EXTERNAL QUALITY ASSESSMENT REPORT

External quality assessment schemes to support European surveillance of Legionnaires' disease in EU/EEA countries, 2022–2023

ECDC TECHNICAL REPORT

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Abbreviations

BAL	Broncho-alveolar lavage
BCYE	Buffered charcoal yeast extract
CFU	Colony-forming unit
EQA	External quality assessment
ELDSNet	European Legionnaires' disease Surveillance Network
EU	European Union
EEA	European Economic Area
ELISA	Enzyme-linked immunosorbent assay
FEPTU	Food and Environmental Proficiency Testing Unit, UKHSA
GVPC	Glycine vancomycin polymyxin B cycloheximide
LUA	*Legionella pneumophila* urinary antigen
MALDI-TOF	Matrix-assisted laser desorption ionisation time-of-flight
MLST	Multilocus sequence typing
MVLA	Multiple-locus variable-number tandem-repeat analysis
NFP	National focal point
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PCR	Polymerase chain reaction
RVPBRU	Respiratory and Vaccine Preventable Bacteria Reference Unit, UKHSA
Sg	Serogroup
SNP	Single nucleotide polymorphism
ST	Sequence type
TALD	Travel-associated Legionnaires' disease
UAT	Urinary antigen test
UKHSA	United Kingdom Health Security Agency
UK NEQAS	United Kingdom National External Quality Assessment Service
WGS	Whole genome sequencing

Executive summary

This report describes the external quality assessment (EQA) scheme for the European Legionnaires' disease Surveillance network, for the detection, isolation, identification, and enumeration of *Legionella* species.

The EQA schemes provide an outbreak scenario with packages of clinical specimens and environmental samples for the participating laboratories to process, according to their technical capacity and protocols.

The purpose of the 2022–2023 exercise was to continue monitoring the accuracy of *Legionella* testing and results reported by individual laboratories and to enable comparison of results between laboratories and within countries across Europe. This report presents an analysis of the results for EU/EEA countries. It also includes results from a survey on the methods/kit information and frequency of testing performed for each method/kit, which was sent to all participating laboratories.

A total of 30 EU/EEA countries were invited to take part via their national focal points (NFP) for Legionnaires' disease. Up to two laboratories per country were selected, based on their involvement in surveillance and the management of public health incidents associated with *Legionella* in their country.

The specimens and samples for the EQA exercise were sent on 24 April 2023. Each package contained 10 specimens representing clinical material (distribution 5582) or 10 samples representing environmental material (distribution 5581). Strains of *Legionella* were provided by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) of the United Kingdom Health Security Agency (UKHSA) and these strains were fully characterised using conventional and molecular methods.

Laboratories only needed to examine the specimens or samples they would routinely test or process and report whether any contained a *Legionella* spp., and then provide identification, enumeration levels, serogroup (Sg) and sequence type (ST), where relevant.

This exercise represented an outbreak associated with a dental practice. The outbreak strain of *Legionella pneumophila* Sg 1, ST2681 was isolated from clinical specimens and environmental samples. This sequence type of *L. pneumophila* is rare and has only been identified in the UK five times since 2018. It has the following allelic profile: 6,10,2,10,13,4,9 and is a single-locus variant of ST501, ST969 and ST2110.

The outbreak strain was present in patients one, three and four. Patients three and five contained a second pathogen that was a non-*Legionella pneumophila*. Patient two contained a non-outbreak strain of *L. pneumophila* and patient six was negative for *Legionella* urinary antigen.

For the clinical distribution, 28 clinical laboratories were sent the package of specimens and all returned a result. Overall, concordance with the intended results was very good (96.2%) for the correct identification of *L. pneumophila* in clinical specimens. However, concordance was much lower (65.4%) for the second non-*L. pneumophila* included in the patient three specimen, as well as for the patient five specimen (85.2%). Concordance was good (85.4%) for reporting the serogroup, with most laboratories performing serogrouping. However, concordance reduced dramatically when a non-Sg 1 strain was included. Fewer laboratories reported sequence type results, with up to 16 laboratories entering a result in this field. Concordance was excellent (98.5%) for urinary antigen testing.

In total, 24 environmental laboratories were sent the package of samples and all returned a result. All 24 laboratories reported a result for isolation and identification, with up to 23 reporting the serogroup at least once. Up to 24 laboratories reported an enumeration count and up to 13 reported a sequence type. For molecular methods, up to 15 laboratories analysed the samples for *L. pneumophila* and up to 14 analysed for *Legionella* spp. The overall isolation performance for culture was very good (88.3% average across all 10 samples) and correct identification when a *Legionella* spp. was present was also excellent (98.4%).

