EURL-PH-LEGI

EU Reference Laboratory for public health in the field of Legionella

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Deliverable name: Detailed EQA laboratory and reporting protocol plans 1

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Document overview

Deliverable number	D6.2
Deliverable name	Detailed EQA laboratory and reporting protocol plan 1
Work package number / name	WP6: Improving and assessing quality
Task number / name	T6.1: Provision of external quality assessment schemes for the detection, isolation, and characterisation of *Legionella* spp. from clinical and environmental specimens
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Lead Beneficiary	Hospices Civils de Lyon
Project website	www.eurl-legi.eu

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LIST OF ACRONYMS

CFU: colony-forming units

cgMLST: Core Genome Multi-Locus Sequence Typing

EU: European Union

EEA: European Economic Area

ECDC: European Centre for Disease Prevention and Control

ELDSNet: European Legionnaires' Disease Surveillance Network

EQA: External Quality Assessment

EURL: European Union Reference Laboratory

EURL-PH-LEGI: European Union Reference Laboratory for Public Health - Legionella

HCL: Hospices Civils de Lyon

IQLS: International Quality Laboratory Services

ISO: International Organization for Standardization

LD: Legionnaires' Disease

NFP: National Focal Points

NRL-L: National Reference Laboratory for Legionella

spp: species

SBT: Sequence-Based Typing

SNP: Single nucleotide polymorphism

SOP: Standard Operating Procedure

ST: Sequence Type

PT: Proficiency testing

QC: Quality Control

PCR: Polymerase Chain Reaction

qPCR: Quantitative PCR

UFC/L: Colony-Forming Units per Liter

UG/L: Micrograms per Liter

WGS: Whole Genome Sequencing

Introduction

Legionnaires' Disease (LD) is one of the five infectious diseases, along with invasive influenza, tuberculosis, HIV/AIDS and pneumococcal disease, having the highest individual and population burden in EU/EEA countries (Cassini et al. 2018, Eurosurveillance). Since 2011, an increasing trend has been observed with the current annual notification rates reaching the observed value of 2.4 cases per 100,000 population in 2021 in the EU/EEA. Legionnaires' disease surveillance has been carried out at the European level since 1987, firstly through a dedicated surveillance network funded by the European Commission and then, since April 2010, through the European Legionnaires' Disease Surveillance Network (ELDSNet). Legionnaires' disease is a statutorily notifiable disease in all EU/EEA countries, but is thought to be under-reported for the following reasons:

- it is underdiagnosed by clinicians, who may not test patients for Legionnaires' disease before empirically prescribing antibiotics likely to cover *Legionella* spp.;
- some healthcare professionals may fail to notify cases to health authorities.

However, under-ascertainment and differences in laboratory practice may also partly explain the variations in notification rates observed among EU/EEA countries. A laboratory's role during Legionnaires' disease outbreaks includes identifying and characterizing the pathogen from clinical specimens and/or environmental samples to support the epidemiological investigation, patient treatment/management, and source control.

The EURL in public health on *Legionella* (EURL-PH-LEGI) will implement Regulation (EU) 2022/2371 of the European Parliament and of the Council of 23 November 2022 on serious cross-border threats to health, in particular relating to *Legionella*. The main role of the EURL-PH-LEGI will be to promote good practices and provide support to EU/EEA Member States on diagnostics, testing and typing methods, to ensure reliability and comparability of data and capacity strengthening, aiming for a high-quality surveillance, notification, and reporting of cases. The EURL-PH-LEGI is constituted by four national reference laboratories for *Legionella* (NRL-L) from four different Member State countries: France, Italy, Germany, and Portugal. This Consortium has accumulated decades of experience in surveillance, outbreak investigation, and applied research on *Legionella* and LD. They are supported by teams of fully trained staff, microbiologists, and dedicated infrastructures to carry out their activities.

External quality assessment (EQA) programs are crucial for identifying testing errors and their causes at an early stage, which allows prompt corrective actions to ensure future testing accuracy. Furthermore, ISO 15189 accreditation requires participation in EQA programs. Since 2019, the ECDC has been offering a *Legionella* EQA scheme to assess the entire process involved in outbreak investigation. This scheme was evaluated as very useful for laboratories and will be continued on a similar scheme, with the addition of assessment of clinical diagnostic by PCR as well as the possibility for participants to be assessed on Whole Genome Sequencing (WGS) based typing alone by an additional bioinformatic EQA scheme in the program.

