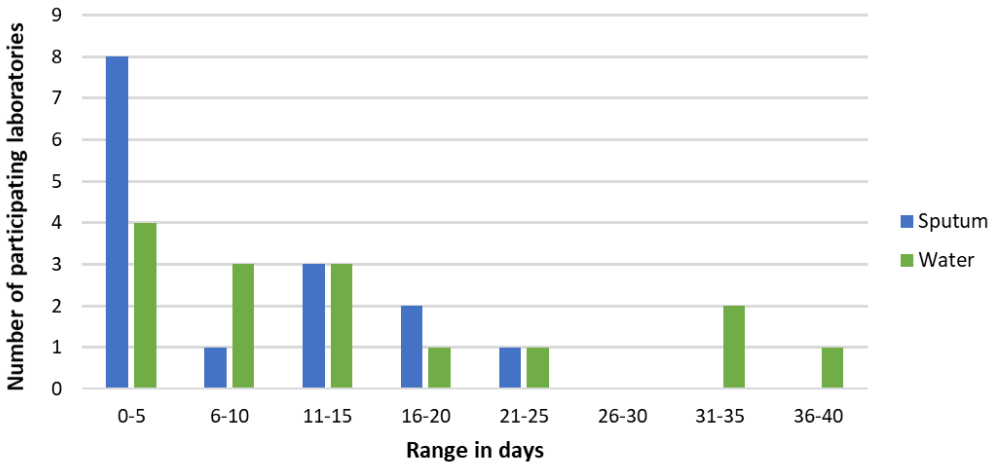
The instructions provided to participants addressed the following:

- How to reconstitute the DNA control;
- How to process the DNA;
- how to report results.

3 Results

Key results of this EQA exercise are given below. The delay between reception of clinical and environmental samples and processing is shown below in Figure 1. On average, participating laboratories processed samples 8 days after reception of the clinical scheme and 14 days after reception of the environmental scheme.

Figure 1. Number of days between receipt of the distribution and processing the samples.



Intended results

The content of the sequences for the bioinformatic scheme is described in Table 2. The contents of the specimens and samples for the clinical and environmental distribution are described in Tables 3 and 4, including the serogroup and sequence type when applicable. DNA control contained *L. pneumophila* serogroup 1 DNA (3 x 10^4 genomic units per tube).

Table 2. Sequence attribution for bioinformatic scheme (2 June 2025)

Strain number	Specimen contents	Sequence type	Quality control	Cluster	Details
1	*L. pneumophila*	259	Pass	No	Water from cooling tower return
2	*L. pneumophila*	93	Pass	Yes	Patient G with high temperature, headache and cough starting July 14 2024
3	*L. Longbeachae*	-	Pass	No	Potting soil from patient E's garden
4	-	-	Fail (contamination)	-	Patient D with pneumonia symptoms starting on April 25, 2024
5	*L. pneumophila*	1	Pass	No	Patient F, cough and muscle pains starting on June 3, 2024
6	*L. pneumophila*	93	Pass	Yes	Patient C with diarrhoea and confusion. Diagnosed with Legionella with signs starting on March 11, 2024
7	*L. pneumophila*	188	Pass	No	Patient H with Legionella infection started on August 27, 2024
8	-	-	Fail (Low number of reads)	-	Water from spa shower
9	*L. pneumophila*	93	Pass	Yes	Patient A with fever and cough starting on February 13, 2024
10	*L. anisa*	-	Pass	No	Water from shower at patient E's home
11	*L. bozemanii*	-	Pass	-	Patient B with fever and nodules in the lungs. Symptoms started on February 28, 2024

12	*L. pneumophila*	93	Pass	No (9 AD / 15 SNP)	Water from a spa pool balance tank
13	*L. pneumophila*	93	Pass	Yes	Patient E with diagnosis on May 20, 2024
14	*L. pneumophila*	23	Pass	No	Patient I with fever, cough, diarrhoea starting on September 23, 2024
15	*L. pneumophila*	93	Pass	Yes	Water from public water fountain in Hotel X

Table 3. Clinical specimens for clinical scheme (22-23 September 2025)

Patient	Specimen number	Type	Specimen contents	Sero-group	Sequence type	Cluster	Details
1	URI-5	Urine	No Legionella	-	-	-	Patient with a history of chronic alcoholism and COPD secondary to tobacco use, presenting with dyspnoea.
	SPU-1	Sputum	*Streptococcus pneumoniae*	-	-	No	
2	URI-3	Urine	*L. pneumophila*	-	-	-	Female patient with a history of hypertension presents with fever, dyspnoea, and altered mental status characterized by incoherent speech.
	SPU-2	Sputum	*L. pneumophila* *L. bozemanii* *Moraxella catarrhalis*	1	23	Yes	
3	URI-2	Urine	No Legionella	-	-	-	Patient with a history of diabetes and obesity presenting with acute respiratory failure. Onset one week ago with fever and sinusitis.
	SPU-3	Sputum	*L. pneumophila* [a] *M. catarrhalis*	3	710	No	
4	URI-12	Urine	*L. pneumophila*	-	-	-	Patient with a history of Lyme disease and hyperthyroidism presents with flu-like symptoms, fever, and somnolence.
	SPU-4	Sputum	*L. pneumophila* *M. catarrhalis*	1	23	Yes	
5	SPU-5	Sputum	*L. longbeachae* [a]	-	-	No	Female patient presenting with confusion, hypovolemia, and inflammatory syndrome. Abdomen is tender on examination.
6	URI-14	Urine	*L. pneumophila*	-	-	-	Patient presents with thoracic pain, fever, and cough. Obese.