When reported results were not concordant with the intended results, the EQA organisers advised laboratories to investigate to determine the root cause. One laboratory reported an incorrect isolation result six times, one laboratory four times, seven laboratories twice and four laboratories once.

Background organisms were included that were relevant to the specimen or sample type in order to simulate a real specimen/sample, but also to challenge the laboratories' processing techniques, such as acid/heat treatment, and to confirm the performance of the selective agar used.

The overall performance for culture-based detection methods was very good for both the clinical (96.2%) and environmental laboratories (88.3%). The overall performance for molecular methods was not calculated due to the low number of laboratories analysing the specimen or samples by this method.

According to the results of the laboratory methods/kit information survey, routine application of molecular methods for environmental samples is not fully implemented. The findings from this EQA exercise suggest that culture remains the preferred method.

The overall conclusion of this 2022–2023 EQA scheme is that both clinical and environmental laboratories demonstrated that they can undertake testing to an acceptable level of at least 80% concordance with the intended results. These data provide assurance, within the limits of EQA design, that EU/EEA laboratories have the ability to undertake effective public health investigations for *L. pneumophila*. Laboratories need to continue to take part in available EQA programmes to maintain up-to-date data on performance and confirm the robustness of testing.

The overall performance in this EQA was very good. There were no significant issues related to species identification, serogroup or sequence type. However, there were issues noted when the specimen or sample contained a non-Sg 1 strain. The methods survey also indicated that few participating laboratories have kits that cover *L. pneumophila* Sg 15 and this may be a limitation.

1 Introduction

Background

Legionnaires' disease is a severe and sometimes fatal form of infection caused by the Gram-negative bacteria, *Legionella* spp. These bacteria are found in freshwater and soil worldwide and can contaminate constructed water systems. There are at least 60 species of *Legionella* and over 20 have been associated with human disease. *Legionella pneumophila* is the most common species isolated both from the environment and from human infections. Based on surface antigens, this species can be divided into at least 16 serogroups, of which *L. pneumophila* serogroup (Sg) 1 is the most common cause of outbreaks. The strains of Sg 1 most commonly associated with disease share a common epitope, as shown by monoclonal subtyping. It is important to be able to routinely differentiate between *L. pneumophila* and other *Legionella* spp. and be able to distinguish Sg 1 from the other serogroups of *L. pneumophila*.

Humans are infected through the inhalation of contaminated aerosols containing *Legionella* bacteria. Legionnaires' disease is classically described as a severe pneumonia that may be accompanied by systemic symptoms and may lead to a fatal outcome. Cases of Legionnaires' disease are mainly reported in older people (>50 years), especially males. Other known risk factors for Legionnaires' disease are smoking, chronic obstructive pulmonary disease, diabetes, immune system compromise, and receipt of transplant or chemotherapy. In Europe, most cases (approximately 70%) are community-acquired and sporadic. About 20% of the cases are travel-related, and identification of the source of infection often requires international collaboration.

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.

Under-ascertainment and differences in laboratory practice may also partly explain the variations in notification rates observed among EU/EEA countries.

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). ECDC coordinates the collation of annual surveillance data on Legionnaires' disease in the EU/EEA through ELDSNet. The resulting surveillance data are available through the 'Surveillance Atlas of Infectious Diseases' on ECDC's website. A second ELDSNet surveillance system focuses on Travel-Associated Legionnaires' disease (TALD) cases.

The aim of TALD surveillance is to detect and communicate on clusters and outbreaks of TALD. The network supports Member States and other countries involved in sharing information and collaborating on response actions to provide better protection from TALD, both domestically and abroad.

TALD surveillance objectives [1] are:

- to rapidly detect cases and clusters of TALD reported in the EU/EEA and affecting European residents, both in their own countries or abroad;
- to disseminate information on TALD and respond in a coordinated fashion;
- to promote awareness of TALD to support primary preventive action and collaborative investigations;
- to assist in detecting and understanding the extent of common-source outbreaks of Legionnaires' disease worldwide by promptly notifying reported travel-related cases and clusters; and
- to reduce the incidence of TALD among EU residents by increasing awareness of active control and prevention measures at accommodation sites.

A laboratory's role during Legionnaires' disease outbreaks includes identifying and characterising the pathogen from clinical specimens and/or environmental samples to support epidemiological investigation, patient treatment/management and source control. Legionnaires' disease cases and environmental findings are reported to the above European surveillance programmes, with cases reported according to agreed case definitions [2].

External quality assessment benefits

The objective of an EQA is to ascertain and assess the level of competency of the participating laboratories in delivering a service to examine clinical specimens and environmental samples for presence and detection of *Legionella* spp.