Objectives

The primary purpose of this EQA program is to determine the accuracy of *Legionella* testing and results reported by individual laboratories, as well as to allow comparison of results between laboratories and within countries across Europe. The results should provide ECDC with information on the laboratories' capabilities to accurately perform *Legionella* testing. This will also increase the confidence in the data submitted for surveillance, help to identify where further support is needed for individual laboratories or countries, and allow laboratories to understand their capabilities if testing demand were to increase due to an outbreak.

The secondary objectives of the 2025 EQA program are:

- 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 background organisms included; and
- to provide data to design wet lab training, webinars, and country visits to improve laboratory performance (after request by concerned countries).

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.

The EQA program will be organized by the National Reference Centre for Legionella in the Hospices Civils de Lyon (France), coordinator of the EURL-PH-LEGI in collaboration with ECDC.

Participants

Overall, 29 EU/EEA countries were contacted to nominate laboratories for their participation in the EQA program 2025. Nominations were made through ECDC National Focal Points (NFPs) for Legionnaires' disease and Operational Contact Point for Microbiology – Legionellosis within ELDSNet. Up to two nominated laboratories per EU/EEA country (to cover clinical specimens and/or environmental samples) can participate. One laboratory could also be nominated to participate in both clinical and environmental examinations if they usually processed both. Participating laboratories should be identified as contributing to national surveillance data or environmental findings shared through ELDSNet surveillance activities.

Participation is free for nominated participants. Nominated laboratories will be sent an email to confirm their consent to participate in the different schemes of the EQA by the 03/05/2025 and a list of participating laboratories will be established. Closer to the deployment of the EQA scheme an email will be sent to them with their registration details to a web interface hosted by our subcontractor IQLS for results submission together with the invitation letter.

If a laboratory withdraws before the shipment of the specimen, ECDC and NFPs will be asked to consider replacing this laboratory with another in the same country. If a laboratory cannot meet the reporting deadline, they will not be considered for any statistical analyses and they will not receive a certificate of participation.

Preparation

On July 3, 2025 a letter of invitation will be sent by email to the nominated laboratories reminding them of the EQAs and the objectives of the program. The letter will also provide an opportunity for the laboratories to confirm their interest in participating and that their details in the system are correct.

The invitation letter to the laboratories will include the following:

- The rationale and objectives of the EQAs;
- Participation criteria;
- Confidentiality agreements;
- Criteria for assigned values and performance evaluation;
- Reporting requirements and timelines (including the minimum requirements for obtaining an EQA certificate);
- Provisions for intellectual property, data ownership, and sharing;
- Descriptions of planned post-EQA outputs such as reports and publications; and
- Information about planned post-EQA participant feedback.

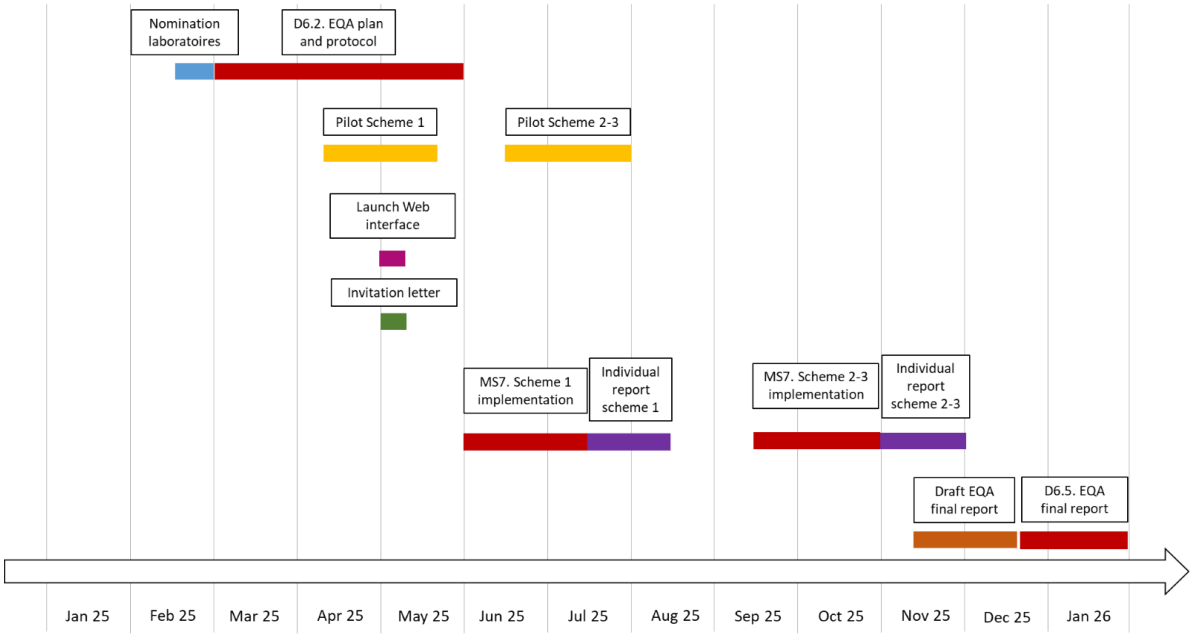
A unique laboratory identification will be created and usernames and passwords generated for each participating laboratory.