[a] *These samples contained non-cultivable* Legionella *spp. but detectable by PCR. The reporting of bacterial growth in the sample was considered correct and laboratories that did so were not negatively scored.*

Table 4. Water samples for environmental scheme (22-23 September 2025)

Sample number	Specimen contents [a]	Serogroup	Sequence type	Source attribution	Details
WAT-1	*Staphylococcus haemolyticus*	-	-	No	Cold water from the aero-refrigeration tower (routine microbiological monitoring)
WAT-2	*L. pneumophila* (5 x 10^3) *Staphylococcus saprophyticus* *Pseudomonas aeruginosa*	1	23	Yes	Hot water from the departure point of the distribution network – Camping B.
WAT-3	*L. anisa* (5 x 10^3) *L. pneumophila* [b] *S. saprophyticus* *P. aeruginosa*	1	143	No	Hot water from the bathroom shower – Patient 4's home
WAT-4	*L. longbeachae* (1 x 10^4) *S. saprophyticus* *P. aeruginosa*	-	-	No	Water samples taken from a sink in the staff break room—which Patient 2 frequently used
WAT-5	*L. pneumophila* [c]	11	New	No	Water sampling from the cold shower located next to the swimming pool from a residential apartment complex

[a] *The levels of Legionella spp. in each sample is shown as approximate colony-forming units (cfu) per litre.*

[b] *This sample also contained low levels of* L. pneumophila *Sg 1 ST143. The reporting of this additional species in the sample was considered correct and laboratories that did so were not negatively scored.*

[c] *This sample contained non-cultivable* L. pneumophila *but was detectable by PCR. The reporting of bacterial growth in the sample was considered correct and laboratories that did so were not negatively scored.*

Scoring applied to the reported results

The individual laboratory reported results were scored for the main examinations, either with a score of zero if not correct, 1 if the result was partial, or 2 if correct. Scores draw attention to differences between a participating laboratory's results and the intended results (or 'assigned values'). Scores help laboratories identify whether there are any problems with their testing.

Bioinformatic scoring

For the sequences/strains, a score of 2 was given for the following:

- For reporting a correct identification of the *Legionella* spp. (when applicable).
- For reporting a correct *Legionella* sequence type (when applicable).
- For correctly reporting the attribution to the cluster (when applicable).
- For correctly reporting the quality of the sequence.

Clinical scoring

For the clinical specimens, a score of 2 was given for the following:

- For reporting a correct urinary antigen result.
- For reporting a correct detection of *Legionella* spp. by PCR.
- For reporting a correct isolation by culture.
- For reporting a correct identification of the *Legionella* spp. (when applicable).
- For reporting a correct *Legionella* serogroup (when applicable).
- For reporting a correct *Legionella* sequence type (when applicable).
- For reporting an intra-laboratory variation under 0.23 Ct (DNA control).

Environmental scoring

For the water samples, a score of 2 was given for the following:

- For reporting a correct detection of *Legionella* spp. by PCR.
- For reporting a correct isolation by culture.
- For reporting a correct identification of the *Legionella* spp. (when applicable).
- For reporting a correct *Legionella* serogroup (when applicable).
- For reporting a correct *Legionella* sequence type (when applicable).
- For reporting an intra-laboratory variation under 0.23 Ct (DNA control).

Results of the bioinformatic scheme

Up to 16 participating laboratories reported results for the sequences. An overview of these results is given in Table 5. Overall performance is given by specimen number and by examination type. Overall performance by specimen was calculated using the mean value across the examinations.

Table 5. Examinations performed and concordance achieved for sequences/strains.

Strain	Anticipated results	Species identification		Sequence type		Quality control		Cluster		Overall performance by specimen
		Nc/Nt	%[a]	Nc/Nt	% [a]	Nc/Nt	% [a]	Nc/Nt	% [a]	%
1	*L. pneumophila* Sg1: ST259	15/16	93.8	10/15	**66.7**	11/16	**68.8**	-	-	**76.4**
2	*L. pneumophila* Sg3: ST93	16/16	100	15/15	100	16/16	100	16/16	100	100
3	*L. Longbeachae*	16/16	100	-	-	14/15	93.3	-	-	96.6
4	QC fail	-	-	-	-	14/16	87.5	-	-	87.5
5	*L. pneumophila* Sg1: ST1	16/16	100	12/15	80	16/16	100	-	-	93.3
6	*L. pneumophila* Sg3: ST93	16/16	100	14/15	93.3	15/16	93.8	15/16	93.8	95.2
7	*L. pneumophila* Sg1: ST188	16/16	100	14/15	93.3	15/16	93.8	-	-	95.5
8	QC fail	-	-	-	-	12/16	**75.0**	-	-	**75.0**
9	*L. pneumophila* Sg3: ST93	16/16	100	15/15	100	16/16	100	16/16	100	100
10	*L. anisa*	14/16	87.5	-	-	11/15	**73.5**	-	-	80.5
11	*L. bozemanii*	6/16	**37.5**	-	-	8/15	**50.0**	-	-	**43.8**
12	*L. pneumophila* Sg3: ST93	16/16	100	15/15	100	16/16	100	12/16	**75.0**	93.4
13	*L. pneumophila* Sg3: ST93	16/16	100	15/15	100	15/16	93.8	16/16	100	98.5
14	*L. pneumophila* Sg1: ST23	16/16	100	9/15	**59.4**	16/16	100	-	-	86.5
15	*L. pneumophila* Sg3: ST93	16/16	100	15/15	100	15/16	93.8	16/16	100	98.5
Overall performance		-	93.8	-	89.3	-	88.2	-	94.8	-

Nc/Nt: number of laboratories achieving a score of 2 over all the laboratories participating in this examination.

%[a]: Percentage of results that were concordant or partial for each examination based on a pooled total of all the participating laboratories' reported results.

STRAIN 1 – the specimen contained *L. pneumophila* Sg1: ST259. There was a good concordance with intended results, with 93.8% (15/16) of participants reporting the correct species, but only 66.7% (10/15) reporting the correct sequence type. Five participants were not able to determine the ST (i.e., "Not found"). Overall quality control was correctly assessed by 68.8% (11/16) of participants. One laboratory did not perform species identification because of quality control failure.

STRAIN 2 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 100% (15/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by 100% (16/16) of participants. All the participants identified this strain as part of the cluster (16/16) with 0 allelic differences or SNP.

STRAIN 3 – the specimen contained *L. longbeachae*. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species. Overall quality control was correctly assessed by 93.3% (14/15) of participants. One participant did not assess the quality of the sequence.