An EQA offers the following benefits:

- provides laboratories with an insight into their performance;
- helps improve local standards;
- reveals unsuspected areas of difficulty;
- provides an educational stimulus for improvement;
- checks the efficacy of internal quality control procedures;
- demonstrates a commitment to quality to colleagues and customers;
- provides a method performance evaluation;
- provides independent evidence of performance for accreditation bodies; and
- enables participating laboratories to monitor, evaluate, and improve their own performance and training needs, since dealing with discrepant EQA results will improve testing performance which, in turn, would directly improve the management of public health incidents and clinical service.

A comprehensive quality assurance system will cover such areas as provision and control of standard operating procedures, education, and training; planned maintenance and calibration of equipment; and the monitoring of response times. Many laboratories are formally accredited to acknowledge compliance with defined objectives and quality standards, such as those detailed in ISO 17025:2017 or ISO 15189:2012.

Results of consistently excellent quality can be expected only when all the components of a quality system are in place.

External quality assessment exercise, 2022–2023

The purpose of this EQA exercise was 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. This report presents an analysis of the results.

The results provide ECDC with information on the laboratories' capabilities to accurately perform *Legionella* testing. They also bolster 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 own capabilities if testing demand were to increase due to an outbreak.

The overall objectives of the 2022–2023 EQA 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, levels and background organisms included; and
- to provide individual technical support to laboratories as a follow-up to the exercise, if requested by the countries.

2 Study design and methods

Organisation of the external quality assessment

This EQA exercise was organised by the Food and Environmental Proficiency Testing Unit (FEPTU) of UKHSA and the United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology, in collaboration with the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) of UKHSA and ECDC, as part of an ECDC Framework contract (ECDC/2019/024).

The EQA exercise was for laboratories nominated through ECDC National Focal Points (NFPs) for Legionnaires' disease within ELDSNet. Up to two nominated laboratories per EU/EEA country (to cover clinical specimens and/or environmental samples) could participate.

The selected laboratories were involved in the management of public health incidents in their country and/or were undertaking expert reference testing for specialised examinations. Each laboratory received a unique identifier, a username and a password. This allowed the laboratory to return results and view individual reports through a secure web portal. Anonymised results were also provided to ECDC.

Both FEPTU and UK NEQAS for Microbiology are accredited EQA providers under ISO/IEC 17043:2010 (Conformity assessment – General requirements for proficiency testing) and all these principles and practices were applied to the exercise:

- ECDC NFPs for Legionnaires' disease were asked to propose up to two laboratories per country to take part in the exercise; 30 EU/EEA countries were contacted. One laboratory that undertakes clinical examination of specimens and one that examines environmental samples was requested. One laboratory could also be nominated to participate in both clinical and environmental examination, if they usually processed both. Participating laboratories needed to be contributing to national surveillance data or environmental findings shared through ELDSNet surveillance activities.
- The EQA organiser sent a letter of invitation to the nominated laboratories informing them of the EQA and the objectives of the exercise. The letter also provided an opportunity for the laboratories to confirm their interest in participating and that their details in the system were correct.
- The packages of specimens/samples were sent to participating laboratories on 24 April 2023. The two distributions comprised a total of 20 simulated specimens and samples: 10 representing clinical specimens and 10 representing environmental samples. Specimen and sample design and format was agreed in advance with ECDC and UKHSA *Legionella* experts.
- UKHSA undertook testing of the specimens and 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 characterisation tests (serogrouping and sequence-based typing) were also undertaken.
- UKHSA also ran a separate survey on the methods/kit information and frequency of testing performed for each method/kit, which was sent to all participating laboratories.
- This EQA exercise simulated an outbreak associated with a dental practice. The outbreak strain was *L. pneumophila* Sg 1 and ST2681.

A total of 24 environmental laboratories and 28 clinical laboratories from 29 EU/EEA countries participated, all of which submitted results (Table 1).

In total, 23 of the 29 participating countries tested both clinical specimens and environmental samples.

Table 1. EU/EEA countries participating in the clinical/environmental EQA exercise, 2022–2023

Country	April 2023 distribution		
	Participated in the clinical EQA (distribution 5582)	Participated in the environmental EQA (distribution 5581)	Number of participating laboratories per country
Austria	Yes	Yes	2
Belgium	Yes	Yes	2
Bulgaria	Yes	Yes	2
Croatia	Yes	Yes	2
Cyprus	Yes	Yes	2
Czechia	Yes	Yes	2
Denmark	Yes	Yes	2
Estonia	Yes	Yes	2
Finland	Yes	Yes	2
France	Yes	Yes	2
Germany	Yes	Yes	2
Greece	Yes	Yes	2
Hungary	Yes	Yes	2
Iceland	Yes	No	1
Ireland	Yes	Yes	2
Italy	Yes	Yes	2
Latvia	Yes	Yes	2
Liechtenstein	Yes	Yes	2
Lithuania	Yes	No	1
Luxembourg	No	No	0
Malta	Yes	No	1
Netherlands	Yes	Yes	2
Norway	Yes	Yes	2
Poland	No	Yes	1
Portugal	Yes	Yes	2
Romania	Yes	Yes	2
Slovakia	Yes	No	1
Slovenia	Yes	Yes	2
Spain	Yes	No	1
Sweden	Yes	Yes	2