Methodology

Three schemes are proposed for the first EQA campaign 2025:

- Scheme 1: Outbreak investigation using bioinformatics methods
- Scheme 2: Outbreak scenario using diagnostic clinical methods
- Scheme 3: Outbreak scenario using water samples for Legionella testing

Figure 1: Timeline of the bioinformatic and clinical/environmental scheme. In red are mandatory deliverables and milestones.



1. Referee laboratory

Annex B of the norm ISO/IEC 17043:2023 suggests that the use of a referee or expert laboratory may be appropriate for determining the assigned value of a proficiency test item, particularly when other methods (e.g., consensus or certified materials) are not suitable. A referee laboratory can also enhance the credibility of an EQA program and help ensure the reliability and validity of assigned values. The referee laboratory is expected to receive the samples under the same conditions as participants and to analyze them as routine samples. Additionally, it should provide the EQA organizer with information on sample recovery as well as general feedback, support and expert comments. For the 2025 EQA schemes, our designated referee laboratory will be the National Reference Laboratory (EOLAB) in Switzerland.

2. Scheme 1: Outbreak investigation using bioinformatics methods

On June 2, the EURL-PH-LEGI will launch a bioinformatics proficiency testing (PT) scheme based on whole genome sequence data of 15 Illumina short read sequences (in fastq format). The following list of sequenced strains was designed by the EQA working group and approved by ECDC:

1. *L. longbeachae*
2. *L. anisa*
3. *L. bozemanii*
4. Lp1 ST23
5. Lp1 ST259
6. Lp1 ST93 cluster reference
7. Lp1 ST93 cluster
8. Lp1 ST93 cluster
9. Lp1 ST93 cluster
10. Lp1 ST93 cluster
11. Lp1 ST93 outside cluster
12. Lp1 ST188 (not antimicrobial resistant)
13. Lp1 ST1
14. Multiple *Legionella* (QC control fail by contamination)
15. Lp Incomplete ST (QC control fail, low read count)

Reads will be provided through a secured server, and results will be collected on the web interface. Participants will have to quality control, perform species identification, sequence-based typing, and cluster analysis by tool of choice

(e.g. cgMLST or SNP). Strain 6 to 10 are expected to form one genomic cluster. Participating laboratories will be asked to report the software/methods they used. They will have 6 weeks to return their results. At the end of the deadline for result reporting, an email will be sent with expected results to participating laboratories. An intermediate individual report on the results of the bioinformatic scheme will be issued for the participating laboratories in August. We expect laboratories to be challenged also by sequences of poor quality that should fail a QC control and from *Legionella* non *pneumophila*.

3. Scheme 2: Outbreak scenario using diagnostic clinical methods

An outbreak scenario will be provided to the participants together with a clinical description of the patients.

3.1. Urine

Patient urines were collected, tested for homogeneity and stability, and will be shipped to participants at 4°C. The distribution presented in Table 1 was agreed upon by EQA working group and approved by ECDC.

Table 1: Composition of the urine sample

Patient	Specimen type	Format	Content
1	Urine	Liquid	*L. pneumophila*
2	Urine	Liquid	*L. pneumophila*
3	Urine	Liquid	Negative
4	Urine	Liquid	*L. pneumophila*
5	Urine	Liquid	Negative

Participating laboratories will be asked to perform urinary antigen testing and to report the kit they used.

3.2. Simulated sputum

Commercially available artificial sputum (Research Center Borstel – Leibniz Lung Center) spiked with determined concentrations of *Legionella* spp. and/or flora were tested for stability and homogeneity. Artificial sputum mimics respiratory samples, especially their viscosity. The distribution presented in Table 2 has been agreed upon by the EQA working group and approved by ECDC.