STRAIN 4 – the specimen showed a high number of small contigs and a total length above what is expected from *L. pneumophila.* This was due to the presence of genetic material from two *Legionella* species. Overall quality control was correctly assessed by 87.5% (14/16) of participants.

STRAIN 5 – the specimen contained *L. pneumophila* Sg1: ST1. There was a very good concordance with intended results, with 100% (16/16) of participants reporting the correct species, but only 80.0% (12/15) reporting the correct sequence type. Three laboratories could not determine the ST (i.e., "Not found"). One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by all participants (16/16).

STRAIN 6 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 93.3% (14/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"), and one was not able to determine the ST (i.e., "Not found"). Overall, quality control was correctly assessed by 93.8% (15/16) of participants. In the outbreak scenario, 93.8% of the participants identified this strain as part of the cluster (15/16) with 0 allelic difference or SNP.

STRAIN 7 – the specimen contained *L. pneumophila* Sg1: ST188. There was a very good concordance with intended results, with 100% (16/16) of participants reporting the correct species and 93.3% (14/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by 93.8% (15/16) of participants.

STRAIN 8 – the specimen showed a low read count (<200k). Overall quality control was correctly assessed by 75% (12/16) of participants.

STRAIN 9 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 100% (15/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by all participants (16/16). All the participants identified this strain as part of the cluster (16/16) with 0 allelic differences or SNP.

STRAIN 10 – the specimen contained *L. anisa*. There was a good concordance with intended results, with 87.5% (14/16) of participants reporting the correct species. Overall quality control was correctly assessed by 73.3% (11/15) of participants. One participant did not control the quality of the sequence (i.e., "Not examined").

STRAIN 11 – the specimen contained *L. bozemanii*. There was a poor concordance with intended results, with only 37.5% (6/16) of participants reporting the correct species. Overall quality control was correctly assessed by only 50% (8/15) of participants. One participant did not control the quality of the sequence (i.e., "Not examined").

STRAIN 12 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 100% (15/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall, quality control was correctly assessed by all participants (16/16). However, only 75% of participants (12/16) correctly identified that this strain did not belong to the outbreak (according to a 5 AD threshold).

STRAIN 13 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 100% (15/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by 93.8% of the participants (15/16). All participants (16/16) correctly identified that this strain belongs to the outbreak with 0 AD or 1 SNP.

STRAIN 14 – the specimen contained *L. pneumophila* Sg1: ST23. There was a good concordance with intended results, with 100% (16/16) of participants reporting the correct species. Nine participants reported the correct sequence type, and one participant gave a partial result. Two participants were not able to determine the ST (i.e., "Not found"), and three reported an erroneous ST. Finally, one participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by all participants (16/16).

STRAIN 15 – the specimen contained *L. pneumophila* Sg3: ST93. There was an excellent concordance with intended results, with 100% (16/16) of participants reporting the correct species and 100% (15/15) the correct sequence type. One participant did not perform ST determination (i.e., "Not examined"). Overall quality control was correctly assessed by 93.8% of the participants (15/16). All participants (16/16) correctly identified that this strain belongs to the outbreak with 0 allelic difference or 1 SNP.

Results of the clinical scheme

Up to 17 participants reported results for the DNA control. Up to 22 participants reported results for the urine specimens, and up to 23 participants reported results for the simulated sputum specimens. An overview of these results is given in Tables 6, 7, and 8. Overall performance is given by specimen number and by examination type. Overall performance by specimen was calculated using the mean value across the examinations.

DNA control

Table 6. Examinations performed and concordance achieved for DNA control.

Specimen contents	*Legionella* spp.		*L. pneumophila*		*L. pneumophila* sg 1		Overall performance
	Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	%
L. pneumophila	9/13	**69.0**	8/13	**62.0**	5/6	83.0	**71.0**

Nc/Nt: number of laboratories achieving a score of 2 over all the laboratories participating in this examination.

%[a]: Percentage of results that were concordant or partial for each examination based on a pooled total of all the participating laboratories' reported results.

DNA control – Concordance with intended results was satisfactory, with 69% of participants for *Legionella* spp., 62 % of participants for *Legionella pneumophila,* and 83% of participants for *L. pneumophila* serogroup 1 reporting an intra-laboratory variation under 0.23 Ct.

Urine specimens

Table 7. Examinations performed and concordance achieved for urine specimen.

Specimen number	Specimen contents	Urinary antigen	
		Nc/Nt	%
URI-5	No Legionella	21/22	95.0
URI-3	*L. pneumophila*	19/22	86.0
URI-2	No Legionella	22/22	100
URI-12	*L. pneumophila*	21/22	95.0
URI-14	*L. pneumophila*	20/22	91.0
Overall performance		-	94.0

Nc/Nt: number of laboratories achieving a score of 2 over all the laboratories participating in this examination.

%: Percentage of results that were concordant over the total number of participating laboratories reporting results.

URI-5 – The specimen was negative for *L. pneumophila* urinary antigen. Performance was excellent, with 21/22 (95.0%) of participating laboratories returning a result for this specimen reporting the correct result. One participant reported that the specimen was positive for urinary antigen.

URI-3 – The specimen was negative for *L. pneumophila* urinary antigen. Performance was good, with 19/22 (86.0%) of participating laboratories returning a result for this specimen reporting the correct result. Three participating laboratories reported that the specimen was negative for urinary antigen.

URI-2 – The specimen was negative for *L. pneumophila* urinary antigen. Performance was excellent, with 22/22 (100%) of participating laboratories returning a result for this specimen reporting the correct result.

URI-12 – The specimen was negative for *L. pneumophila* urinary antigen. Performance was excellent, with 21/22 (95.0%) of participating laboratories returning a result for this specimen reporting the correct result. One participant reported an inconclusive result for urinary antigen.

URI-14 – The specimen was negative for *L. pneumophila* urinary antigen. Performance was very good, with 20/22 (91.0%) of participating laboratories returning a result for this specimen reporting the correct result. Two participants reported that the specimen was negative for urinary antigen.

Simulated sputum specimens

Table 8. Examinations performed and concordance achieved for simulated sputum specimen.