The packages of specimens/samples were dispatched in approved United Nations containers that included an EQA protocol. This protocol contained information on the specimen/sample details, instructions on how to process the safety data information, and instructions on how to enter the results online. The information was also available to all participants electronically and to NFPs for Legionnaires' disease in ELDSNet via the UK NEQAS web portal.

A dedicated page was available on the UK NEQAS website for laboratories to enter and submit their results. Laboratories could access instructions for using the secure web portal and download the protocol via the web page. Detailed instructions were included on how to access the secure website using the username and password provided. The deadline for final submission of results was stated on the protocol. For convenience, a copy of the online reply form was available for laboratories to download to facilitate the manual recording of test results prior to submission via the website. Laboratories were allowed eight weeks (56 days) from the date of dispatch to examine the specimens/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.

Eight weeks after the dispatch date, the web platform was closed for results submission and the intended results were published on the UK NEQAS website. Laboratories were notified by email that the intended results were available for viewing. Individual reports were made available in September 2023.

After the web platform was closed for results submission, the survey regarding method/kit information was sent electronically to all the laboratories that took part in the EQA exercise. A summary of the findings is presented in the Annex.

From 6–19 October 2023, ECDC conducted a short online survey to obtain feedback on the EQA exercise and to enable the laboratories to suggest improvement for future EQAs organised by ECDC.

Certificates of participation were sent electronically to the laboratories on 14 July 2023. A hard copy of the certificate was available by request.

Exercise scenario and specimen/sample design

The strains selected for the exercise were chosen in consultation with UKHSA *Legionella* experts in clinical and environmental microbiology. Specimens and samples were designed in collaboration with the UKHSA, UK NEQAS and an ECDC expert.

Strains of *Legionella* were provided by RVPBRU as fully characterised isolates. Commensal/background flora was taken from a bank of organisms held by the EQA organisers and these strains were fully characterised using conventional methods and an analytical profile index system.

All packages were dispatched at ambient temperature, 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 specimen or sample. This included the identification of the *Legionella* spp., serogroup, sequence type and enumeration results, where applicable. The report also provided an overall performance result (i.e. the percentage of results that were concordant) for each examination based on a pooled total of all the laboratories' reported results.

Distribution, 24 April 2023

Specimens and samples were prepared and quality-controlled by the EQA organisers and the packages were dispatched as distributions 5582 (clinical) and 5581 (environmental).

Clinical specimens were taken from six patients with suspected symptoms indicating Legionnaires' disease (sputum, Broncho-alveolar lavage (BAL) and urine specimens).

Five environmental samples were supplied to represent an outbreak associated with a dental practice. These included water samples provided from:

- a handpiece from an ultrasonic scaler;
- a handwash basin (hot outlet in dentist's treatment room);
- plastic tubing from a water reservoir;
- a handwash basin; and
- a dental cart.

The remaining five water samples were from routine monitoring. These were taken from:

- a shower located by a swimming pool;
- the balance tank of a spa pool;
- a shopping centre drinking water fountain;
- the mains supply of a shopping centre; and
- the hot and cold system at a spa.

The outbreak strain of *L. pneumophila* Sg 1, ST2681 used for this exercise was isolated from clinical and environmental samples. This strain is rare and has only been identified in the UK five times since 2018. This ST has the following allelic profile: 6,10,2,10,13,14,9 and is a single-locus variant of ST501, ST969 and ST2110.