Table 2: Composition of sputum sample

Patient	Content	Sg	ST	Quantity (CFU/L)	Comment
1	Negative + *Haemophilus influenza*				Discordant pair urine/sputum
2	*L. pneumophila* + *L. bozemanii* + *Streptococcus mitis + Moraxella catarrhalis*	1 -	23 -	6.0x10e3 2.0x10e2	Promote phylogeny Dual infection
3	*L. pneumophila* + *Streptococcus mitis + Moraxella catarrhalis*	3	710	3.0x10e2	Other serogroup
4	*L. pneumophila* *Streptococcus mitis + Moraxella catarrhalis*	1	23	1.0x10e3	Promote phylogeny
5	*L. longbeachae*	-	-	3.0x10e3	*L. spp*

Participants will be asked to perform detection, isolation, and characterisation of *Legionella* spp. as well as subtyping (including molecular typing) on simulated sputum samples. Participants should perform detection and characterisation according to their routine methods (culture and/or PCR) and return Not examined' or NE if they do not routinely perform a specific examination. Participants will be given the option to report quantification by culture (CFU) and by PCR (Ct). Finally, they will be invited to conduct strain comparison analyses between clinical isolates but also environmental samples (if they participate to Scheme 3). To this end, they should provide a conclusion either based on cgMLST (the size of the cgMLST scheme used for the analyses will be collected on the online platform) or based on phylogeny.

4. Scheme 3: Outbreak scenario using water samples for Legionella testing

An outbreak scenario will be provided to the participants together with a description of the water sampling conditions.

4.1. Simulated water samples

Lenticuled pellets are a well-established method to simulate water samples. Lenticuled pellets were therefore prepared, and stability and homogeneity of the samples were established. The distribution presented in Table 3 was agreed upon by the EQA working group and approved by ECDC.

Table 3: Composition of water sample

Specimen	Content	Sg	ST	Quantity (CFU/L)	Comment
Water 1	Negative + *Pseudomonas aeruginosa* + *Staphylococcus saprophyticus*				
Water 2	*L. pneumophila* + *Pseudomonas aeruginosa* + *Staphylococcus saprophyticus*	1	23	5.0x10e3	Low concentration, outbreak
Water 3	*L. pneumophila* + *L. anisa* + *Pseudomonas aeruginosa* + *Staphylococcus saprophyticus*	1	143	8.0x10e4 4.0x10e5	Dual infection, average concentration
Water 4	*L. thaurensis* + *Pseudomonas aeruginosa* + *Staphylococcus saprophyticus*	-	-	1.0x10e4	*L. spp*
Water 5	*L. pneumophila*	11	New	7.0x10e5	Other sg, high concentration

Participants laboratories will be asked to perform detection and enumeration by culture and PCR as well as subtyping (including molecular typing) on simulated water samples. In addition, they will have the opportunity to report quantification result based on qPCR (UG/L) and culture (CFU/L). Optionally, if they participate to both clinical and environmental, participants will be given the opportunity to report results of strain comparison analyses between clinical and environmental samples (if participating to Scheme 2). To this end, they should provide a conclusion either based on cgMLST (the size of the cgMLST scheme used for the analyses will be collected on the online platform) or based on phylogeny.

4.2. Standard DNA

A certified standard range from standard reference material of *Legionella* DNA developed by NRL-L Lyon (https://teamhcl.chu-lyon.fr/cnr-legionelles) to evaluate the performance of PCR methods for detection and diagnosis. This quantitative reference material will be sent to serve as a primary measurement of the performance of *Legionella* qPCR.

PCR performance will be evaluated through the evaluation of the standard curve efficiency (6 dilutions in series) and the intra-laboratory standard deviation. Storage, rehydration, and dilution protocol will be provided. The slope of the calibration function must be between -4.115 and -2.839.

Participating laboratories will be asked to report the PCR kit used and, if in-house PCR is performed, laboratories should report a brief protocol.

DNA Standard will be provided to clinical labs that do not participate to Scheme 3 as well.

5. EQA protocol

Instructions will be provided to participants and will include:

- How to store samples upon reception;
- Specific consideration when analysing artificial sputum samples
- How to resuspend the lenticuled pellets to simulate water samples.
- How to inoculate the appropriate media with the appropriate incubation conditions to isolate any potential pathogens;
- Information on reporting results (absence or presence of *Legionella pneumophila* or other species).

For more information see Annex A: "EQA Protocol – Guide for Participants".

Distribution

A subcontractor, IQLS (Lyon, France), will support HCL for EQAs distribution, especially parcel shipping.