Number	Specimen contents	PCR detection		Isolation		Identification		Serogroup		Sequence type		Overall performance by specimen
		Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	%
SPU-1	No *Legionella*	17/20	85.0	20/21	95.0	-	-	-	-	-	-	90.0
SPU-2	*L. pneumophila* Sg1 ST23 *L. bozemanii*	15/19	84.0	17/20	85.0	10/18	78.0	14/14	100	9/11	86.0	87.0
SPU-3	*L. pneumophila* Sg3 ST143	17/19	89.0	18/21	85.7	7/7	100	2/6	**33.3**	7/7	100	81.7
SPU-4	*L. pneumophila* Sg1 ST23	17/19	89.5	10/21	**47.6**	11/11	100	12/12	100	9/10	90.0	86.0
SPU-5	*L. longbeachae*	13/20	**65.0**	18/21	85.7	5/5	100	-	-	-	-	83.6
Overall performance		-	82.5	-	79.8	-	94.5	-	77.8	-	92	-

Nc/Nt: number of laboratories achieving a score of 2 over all the laboratories participating in this examination.

%[a]: Percentage of results that were concordant or partial for each examination based on a pooled total of all the participating laboratories' reported results.

SPU-1 - Microbiological results were not in favour of Legionellosis. There was an excellent concordance with the intended results, with 85% (17/20) of participants reporting that no *Legionella* spp. DNA was detected by PCR. One participant reported that the specimen was positive for *Legionella* spp., and two laboratories reported positivity for *L. pneumophila*. Among laboratories, 95% (20/21) reported no growth by culture.

SPU-2 - the specimen contained *L. bozemanii* and *L. pneumophila* Sg1: ST23. There was a very good concordance with the intended results, with 89.5% (17/19) of participants reporting detection of *Legionella* spp. (score 1 – two laboratories) or *Legionella pneumophila* (score 2 – 15 laboratories) DNA by PCR, and 85% (17/20) reporting growth by culture. Ten participants (55.6%) correctly identified the co-infection (scored 2), and 8 participants (44.4%) identified at least one species (score 1), with 5 laboratories reporting isolation of *L. bozemanii* and 3 reporting *L. pneumophila*. Among the 14 participants who determined the serogroup, they all identified it correctly. For sequence-based typing, 91% (10/11) identified the correct sequence type (including one partial ST23, score 1). One reported another sequence type (2; 3; 9; 10; 93; 1; 6).

SPU-3 - the specimen contained *L. pneumophila* Sg3: ST710. There was a very good concordance with 89.5% (17/19) of participants reporting detection of *L. pneumophila* DNA by PCR. Two participants reported that they did not detect *Legionella* spp. DNA. Among participating laboratories, 85.7% (18/21) reported no growth by culture. Growth was also considered an acceptable result, as it may reveal some stochastic variation in the preparation of the sample. Seven participants (100%) correctly identified the species, including 4 in the absence of a strain. Among the six participants who determined the serogroup, only two identified it correctly (including one in the absence of strain). Two laboratories reported serogroup 1, one laboratory reported serogroup 2, and one laboratory reported serogroup 6. For sequence-based typing, 100% (7/7) of participants identified the correct sequence type, including 4 in the absence of strain.

SPU-4 - the specimen contained *L. pneumophila* Sg1: ST23. There was a good concordance with the intended results, with 94.7% (18/19) of participants reporting detection of *Legionella* spp. (score 1 – one laboratory) or *L. pneumophila* (score 2 – 17 laboratories) DNA by PCR. Regarding the culture, 47.6% (10/21) reported growth by culture. As the quality control performed by the EQA organizer showed a decrease in positivity over time, participants reporting no growth were not evaluated, as it may have been a property of the control material. Eleven participants (100%) correctly identified the species (including one in the absence of a strain). Among the 12 participants who determined the serogroup, they all identified it correctly (including one in the absence of strain). For sequence-based typing, 90% (9/10) of participants identified the correct sequence type, including one in the absence of strain. One reported another sequence type (2; 3; 9; 10; 93; 1; 6).

SPU-5 - the specimen contained *L. longbeachae*. There was a good concordance with the intended results, with 65% (13/20) of participants reporting detection of *Legionella* spp. DNA by PCR. Seven participants reported not detecting *Legionella* spp. DNA. Regarding the culture, 85.7% (18/21) reported no growth by culture. Growth was also considered an acceptable result, as it may reveal some stochastic variation in the preparation of the sample. Five participants (100%) correctly identified the species (including two in the absence of a strain).

Results of the environmental scheme

Up to 20 participants reported results for the simulated water samples. Enumeration values were not evaluated for this scheme as the number of participants returning a result was insufficient for a quantitative analysis. An overview of the results is given in Table 9. Overall performance is given by specimen number and by examination type. Overall performance by specimen was calculated using the mean value across the examinations.

Table 9. Examinations performed and concordance achieved for simulated water specimen.

Specimen number	Specimen contents	PCR detection		Isolation		Identification		Serogroup		Sequence type		Overall performance by specimen
		Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	Nc/Nt	%[a]	%
WAT-1	No *Legionella*	6/7	85.7	20/20	100	-	-	-	-	-	-	92.5
WAT-2	*L. pneumophila* Sg1 ST23	7/7	100	19/20	95.0	20/20	100	20/20	100	8/9	88.9	96.8
WAT-3	*L. pneumophila* Sg3 ST143 *L. anisa*	5/7	**71.0**	9/20	**45.0**	8/10	90.0	2/2	100	-	-	76.5
WAT-4	*L. longbeachae*	6/7	85.7	16/20	80.0	11/16	78.0	-	-	-	-	81.2
WAT-5	*L. pneumophila* Sg11 ST new	7/7	100	13/20	**65.0**	9/9	100	2/6	**58.0**	-	-	80.8
Overall performance		-	88.5	-	77.0	-	92	-	86	-	88.9	-

Nc/Nt: number of participants achieving a score of 2 over all the participating laboratories in this examination.

%[a]: Percentage of results that were concordant or partial for each examination based on a pooled total of all the participants' reported results.