Other strains included in these distributions were:

- ***L. pneumophila* Sg 1, ST2210** – this sequence type is rare and has only been identified in the UK four times since 2015. It has only been isolated from clinical samples from community-acquired and travel-associated cases. This ST has the following allelic profile: 6,10,2,10,13,4,9. It is a single-locus variant of ST501, ST969, ST1608 and ST2681.
- ***L. pneumophila* Sg 1, ST47** – this sequence type has been isolated from patients and domestic water systems. It has been isolated from clinical and environmental samples in several countries across Europe and Canada. Genomic differences have been identified for differing ST47 variants, including recombination events. It can be detected using the PCR described by Mentasti et al [3]. All *L. pneumophila* ST47 strains identified to date belong to Sg 1. This ST has the following allelic profile: 5,10,22,15,6,2,6.
- ***L. pneumophila* Sg 15 ST336** – this sequence type is rare and was first isolated from a clinical sample in the United States (US) in 1981. This unique strain has only been identified three times, according to the ST database. The second strain with the same ST, identified as *L. pneumophila* Sg 3, was isolated from an environmental sample in Kuwait in 2013. This ST has the following allelic profile: 11,14,16,25,7,13,24. It is a single-locus variant of ST681, ST1065, ST1118 and ST1334.
- ***L. pneumophila* Sg 5, ST728** – although Sg 3 is the most common serogroup associated with this sequence type, other serogroups (Sg 1, Sg 5 and Sg 6) have also been identified as ST728. This sequence type of *L. pneumophila* has been isolated from clinical and environmental samples since 2009. It has been associated with community-acquired, nosocomial and travel-related cases and has been isolated from several countries across Europe including Italy, Greece, Czechia, Denmark, and the UK. This ST has the following allelic profile: 2,10,3,28,9,4,3.

- ***L. pneumophila* Sg 6, ST68** – this sequence type is mainly identified as *L. pneumophila* non-Sg 1 strain (including Sg 6). It has been isolated from clinical and environmental samples in several countries across Europe, as well as India and Canada. This ST has the following allelic profile: 3,13,1,28,14,9,3.
- ***Legionella sainthelensi* Sg 2** – this is a non-auto-fluorescing species first isolated from spring water in the vicinity of Mount Saint Helens, Washington. A second serogroup of *L. sainthelensi* was identified by Benson et al. in 1990. Since their discovery, both serogroups of *L. sainthelensi* have been reported as aetiological agents of Legionnaires' disease, including outbreaks in nursing homes [4,5,6].
- ***Legionella bozemanii*** – this was first isolated from lung tissue in 1959. The isolate came from a healthy scuba diver in Florida who had fatal bronchopneumonia. This species consists of two serogroups, both of which have been reported to cause Legionnaires' disease. A distinguishing characteristic of this species is its blue-white autofluorescence when viewed under ultraviolet light. *L. bozemanii* is within the top five most common *Legionella* spp. isolated from water distribution systems [7,8,9,10].
- ***Legionella longbeachae*** – this was first isolated from the respiratory tracts of patients with pneumonia in 1982. A second serogroup of *L. longbeachae* was isolated from the post-mortem lung tissue of a patient with pneumonia. *L. longbeachae* infections are associated with exposure to soil, compost, and potting mixes. Human infection from *L. longbeachae* is particularly common in Australia and New Zealand, but cases have been documented in other countries, including the US, Japan, Greece and the UK [11,12,13].

Clinical specimens

For each distribution, 10 clinical specimens were prepared: three simulated sputum, two BALs and five liquid urine specimens. An overview of these is provided in Table 2.

Participating laboratories were asked to provide an organism identification, serogroup and sequence type (simulated sputum and BAL specimens) and *L. pneumophila* urinary antigen (LUA result) (urine specimens). Simulated sputum and BAL specimens were prepared in a lyophilised format. The freeze-dried specimen matrix was composed of inositol serum broth with variable concentrations of the pathogen *L. pneumophila* or other species. To simulate authentic clinical material, the freeze-dried vials contained a strain of the pathogen and commensal flora commonly isolated from lower respiratory tract infections. The serogroup and species of *Legionella* used were approved by the commissioned experts at the UKHSA. Participants' results were analysed and considered 'concordant' if the reported categorisation agreed with the UKHSA reference laboratory (RVPBRU) interpretation.

The yield of the pathogen after reconstitution of the lyophilised vials ranged between 10^2–10^4 colony-forming units (cfu) per mL. The yield of the commensal flora following lyophilisation ranged between 10^2–10^3 cfu/mL.

The instructions provided to participants addressed the following:

- how to reconstitute lyophilised specimens with 1 mL of nutrient broth (the pellet had to be fully dissolved in the liquid media to attain a homogenous suspension);
- how to inoculate the appropriate media with the appropriate incubation conditions to isolate any potential pathogens; and
- how to report results (absence or presence of *L. pneumophila* or other species).

The simulated sputum specimens were examined using the national documents SMI ID18 Identification of *Legionella* spp. and SMI B 57 Investigation of bronchoalveolar lavage, sputum and associated specimens. This is in accordance with the requirements for clinical laboratories accredited to ISO 15189:2012 and ISO 15189:2022 (Medical laboratories – Requirements for quality and competence).

Environmental samples

For each distribution, 10 environmental samples were prepared as LENTICULE® discs. An overview of these samples is provided in Table 3.