1. Scheme 1

The Scheme 1 will be launched on June 2, 2025, on a server and by email. Therefore, it doesn't require logistic arrangements. An email will be sent to participants containing:

- A dispatch letter
- Specific instructions

2. Scheme 2-3

We envision that Scheme 2-3 will be launched on the week of September 22, 2025. To the extent possible, the shipment will be made on September 23 to ensure parcels will arrive before the weekend.

Each shipment will include the following essential documents:

- EQA Protocol – A comprehensive guide for participants
- Manual for the web interface
- Dispatch letter
- Specific instructions for each sample
- Safety data sheet (when applicable)
- Scoring information
- Commercial invoice (when necessary)

All samples will be shipped at Room temperature and 4°C, except for the DNA standard, which will be maintained at -20°C.

To guarantee secure and timely delivery, we have implemented the following logistical arrangements:

- **Courier Services**: We have chosen FedEx, a reliable courier service experienced in handling biological samples under the UN3373 standard. FedEx offers temperature-controlled shipping options, ensuring the required temperatures (especially 4°C and -20°C) are maintained throughout transit within EU countries.
- **Packaging**: We will utilise insulated triple packaging with gel packs, eutectic plates, and change phase materials to maintain the necessary temperatures. Before shipment, the HCL will conduct tests on the selected packaging to ensure durability and temperature stability during transit.
- **Tracking and Monitoring**: FedEx provides real-time tracking, allowing us to continuously monitor all shipments' location and condition.

Instructions for Completion (Minimum Requirements)

Participating laboratories are expected to process samples and sequences **as per own routine protocols**, i.e. choose the adequate bioinformatic or laboratory methods for detection/identification of *Legionella* species for the respective sample/sequence provided.

Participants will be requested to report positivity, organism identification, serogroup, and sequence type (simulated sputum and water samples) and *L. pneumophila* urinary antigen result (urine specimens). Clinical samples should be investigated by PCR and/or culture. Direct Fluorescent Antibody testing is outside of the scope of this EQA as this method is known for its low sensitivity.

Water samples should be tested according to the international method ISO 11731:2017 (Water quality – Enumeration of Legionella) for water, sludge, and swab samples. However, results from laboratories which do not use the ISO method will be analysed separately. The evaluation of the enumeration of colony-forming units for simulated water samples will be assessed after the exercise to ensure its relevance.

Participants need not to report on the background flora included. In addition, participants will be asked to provide further information on methods used for this EQA exercise, but this will not be evaluated. If the participant cannot examine the samples or if they do not routinely perform the examinations, they can return their results as 'Not examined'. Detection of the outbreak and identification of the outbreak source will not be scored.

The submission of participants' results will be realized through a web platform.

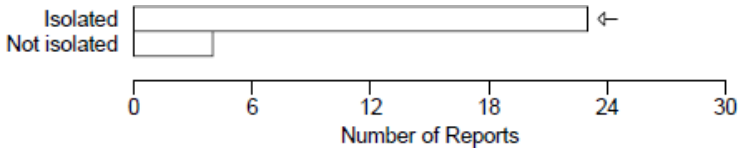
Laboratories will be given 6 weeks from the date of dispatch to perform the test and submit their results on the platform. After this date, the web platform will be closed for results submission and the expected results will be published on the EURL secured Sharepoint.

Data Analysis

1. Qualitative analysis

The performance will be first assessed by the **number and percentage of laboratory reporting the assigned value** calculated for each specimen and each test (example in Figure 2). Significant patterns will be identified by comparing the results obtained for different specimens or different methods.

Figure 2: Example of graphs used to show participant performance. Arrow indicates the participating laboratory reported result.



A scoring system reflecting clinical importance will be used (Table 4). A correct result (in agreement with the interpretive comment) is given a score of 2. A score of 1 to 0 is assigned for incorrectly identified results, where 0 represents a gross misclassification of the result. A negative result for a positive sample is given a score of 0. Equivocal comments during further investigation (species, sg, ST, cgMLST) for a positive sample are given a score of 1.

Table 4: Interpretation of the scoring system

Score	Interpretation
2	Good
1	Warning
0	Unacceptable

The score will be used for basic statistical analyses such as standard deviation and ranking. Performance rating is a form of ranking that compares other labs examining the same specimens. It will be expressed as the number of standard errors by which the cumulative score lies above or below the mean for all laboratories. A performance rating of more than 1.96 x standard error below the mean indicates possible poor performance.