WAT-1 – the specimen did not contain *Legionella* spp. There was an excellent concordance with the intended results, with 86% (6/7) of participants reporting that no *Legionella* spp. DNA was detected by PCR, and 100% (20/20) reported no growth by culture. One participant reported that they detected *Legionella* spp. DNA.

WAT-2 - the specimen contained *L. pneumophila* Sg1: ST23. There was an excellent concordance with the intended results, with 100% (7/7) of participants reporting detection of *L. pneumophila* DNA by PCR, and 95% (19/20) reporting growth by culture. All participants (100%) correctly identified the species and the serogroup (including 1 in the absence of strain). For sequence-based typing, 89% (8/9) identified the correct sequence type. One reported another sequence type (2; 3; 9; 10; 1; 1; 6).

WAT-3 - the specimen contained *L. anisa* and low amounts of *L. pneumophila* Sg1: ST143. There was a good concordance with the intended results, with 71% (5/7) of participants reporting detection of *L. pneumophila* DNA by PCR, and 45% (9/11) reporting growth by culture. Two laboratories reported that they did not detect *Legionella* spp. DNA and 11 laboratories did not isolate *Legionella* spp. As the quality control performed by the EQA organizer showed a decrease in positivity over time, participants reporting no growth were not evaluated, as it may have been a property of the control material. Eight participants (80%) correctly identified one of the species (score 2, including 1 in the absence of a strain). Six participants reported *L. anisa*, two participants reported *L. pneumophila,* and two participants established that it was *Legionella* spp. (score 1). Among the two participants who identified *L. pneumophila*, they identified the serogroup correctly (including one in the absence of strain). None of the participants determined the sequence type.

WAT-4 - the specimen contained *L. longbeachae*. There was a good concordance with the intended results, with 86% (6/7) of participants reporting detection of *Legionella* spp. DNA by PCR. One participant reported that they detected *L. pneumophila* DNA. Regarding the culture, 80% (16/20) reported growth by culture. Eleven participants (73%) correctly identified the species (score 2), and 3 established that it was *Legionella* spp. (score 1). Two participants reported that they isolated *L. pneumophila*.

WAT-5 - the specimen contained low levels of non-cultivable *L. pneumophila* Sg11 (new ST). There was a good concordance with the intended results, with 100% (100/100) of participants reporting detection of *L. pneumophila*. DNA by PCR. Regarding the culture, 65% (13/20) reported no growth by culture. Growth was also considered an acceptable result, as it may reveal some stochastic variation in the preparation of the sample. Nine participants (100%) correctly identified the species (including two in the absence of a strain). Among the six participants who determined the serogroup, 2 (33%) identified it correctly (score 2), and 3 (50%) identified the strain as belonging to the serogroup 2 to 14 (score 1). One participant reported that they identified a serogroup 2.

4 Discussion

General

The bioinformatic scheme of the 2025 EQA exercise represented an outbreak associated with a hotel. The outbreak strain of *L. pneumophila* sg 3, ST93, was observed in clinical specimens and environmental strains. This strain is reported sporadically in France, both in clinical and environmental isolates. The clinical and environmental schemes of the 2025 EQA exercise represented an outbreak associated with a campground. The outbreak strain of *L. pneumophila* sg 1, ST23 was isolated from clinical and environmental samples/specimens. This strain is the most prevalent and most causative ST for outbreaks in Japan and Europe and is endemic in France.

Participating laboratories' overall performance for **whole genome sequences** was very good. There were no significant issues arising for species identification, sequence type, and cluster identification. It was, however, noted that participants performed poorly for the identification of non-*L. pneumophila* species and especially *L. bozemanii*. In addition, the determination of the sequence type where two copies of the *mompS* gene can be found was challenging for the participants. Both discrepancies can be explained by the use of non-optimal software for the determination of *Legionella* species and sequence types.

For clinical samples, performance was very good; only a few participants tested the intra-laboratory variation using PCR; therefore, conclusions are not robust on these examinations. The majority of participants for clinical specimens identified the pathogen, serogroup, and sequence type. There was a significantly lower concordance when an Sg 2-14 was included. However, this can be explained by the absence of isolated strains and the unavailability of molecular tools for detailed serogrouping in this context. In addition, the determination of the sequence types with two *mompS* copies was more challenging for the participants. Finally, performance was below average for the detection of *L. longbeachae* DNA by PCR.

Overall performance **for environmental samples** was good. In general, there were no significant issues arising for species identification, serogroup, or sequence type. It was, however, noted that the number of participants reporting a correct species for non-*L. pneumophila* species, as well as a correct serogroup of the *L. pneumophila* 2-14 was lower. This is explained by the fact that the ISO 11731:2017 requires that suspected colonies are identified as at least *L. pneumophila*. Sequence type was investigated for less than half the participants. The determination of the sequence type with a two *mompS* copies was more challenging for the participants. When the concentration of *Legionella* spp. in a sample was low, some participants failed to isolate the organism, which could be because the concentration was below the detection limit for the method they used.

While validation studies had shown that preservation techniques ensured the stability of the organisms during transit to the EU/EEA countries, issues emerged with one simulated sputum (SPU-4) and one water sample (WAT-3). Lack of homogeneity and decrease in viability were observed during the distribution and quality control. Participants were not penalized by these issues. To maintain parameters of homogeneity, stability, and viability, improvements will be made to technologies used for preserving organisms/levels of organisms for the next EQA campaigns.

Bioinformatic discussion

The bioinformatic aspect of this EQA was a qualitative exercise designed to assess whole genome sequences. The scheme contained 15 Illumina paired read sequences. The exercise was designed to mimic an outbreak. The panel of sequences was used to ascertain species determination, sequence type, control of sequence quality, and cluster identification.

Species identification

- Overall performance was excellent (93.8%) for the correct identification of *L. pneumophila* species.
- Performance was lower for the identification of non-*L. pneumophila* species, especially for *L. bozemanii*
- When reported, the bioinformatic tools were not always optimal for *Legionella* species determination.

Sequence type determination

- Overall performance was very good (89.3%) for the correct sequence type determination.
- A lower concordance was obtained for the determination of sequence types with two *mompS* copies, such as ST1 and ST259.