Preparing samples as LENTICULE® discs has been extensively validated and proven to preserve organisms over long periods of time. Samples were tested in the FEPTU 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).

The simulated sample designs included a selection of the following to make the 10 samples required: water taken from various sites such as cooling towers, hot and cold water systems, spa pools and swab samples.

The samples positive for *Legionella* spp. contained bacteria at varying levels from $<10^2$–10^5 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.

Samples were authorised for inclusion in the distribution if:

- they were homogeneous;
- they passed quality control testing prior to the distribution date; and
- their contents matched those obtained from RVPBRU for identification, serogroup and sequence type.

Samples were quality-controlled in the same ways they would have been by the participating laboratories. This step involved rehydrating and culturing a neat sample onto glycine-vancomycin-polymyxin-cycloheximide agar (GVPC), following heat and acid treatment. Agar plates were aerobically incubated for up to 10 days at 37°C and read on Days 3, 6 and 10. Any suspected *Legionella* spp. was ascertained by means of confirmatory testing.

Background flora selected for inclusion in the samples were those that would compete with the *Legionella* spp. in the sample. During processing for the isolation of *Legionella*, heat and acid treatment is employed to kill competing organisms (if done correctly).

Homogeneity and stability results were analysed using local robust statistics to ensure suitability for use and that defined criteria were met.

Results for environmental samples were analysed according to ISO 13528:2015 (Statistical methods for use in proficiency testing by inter-laboratory comparison). For enumeration values, the participants' median was used as the assigned value and the intended range was calculated using robust statistics (5th and 95th percentiles).

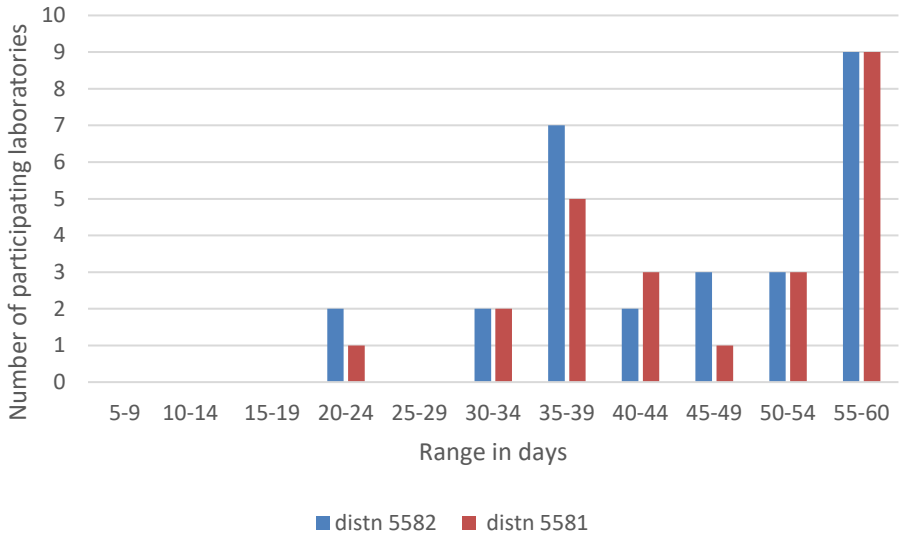
Data were displayed graphically. Detected/not detected, the serogroup and the sequence type results were analysed against the intended results, which were based on RVPBRU confirmation. For molecular examination, the samples were examined according to the procedures in ISO/TS 12869:2019 (Water quality – Detection and quantification of *Legionella* spp. and/or *Legionella pneumophila* by concentration and genic amplification using a quantitative polymerase chain reaction (qPCR)).

3 Results

Key results of this EQA exercise are given below. The findings of the survey on methods/kit information can be found in the Annex.

The clinical distribution (5582) and the environmental distribution (5581) were dispatched on 24 April 2023. Participating laboratories reported results via the United Kingdom National External Quality Assessment Service (UK NEQAS) website. The turnaround time of reporting results for all the exercises was analysed and is shown in Figure 1.

Figure 1. Number of days between receipt of the distribution and reporting of results



distn: distribution.

Intended results for the 2022–2023 exercise

The contents of the specimens and samples for the clinical and environmental distributions are described in Tables 2 and 3, including the serogroup and sequence type when *L. pneumophila* was present.