2. Quantitative analysis

The difference between participant value and assigned value will be expressed as a percentage of the assigned value D_i%. Our criteria of performance will be set to 25%, if the participant D_i% is superior to this threshold, the result will be unacceptable.

In addition, the z score will be calculated according to the difference between the results of the participant and the assigned value and the standard deviation calculated on the results of all participants in the EQA program. The performance will be evaluated according to conventional criteria as follows:

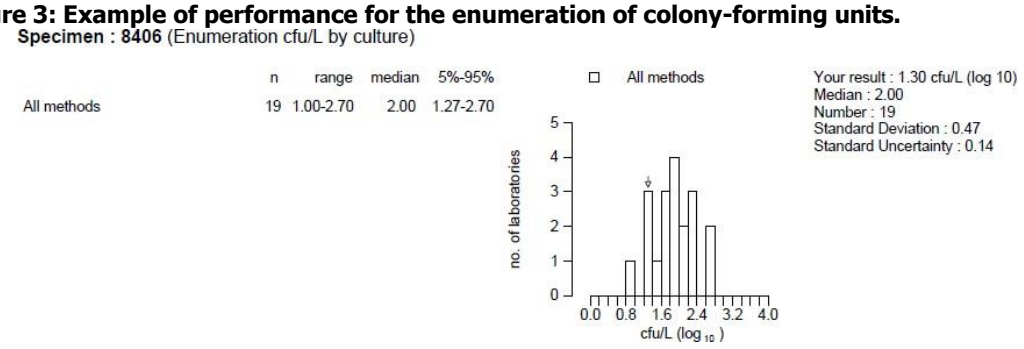
- $|z_i| \leq 2{,}0$: Acceptable results
- $2{,}0 \leq |z_i| \leq 3{,}0$: Warning
- $|z_i| \geq 3{,}0$: Unacceptable results

The following performance indicators will be considered for the EQA scheme:

- Bias as calculated from the linear regression analysis of the laboratory results against the assigned values.
- Imprecision as calculated based on the standard deviation

Performance graphs will still be provided with the number of responses, the range, the median and the expected range (5-95 percentile) for participant results (Figure 3). In addition, standard deviation and standard uncertainty will be calculated (1,25x SD/√n).

Figure 3: Example of performance for the enumeration of colony-forming units.



At least 20 reported results are required for a parameter to conduct a robust statistical evaluation. In the event of a lack of data to robustly calculate a laboratory's performance, the test will not be scored and participants informed.

Reporting of Results

1. Reports to Participants

The report **header** will contain the identifier of the laboratory, the distribution/scheme number, and the dispatch date. **The first section** will describe the intended results and report the results registered by the participant together with their score. Scores will be explained in a dedicated box. **A second section** will give a summary of the EQA scheme with notably the number of sets of specimens distributed and the number of participants that returned results. A summary of results obtained by the participants for each specimen will be provided and results of the participant will be compared to the results of all participants using percentages and graphs. Individual performance indicators will be provided as well as a comparison to assigned values. A **third section** will contain information on the different species and ST used in the PT schemes. A **last section** will contain references, acknowledgments, any subcontracted areas (shipping), and the contact of the organiser to serve for further inquiries. **The following warning will be on each individual report: "This scheme is not ISO/IEC 17043:2010 accredited; however, all principles and practices of the standard are followed in the delivery of this scheme**".

A certificate will be issued if the laboratory has returned all mandatory results of the respective EQA scheme they have participated in within the given timeframe and achieved the minimum criteria. Certificates of participation will be designed according to the ECDC template and will contain:

- Logos: ECDC, EURL, EU Funded
- Name of the EURL and of the relevant ECDC disease network (ELDSNet)
- Awarded to: name of the laboratory
- Attesting participation in: set of tests realized
- External Quality Assessment scheme: name of the scheme 1, 2 and/or 3
- Date of the schemes
- The certificate will not include any results assessment or scoring.

Reports and certificates will be delivered on the online platform. Participants will be alerted by email that their individual report and certificates are available on the platform using their username and password.

Follow-up support and resources will be automatically provided to laboratories flagged as underperforming. All participants can request a follow-up discussion on their overall performance with the EQA organizer. The EQA provider will promote participation in wet-lab training or application for *in situ* country visits when deemed relevant. Participants will not be given the option to dispute or clarify their results.

2. Publications

Scientific publications might be prepared based on the aggregated and anonymised EQA data if they do not breach confidentiality. This will be considered if the results convey recommendations of public health relevance. The EQA technical working group will decide on the pertinence of such a publication based on their expertise in the field. Before starting the publication process, the EQA working group will search for the agreement of relevant stakeholders and ECDC.