Cluster identification

- All the participants correctly identified the strains belonging to the outbreak.
- Identification of sequences outside of the cluster but genetically close was challenging, suggesting a lack of a clear threshold to establish outbreaks.

Determination of Illumina sequences quality

- Overall performance was very good (88.2%) for the correct identification of sequences of poor quality.
- The determination of the sequence quality for non-*L. pneumophila* species was challenging.

Clinical discussion

The clinical aspect of this EQA was a qualitative exercise designed to assess simulated sputum and urine specimens. The scheme contained four paired (sputum/urine) simulated specimens and two single specimens (sputum and urine). The paired specimens were designed to mimic an outbreak. Simulated sputum and urine specimens were used because a recent EURL-PH-LEGI survey identified these as the most commonly described specimen types.

Urinary antigen testing to detect *L pneumophila*

- Overall concordance was excellent (94%) for the correct reporting of urinary antigen testing results.
- Concordance with intended results was even higher on the negative specimen.

Intra-laboratory variation by PCR

- Few laboratories participated in the examination of the DNA control but overall performance was good (71%) with most laboratory showing an intra-laboratory variation below 0.23 Ct.
- Fewer participants perform PCR specific for *L. pneumophila* serogroup 1, therefore the results might not be robust for interpretation.

Detection by PCR

- Overall performance was good on sputum specimens (82.5%) for the correct detection of *Legionella* spp. or *L. pneumophila*.
- Concordance was lower for the detection of non-*L. pneumophila* species by PCR.

Species identification

- Overall concordance was excellent (94.5%) for the correct identification of *L. pneumophila*. This is consistent with previous EQA exercise results.
- Concordance was lower for the identification of *L. pneumophila* when specimens contained two *Legionella* spp.

Serogroup determination

- Overall concordance was excellent for the correct determination of the serogroup for all Sg 1 strains.
- There was significantly reduced concordance (33.3%) for correct serogroup determination when the specimen included an Sg 2-14 *L. pneumophila*.

Sequence type determination

- Overall concordance was excellent (92%) for the correct determination of the sequence type, higher than in previous EQA.
- A lower concordance with intended results was obtained for ST with a double copy of the *mompS* gene.

The source of infection can be identified by comparing clinical and environmental *L. pneumophila* isolates using various typing methods. These include molecular techniques such as polymerase chain reaction (PCR), core genome multilocus sequence typing (cgMLST), single nucleotide polymorphism (SNP) assays. The attribution of the specimen to a cluster was not evaluated in this exercise.

Environmental discussion

The environmental aspect of this EQA was a qualitative exercise designed to assess five simulated water samples. The environmental samples were used to ascertain the presence or absence of *Legionella* spp. and identification from species to sequence type level. There was also an option to report enumeration, but it was not evaluated in the 2025 exercise.

Detection by PCR

- Few participants analysed the samples using molecular methods, consistent with previous EQAs. Molecular testing for *Legionella* in water and environmental samples is not widely adopted, with only a limited number of commercial laboratories offering it routinely. Additionally, detecting DNA from non-viable *Legionella* cells has limited public health relevance.
- The overall concordance for identifying *L. pneumophila* in the context of co-infection using molecular methods was lower (71%) that in mono-infections.

Isolation

- The overall concordance was good (77%) for correct isolation results.
- The concordance for WAT-3 and WAT-5 was lower than for other samples. The reason for this could be that the low concentration of *Legionella* spp. in the sample was below the lowest detection limit for the methods used by the laboratories.
- The most common isolation media used were GVPC and/or BCYE as recommended in the ISO11731:2017.

Species identification

- The overall concordance was very good (92%) for correct identification results.
- The concordance for the correct identification of *Legionella* spp. was lower (78.0%). The concentration of *Legionella* spp. is crucial for assessing control measures in water systems. While *L. pneumophila* is most common, other species can cause infections, especially in hospitals. However, the EQA organisers note that national guidelines may focus only on *L. pneumophila*.

Serogroup determination

- Overall concordance was good (86%) for the correct determination of the serogroup.
- There was significantly reduced concordance (58%) with intended results when the specimen included an Sg 11 *L. pneumophila*.

Sequence type determination

- Only one sample was considered for sequence typing, as the bacterial concentration in the remaining samples might have been too low to obtain an isolate. However, concordance was very good (88.9%) for sequence type determination.

Limitations of this EQA exercise

This EQA exercise assessed only the analytical and post-analytical stages of testing; the pre-analytical stage was not evaluated. Pre-analytical factors, such as patient demographics, sample type and volume, appropriate tests, and suitable containers, were pre-determined for this panel. Participation was limited to a maximum of two laboratories per EU/EEA country, restricting the cohort to those who received and returned the panel.

A period of six weeks was given for laboratories to return results. This period was allocated to allow sufficient time for the panel to arrive at the laboratories via air freight. The time allowed was meant to be greater than the expected turnaround time for investigation and return of results. We observed that participants waited an average of 7 (clinical scheme) to 14 days (environmental scheme) to process the samples (Figure 1). This observation was unexpected as the participants are instructed to process samples as they would routinely. This may have had a negative impact on the reported results if the control material was not stored properly before being processed.

The reporting system could not capture preliminary results, which some laboratories provide. The clinical specimens sent in liquid format aimed to represent the matrix of an authentic liquid purulent sputum that would normally be received by a diagnostic laboratory. The specimens distributed for the detection of urinary antigens were authentic, unspiked clinical liquid urines.

For the environmental samples, once a lenticule pellet was rehydrated, it would constitute one liter of water, which would not be representative of the chemical constituents normally found in real samples. While ringer tabs were provided for the rehydration solution, some participants reported using tap water. This was shown to be less efficient in recovering the bacteria in our validation studies. For environmental samples, the enumeration results could not be evaluated due to an insufficient number of data sets returned for quantitative analysis.

5 Conclusions

Laboratories from 26 EU/EEA countries performed well in this year's EQA exercise for the **identification of *Legionella* spp.** using bioinformatic methods (93.8% overall concordance with intended results), clinical methods (94.5% overall concordance), and environmental methods (92% overall concordance).