Table 2. Clinical specimens 8415–8424, provided in distribution 5582 (24 April 2023)

Specimen number	Patient	Specimen	Specimen type	Specimen contents	Serogroup	Sequence type	Details
8415	1	1	Sputum	*Legionella pneumophila* *Neisseria sicca* *Streptococcus mitis*	1	2681	Muscle pains and headache in a 42-year-old dentist
8416		2	Urine	*Legionella pneumophila*	1	2681	
8417	2	1	BAL	*Legionella pneumophila* *Streptococcus oralis* *Streptococcus mitis*	1	2110	Pneumonia in a 64-year-old male, following dental implant one week prior
8418		2	Urine	*Legionella pneumophila*	1	2110	
8419	3	1	Sputum	*Legionella pneumophila* *Legionella bozemanii* *Streptococcus mitis* *Streptococcus oralis*	1	2681	Diarrhoea and confusion in a locum dental hygienist
8420		2	Urine	*Legionella pneumophila*	1	2681	
8421	4	1	Sputum	*Legionella pneumophila* *Streptococcus salivarius* *Streptococcus oralis*	1	2681	High temperature, fever and chills in a receptionist working at a dental practice
8422		2	Urine	*Legionella pneumophila*	1	2681	
8423	5	1	BAL	*Legionella pneumophila* *Legionella longbeachae*	15	336	Fever, cough, and shortness of breath for 10 days
8424	6	1	Urine	No *Legionella*	NA	NA	Locum dentist with prolonged headache and cough

NA: not applicable.

Table 3. Environmental samples 8405–8414, provided in distribution 5581 (24 April 2023)

Sample number	Sample type	Sample contents[a]	Serogroup	Sequence type	Comments
8405	Water from a handpiece from an ultrasonic scaler	*Legionella pneumophila* ($4.2x10^3$) *Brevundimonas vesicularis* *Acinetobacter junii*	1	2681	Samples were taken as part of one outbreak investigation.
8406	Water from a handwash basin (hot outlet in dentist's treatment room)	*Legionella pneumophila* ($3.0x10^2$) *Citrobacter braakii* *Staphylococcus saprophyticus*	5	728	
8407	Swab taken from the plastic tubing from a water reservoir	*Legionella pneumophila* ($3.0x10^2$) *Acinetobacter junii* *Pseudomonas aeruginosa*	1	2681	
8408	Water from a handwash basin	*Microbacterium luteolum* *Enterococcus faecium*	NA	NA	
8409	Water from a dental cart	*Legionella pneumophila* ($2.4x10^3$) *Aerococcus viridans*	1	2681	
8410	Water from a shower located by a swimming pool	*Legionella sainthelensi* ($2.5x10^2$) *Microbacterium luteolum* *Aerococcus viridans*	2	NA	Samples were taken as part of routine monitoring of water quality.
8411	Water from the balance tank of a spa pool	*Legionella pneumophila* ($8.4x10^2$) *Pseudomonas lundensis* *Aeromonas hydrophila*	1	47	
8412	Water from a shopping centre drinking water fountain	*Staphylococcus saprophyticus* *Microbacterium luteolum*	NA	NA	
8413[b]	Water from the mains supply of a shopping centre	*Legionella pneumophila* ($2.3x10^2$) *Roseomonas aestuarii*	15	336	
8414	Water from the hot and cold system at a spa	*Legionella pneumophila* ($2.3x10^4$) *Legionella bozemanii* ($7.9x10^4$) *Citrobacter braakii* *Aerococcus viridans*	6	68	

NA: not applicable.
[a] *The levels of* Legionella *spp. in each sample is shown as approximate colony-forming units (cfu) per litre.*
[b] *This sample also contained extremely low levels of* L. pneumophila *Sg 6 ST68. This specific strain would only be identified if numerous colony picks were done and tested. The reporting of this additional species in the sample was considered correct and laboratories that did so were not negatively scored.*

Scoring applied to the reported results

All of the reported results were scored for the main examinations, either with a score of zero if not correct or two if correct (a score of one was not possible). 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.

Clinical scoring

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

- For reporting a correct identification of the *Legionella* spp.
- For reporting a correct *Legionella* serogroup.
- For reporting a correct urinary antigen result.

Environmental scoring

For the environmental samples, a score of two was given for the following:

- For reporting a correct isolation result.
- For reporting a correct identification of the *Legionella* spp.
- For reporting a correct serogroup.

Clinical distribution 5582 results

Up to 26 laboratories reported results for the simulated sputum specimens and up to 27 laboratories reported results for the urine specimens. An overview of these results are given in Table 4. Overall performance (i.e. a pooled percentage of concordant results) is given by specimen number and by type of examination. Overall performance by specimen was calculated using the mean value across a maximum of the three examinations.