3. Reports to ECDC

A technical report based on anonymised and aggregated data summarising the results of the performance of the participating countries will be delivered. The report will include needs for training or capacity building and recommendations for such activities. EQA findings that point to limitations in the methods routinely used for laboratory-based surveillance or event confirmation (e.g. technical inconsistencies, harmonisation gaps) will be particularly highlighted as these technical problems may compromise the accuracy and comparability of surveillance data. The format will be following the template provided by ECDC and the general plan below:

- Introduction
- Study design and method
- Results
- Discussion
- Conclusions
- Recommendation
- References

A draft report will be shared with ECDC, one month after completion of the program for revision. The final report containing anonymised and aggregated data should be published within 3 months after completion of the annual EQA program. The deadline for the present EQA is the 31 of January 2026.

Participant Survey

The purpose of the survey will be to obtain feedback from the participants on the EQA program. It also aimed to enable the laboratories to suggest improvements for any future EQAs organised for the ECDC disease network on *Legionella*.

Key topics to be covered in the survey will be:

- Importance for diagnostic capability
- Satisfaction with the format of the individual report, outbreak scenario, and the online platform
- Corrective actions undertaken based on results
- The usefulness of the program for accreditation/licensing purposes
- Suggestions for future EQAs
- Need for additional training activities
- Additional feedback for the EQA organizer

The feedback evaluation survey will be sent to all participating laboratories on the web interface used for result submission and will be open from 15th November to 15th December. The analyses will be conducted by the EQA manager. The response rate will be calculated, and the analysis will follow a qualitative approach. Anonymised results of this survey shall be shared with ECDC.

Data Management, Ownership, and Sharing

All the SOP and documentation related to the organisation of the EQA will be attributed a unique identifier and will be uploaded onto the quality management system (Kalilab) in place in the HCL. Documents will be reviewed and updated regularly if needed. The different versions of the documents will be tracked in the quality management system.

The HCL as EQA organiser is the owner of all EQA data. EQA-related data will be stored on secured servers with limited access (only expert biologists at the national reference laboratory of the HCL).

Nominative data will be additionally protected by a password. Nominative and anonymised data will be stored for 10 years after the end of the contract of the HCL as an EQA organiser. After this date, nominative data will be destroyed from the HCL server. Nominative data will not be shared with any third parties, anonymised data might be shared with third parties for research purposes solely after the publication of the final report and on the decision of the EQA working group and the agreement of other stakeholders including ECDC. A data privacy statement will be attached to any communication requiring the collection of individual data.

Aggregated data will be publicly available and therefore will be stored indefinitely. They will be shared with ECDC for the publication of the EQA report. Upon request, aggregated data will be shared with third parties after publication of the final EQA report and solely on the decision of the EQA working group and the agreement of other stakeholders including ECDC and participants.

ANNEX A: EQA Protocol for ECDC External Quality Assessment (EQA) scheme supporting the surveillance of Legionnaires' disease at European level.

Protocol valid for distribution September 2025.

Introduction

The European Reference Laboratory in Public Health for *Legionella* (EURL-PH-LEGI) has been commissioned by the European Commission and the European Centre for Disease Prevention and Control (ECDC) to deliver a *Legionella* External Quality Assessment (EQA) scheme yearly.

The objective of this service is to deliver an EQA to support the laboratory surveillance of Legionnaires' disease, to ensure high quality laboratory diagnostic capability, sequence-based typing, and environmental investigation in the European Legionnaires' Disease Surveillance Network (ELDSNet) affiliated laboratories in EU/EEA and EU enlargement countries.

EQAs help to identify issues in laboratory processes (pre-, post-analytical and analytical), and highlights where quality improvements can be made to either diagnostic testing capabilities or examination of environmental samples or reporting.

The objectives of this EQA exercise are to:

- assess the ability of participating laboratories to correctly perform the tests for *Legionella* examination
- assist laboratories to identify specific technical gaps and errors for targeted corrections or corrective actions in their testing ability
- use results from EQA to support capacity-building

EURL-PH-LEGI coordinates the preparation and quality control of the sample panel, organise the logistics and subsequent analysis of the EQA result data. For logistics and shipment, the EURL-PH-LEGI is supported by IQLS (https://iqls.net/).

The documentation in Annex B section provides all the information you need to process this EQA distribution. A hard copy of these documents will also be included in the shipment box that will be sent to your laboratory with the samples.