In this EQA exercise, laboratories examined specimen and sample types that they routinely test. For the clinical specimens, 21 participants examined the sputum and 22 participants examined the urine specimens. For the environmental samples, 20 participants examined water samples. On average, both the clinical and environmental laboratories reached 87% if the full scores for the results they submitted across all schemes and all samples. However, there are a few laboratories that fall short of this threshold. Given the implications of low scoring on clinical treatment and surveillance data completeness, these laboratories should review their methodologies and, eventually, seek EURL-PH-LEGI expertise to identify the root causes, so that improvements can be made. As of January 2026, the EURL-PH-LEGI did not receive request related to the 2025 EQA exercise.

To note that this year's exercise was the first one organized by the EURL-PH-LEGI and that participants are invited to continue taking part in EQA schemes organized by the EURL-PH-LEGI so they can determine their actual ongoing performance with the varying designs of EQA specimens and samples. Although the data provided some assurance of the laboratories' ability to undertake effective public health investigations for *L. pneumophila* and other *Legionella* spp., this should be confirmed by future EQA.

For the **bioinformatic laboratories**, up to 16 reported identification, sequence type, and cluster determination. Identification of non-*L. pneumophila* species remains challenging due to the use of non-optimal tools. However, all the laboratories were able to identify very closely related strains forming the outbreak. This scheme was the first one organized on bioinformatic methods and will be proposed again in the following years.

For the **clinical laboratories**, up to 18 reported an identification, up to 14 reported the serogroup, and up to 11 reported on sequence type. Identification of *Legionella* spp. and determination of the sequence type remains rare in the absence of isolated strains. Isolation in culture remains the gold standard for the characterization of infection caused by *Legionella* spp. MALDI-TOF MS is frequently used to identify isolates to the species level. Most clinical laboratories were able to identify a non-*L. pneumophila* to the species level. Differentiation and typing of strains can be achieved using a range of molecular techniques, including sequence-based typing, whole genome sequencing, and PCR methods. Up to 13 participants examined the DNA control for the *Legionella* spp. and *L. pneumophila* targets. Overall, intra-laboratory variations were acceptable (from 62% to 71%), but only 9 participants examined the *L. pneumophila* serogroup 1 target.

For the **environmental laboratories**, all 20 reported a result for isolation and identification, up to 20 reported a serogroup, and up to 9 reported a sequence type. The overall concordance for isolation using culture was 77%. The reason for this below average performance could be due to the low concentration of *Legionella* spp. in the sample, which may have been below the lowest detection limit for the methods used by these laboratories. To overcome this limitation, isolated strains will be provided in the future to be able to evaluate downstream analyses. Enumeration values were not evaluated in this year's EQA due to a low number of reporting laboratories. For molecular methods, only 7 participants analyzed the samples for *Legionella* spp. and *L. pneumophila* by PCR. Overall performance was good (88.5%), but the results should be interpreted cautiously as a low number of laboratories participated in this examination.

6 Recommendations

Regular participation in EQA schemes is strongly encouraged, as it provides ongoing assurance of result accuracy, highlights performance gaps, and enables targeted improvements in testing capabilities and capacity-building. Engagement in accredited EQA programs throughout the year supports continuous monitoring of laboratory practices across the EU/EEA, ensures reliability under changing environmental conditions, and facilitates timely identification and resolution of genuine performance issues. In addition, the following ongoing areas of actions for laboratories were identified:

- Promote methods that can detect the full range of *Legionella* species from both clinical and environmental samples, supported by EURL-PH-LEGI training, technical guidance, and the laboratory handbook.
- Encourage the use of molecular and sequence-based approaches to determine species and sequence type, even in the absence of clinical or environmental isolates, in line with EURL-PH-LEGI protocols.
- Reinforce the importance of standardised procedures for effective management of public-health events involving *Legionella*, aided by EURL-PH-LEGI technical support and resources.

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Annex 1. Methods information

As part of this EQA exercise, the methods/kit information used to analyse the specimens or samples were collected from participating laboratories. The participants were only asked to provide information on the methods they use in their laboratory, and the fields were not mandatory to fill out. This information was gathered online. The methods data shown are for information only and do not provide an evaluation or associate the data with a failure in the EQA exercise or method/process used. They also do not serve to compare the performance of the various molecular kits/processes. A more detailed reporting of methods will be implemented in the next EQA exercise.

Findings for the bioinformatic scheme

Participants were invited to report on the methods used for each step of the analysis of the sequences. While several participants reported more than one method for certain steps, others did not provide this information. The table below shows the number of participants using a given method over the total number of methods reported for a given step of analysis. A total of 16 participants from EU/EEA countries returned results within the specified period.

Table A1: Methods used for each step involved in the analyses of the sequence included in the bioinformatic scheme

Analysis Steps	Methods	% of participants
QC method	FastQC	33%
	In-house pipeline	20%
	Ridom Seqsphere	13%
	MultiQC	7%
	Kraken2	7%
	Minimap	7%
	QUAST	7%
	INNUca	7%
Bacterial identification	rMLST	29%
	Kraken2	24%
	KmerFinder	18%
	FastANI	12%
	INNUca	6%
	Mash Screen	6%
	MegaBLAST	6%
Sequence type	Legsta	37%
	el_gato	32%
	MOST	16%
	Ridom SeqSphere	11%
	MompsTool	5%
Clustering	Ridom SeqSphere	42%
	ChewBBACA	33%
	Single linkage clustering	8%
	Split Kmer Analysis	8%
	ReportTree	8%
Scheme size	1521	69%
	1443	8%
	1533	8%
	1537	8%
	50	8%

For quality control, 33% of participants reported using FastQC, and 20% used in-house pipelines. The main methods used for bacterial identification were rMLST (29%), Kraken2 (24%), KmerFinder (18%), and FastANI (12%). Here, it is important to note that the databases for Kraken2 and KmerFinder are not always adapted to *Legionella* spp. Even though Legsta was used by 37% of participants, this tool is not optimal for the determination of sequence types with a double copy of the *mompS* gene in comparison to el gato (32%) and MOST (16%). Clustering analyses were mostly based on the analysis of the core gene MLST (cgMLST) using commercial tools like Ridom SeqSphere (42%) or open-access tools like ChewBBACA (33%). Finally, the scheme based on 1521 genes was the most frequently used for cgMLST analyses, even though some laboratories reported other schemes. To compare results in a cross-border outbreak, it is crucial that countries use the same scheme.