The main examinations done will be scored. The allocation of scores is a means of drawing attention to differences between a participant's result and what has been designated as the intended result or the 'assigned value.' Scores may help participants to identify whether there is a problem with their testing. Information on scoring is found in the respective document within the annex B section.

You will receive, on the email address provided to the EQA organizer, your unique laboratory identification and password to access the secure on-line web-based reporting system from IQLS later this week. If you have not received this by next week, please contact us at HCL.eurlphlegi@chu-lyon.fr immediately.

Important information with deadlines

Sample dispatch date: 10 June 2025

This EQA exercise will consist of a panel of 15 simulated samples: 10 samples will be for clinical investigations and 5 samples for water examinations. A laboratory will receive either one or both sample sets based on their registration information.

Samples should be processed as per your routine procedures.

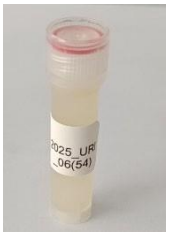
The laboratory will receive uniquely numbered samples and all associated paperwork in an approved box that conforms to the International Air Transport Association (IATA) shipping requirements (see below for more information).

Please make sure you check the safety information carefully before processing the samples as some will contain viable bacteria.

If you do not examine any sample/specimen type, then please destroy these through your normal route of discarding infectious material.

Samples will be received in the following format:

Clinical samples will be sent as sputum or urine in plastic tubes, both are transported at -20°C. Please cofirm upon receipt that the temperature is correct. Urine samples must be stored at -20ºC until ready for processing. Sputum must be processed immediately or stored at -20°C.



Liquid urine in plastic tube

Liquid sputum in plastic

Environmental water samples will be sent as lenticuled pellets in plastic tubes with silica beads. These samples must be stored at -20ºC until ready for processing.

Lenticuled pellets

The following document will be included in your shipment box (see annex b for more information):

- Sample Safety data sheets
- Reply form for the samples your laboratory receives for examination. This form will detail the sample types, and the examination required, each sample will have a unique number which should correspond to the details on the form
- Instruction sheet on how to process the samples
- Scoring information.

Results must be reported by **22 July 2025** through the secure IQLS website including information on the methods used to examine the EQA samples.

Within two weeks after the closing date for result submission, the intended results will be published on the ECON Legionella Sharepoint for you to access and compare to your own results. An email notification will be sent when this is available.

The reported results will be analysed by EURL-PH-LEGI and participating laboratories will be notified when country-specific reports are available on the ECON Legionella Sharepoint for download. Each participating laboratory will be able to see and download only their own report. Included with the report, participants will receive an analysis of their individual results. Reports and individual results analysis should be available 8 weeks after the closing date. Certificate of participation will be issued after each EQA distribution to all participating laboratories who submitted results.

Confidentiality

EURL-PH-LEGI will collect and analyse the results, and report to participating laboratories as described above. Moreover, a summary report will be shared with ECDC containing pseudonymised results. Confidentiality of results is strictly maintained and details of the performance of individual laboratories are never revealed to other individuals, or to any organisation without written permission from the head of the respective participating laboratory.

Authorship

Publication of reports using results from this EQA scheme distribution will be undertaken by ECDC. Authorship for such reports and any other scientific publication arising from the results collated under this EQA scheme will be considered according to the ECDC internal policy (available on ECDC website): ECDC/POL/07.

Contact details

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Annex B

Documentation required to process clinical samples

Documentation name	Brief description of contents	Document file
Legionella clinical report form	Report form containing sample type and clinical details	CLINICAL SAMPLES REPORT FORM
Legionella clinical specimen instructions	Instruction on how to process the samples	CLINICAL SAMPLES INSTRUCTIONS; DNA STANDARD INSTRUCTIONS
Safety data sheet for clinical samples	Information about safety	CLINICAL SAMPLES SAFETY DATA SHEET
Scoring information for clinical samples	Information about scoring	CLINICAL SAMPLES SCORING

Documentation required to process the water samples

Documentation name	Brief description of contents	Document file
Legionella Environmental Samples for outbreak analysis - Report Form	Request form for water samples providing information on sample type, examinations to be undertaken sample type and recording results	ENVIRONMENT REPORT FORM
Instruction sheet for processing water samples for *Legionella* spp.	Instruction on how to process the water samples	ENVIRONMENT INSTRUCTIONS
Safety data sheet lenticuled pellets	Information about safety	ENVIRONMENT SAFETY DATA SHEET
Scoring information on environmental samples	Information about scoring	ENVIRONMENT SCORING