Findings for the DNA control

Participants were invited to report on the methods used for the analysis of the DNA control. While some participants reported more than one method, others did not provide this information. The table below shows the number of participants using a given method over the total number of methods reported. A total of 17 participants from EU/EEA countries returned results within the specified period.

Provider	Kit	% of participants
In house	in house	24%
R-Biopharm	RIDA GENE Legionella	12%
Applied biosystems	SYBR	6%
Biorad	IQCheck Legionella spp and IQCheck Legionella pneumophila	6%
	SsoAdvanced SuperMix	6%
DNA Technology	Legionella pneumophila real-time PCR detection kit	6%
SIA Hydrox	Genesig Real-time PCR detection kit for L.pneumophila	6%
SIA Semetron	GeneProof Legionella pneumophila (LP) PCR Kit	6%
Seegene	Allplex PneumoBacter Assay	6%
DIAGNOTECH	DIAGNOVITAL Respiratory Panel Real Time PCR Kit	6%
Promega	GoTaq® qPCR Master Mix and GoTaq® Probe qPCR	6%
Biomerieux	Argene Legio pneumo/Cc	6%
Qiagen	Qiastat SARS Cov2 respiratory panel	6%

Participating laboratories reported a wide range of PCR kits used for the analysis of the DNA control. The majority of participants (24%) reported using in-house PCR for *Legionella* spp., *L. pneumophila,* and *L. pneumophila* serogroup 1 detection. The kit RIDA GENE *Legionella* from R-biopharm was the most frequently used commercial kit, as reported by the participating laboratories. To note that participants had the choice between using kits for clinical diagnostics or for environmental detection.

Findings for the clinical scheme

Participants were invited to report on the methods used for each step of the analyses of the clinical samples. While several participants reported more than one method for certain steps, others did not provide this information. The table below shows the number of participants using a given method over the total number of methods reported for a given step of analysis. One participant reported using a liquefier against the recommendation of the EQA provider. A total of 21 participants from EU/EEA countries returned results for simulated sputum within the specified period. Information on the kits used for the urinary antigen testing was not collected.

Analysis step	Reagent	% of participants
PCR	In house	53%
	Genesig Real-time PCR detection kit for L.pneumophila	12%
	RIDA GENE Legionella/r-biopharm	12%
	Legionella 4plex kit Generon	6%
	DNA Technology Legionella pneumophila RT-PCR detection kit	6%
	Allplex PneumoBacter Assay, Seegene	6%
	GeneProof, L.p.PCR ki	6%
	TibMolBiol LightMix Modular Legionella spp/pn.	6%
	Qiastat SARS Cov2 respiratory panel	6%
	Argene Legio pneumo/Cc	6%
Culture	BYCE	94%
	GVPC	47%
	BMPA	47%
	MWY	24%
Species identification	Maldi-Tof	35%
	PCR	12%
Sergrouping	Latex agglutination	53%
	Monoclonal antibodies	18%
	PCR	12%
Typing	WGS	35%
	SBT	29%
	Nested SBT	12%

For clinical samples for DNA control, laboratories used in the majority (53%) in-house PCR for the detection of *Legionella* spp. and *L. pneumophila*. Most frequently used commercial kits were those from Genesig and r-biopharm. Culture media used for bacterial isolation from clinical samples were BCYE (94%), followed by GVPC (47%) and BMPA (47%), and lastly by MWY (24%). To identify bacterial species, participants used Maldi-TOF (35%) and PCR (12%) in the absence of isolated strains. Up to 53% of laboratories used latex agglutination to determine the serogroup. Monoclonal antibodies were used by 18% of laboratories and PCR by 12% of laboratories for serogroup determination. Finally, sequence typing is based primarily on sequence-based typing, both by whole genome sequence (35%) and by PCR amplification of the 7 genes (29%). Interestingly, 12% of the laboratories reported using Nested PCR to determine the sequence type directly from the clinical samples in the absence of isolated strains.

Findings for the environmental scheme

Participants were invited to report on the methods used for each step of the analyses of the clinical samples. While several participants reported more than one method for certain steps, others did not provide this information. The table below shows the number of participants using a given method over the total number of methods reported for a given step of analysis. A total of 20 participants from EU/EEA countries returned results for simulated water samples within the specified period.

Analysis step	Reagent	% of participants
PCR	iQ-Check Screening L. spp and iQ-Check screening L. pneumophila	12%
	Legionella spp Diatheva kit	6%
	Microproof	6%
	GoTaq® qPCR Master Mix (Promega) and GoTaq® Probe qPCR (Promega)	6%
Culture	GVPC	94%
	BCYE	88%
	MWY	6%
Species identification	ISO11731	65%
	Maldi-Tof	53%
	WGS	6%
Sergrouping	Latex agglutination	76%
	Monoclonal antibodies	24%
	MAST antiserum	6%
	In house microagglutination	6%
	PCR	6%
Typing	WGS	35%
	SBT	18%

For molecular detection methods, participants used primarily commercial kits, and 12% of the participants reported using the iQ-Check from Bio-Rad. According to the ISO11731:2017, laboratories used GVPC (94%) and BCYE (88%) to isolate *Legionella* spp. from environmental samples. MWY media was only used by 6% of laboratories. Most participants (63%) reported that they used the methodology of the ISO11731:2017 to analyse the water samples. However, the reporting form did not include a specific field to report this information, so this percentage may be underestimated. Environmental laboratories used primarily Maldi-TOF (53%) to identify the bacterial species, whereas whole genome sequencing was used for bacterial identification in 6% of laboratories. Latex agglutination (76%) and monoclonal antibodies (24%) were the most frequent methods used to determine the serogroup. Finally, similar to what was observed for the clinical samples, sequence typing is based primarily on sequence-based typing, both by whole genome sequence (35%) and by PCR amplification of the 7 genes (18%).