Table 4. Examinations performed and concordance achieved for clinical distribution 5582

Specimen number	Contents	Identification		Serogroup		Sequence type		Urinary antigen		Overall performance by specimen
		N	%	N	%	N	%	N	%	%
8415	*Legionella pneumophila* *Neisseria sicca* *Streptococcus mitis*	26/26	100	20/21	95.2	13/14	92.9	NA	NA	96.0
8416	*Legionella pneumophila*	NA	NA	NA	NA	NA	NA	27/27	100	100
8417	*Legionella pneumophila* *Streptococcus oralis* *Streptococcus mitis*	25/26	96.2	23/23	100	14/16	87.5	NA	NA	94.6
8418	*Legionella pneumophila*	NA	NA	NA	NA	NA	NA	27/27	100	100
8419	*Legionella pneumophila* *Legionella bozemanii* *Streptococcus mitis* *Streptococcus oralis*	25/26	96.2	23/23	100	15/16	93.8	NA	NA	95.4
8420	*Legionella pneumophila*	NA	NA	NA	NA	NA	NA	26/26	100	100
8421	*Legionella pneumophila* *Streptococcus salivarius* *Streptococcus oralis*	24/26	92.3	22/22	100	15/16	93.8	NA	NA	96.7
8422	*Legionella pneumophila*	NA	NA	NA	NA	NA	NA	25/25	100	100
8423	*Legionella pneumophila* *Legionella longbeachae*	25/26	96.2	7/22	31.8	8/14	57.2	NA	NA	61.7
8424	No *Legionella* -	NA	NA	NA	NA	NA	NA	25/27	92.3	92.3
Overall performance		NA	96.2	NA	85.4	NA	85.0	NA	98.5	NA

NA: not applicable.

Patient 1

Specimen 8415 – the specimen contained *L. pneumophila* Sg 1: ST2681. There was very good concordance with the intended results, with 26/26 (100%), 20/21 (95.2%) and 13/14 (92.9%) of participating laboratories reporting the correct results for identification, serogroup and sequence type, respectively. One laboratory stated that they didn't examine the sputum, one incorrectly reported Sg 2 and one reported another sequence type (6,10,2,10,13,14,9).

Specimen 8416 – the specimen was positive for *L. pneumophila* urinary antigen. Performance was excellent, with 27/27 (100%) concordance with the intended results from participating laboratories that returned a result. One laboratory reported a negative result through PCR, but reported the overall result as not examined.

Patient 2

Specimen 8417 – the specimen contained *L. pneumophila* Sg 1: ST2110. Performance was good, with 25/26 (96.2%), 23/23 (100%) and 14/15 (87.5%) of participating laboratories reporting the correct identification, serogroup and sequence type, respectively. One laboratory reported the presence of normal flora only, one laboratory reported another sequence type (6,10,2,10,13,4,9) and one laboratory reported ST2681.

Specimen 8418 – the specimen was positive for *L. pneumophila* urinary antigen. Performance was excellent, with 27/27 (100%) of participating laboratories returning a result for this specimen reporting the correct result.

Patient 3

Specimen 8419 – the specimen contained *L. pneumophila* Sg 1: ST2681. Performance was very good, with 25/26 (92.6%), 23/23 (100%) and 15/16 (93.8%) of participating laboratories reporting the correct identification, serogroup and sequence type, respectively. One laboratory reported normal flora only and another reported that the specimen was not examined. One laboratory reported another ST (as 6,10,2,10,13,14,9). Only 17/26 (65.4%) of participating laboratories successfully identified both *L. pneumophila* Sg 1: ST2681 and *L. bozemanii.* For *L. bozemanii*, a result of *Legionella* spp. not *L. pneumophila* was also considered correct.

Specimen 8420 – the specimen was positive for *L. pneumophila* urinary antigen. Performance was excellent, with 100% (26/26) of participating laboratories returning a result for this specimen reporting the correct result.

Patient 4

Specimen 8421 – the specimen contained *L. pneumophila* Sg 1: ST2681. Performance was very good, with 24/26 (92.3%), 22/22 (100%) and 15/16 (93.8%) reporting the correct identification, serogroup and sequence type, respectively. One participating laboratory reported that there was no *Legionella* present, one reported normal flora only and one reported another sequence type (6,10,2,10,12,14,9).

Specimen 8422 – the specimen was positive for *L. pneumophila* urinary antigen. Performance was excellent, with 25/25 (100%) of participating laboratories reporting the correct result.

Patient 5

Specimen 8423 – The specimen contained *L. pneumophila* Sg 15: ST336 and *Legionella longbeachae*. Performance varied, with 25/26 (96.2%), 7/22 (31.8%) and 8/14 (57.2%) reporting the correct identification, serogroup and sequence type, respectively. One laboratory did not isolate either *Legionella* spp. A total of 23/27 (85.2%) of participating laboratories successfully identified both *L. pneumophila* Sg 1: ST2681 and *L. longbeachae.* For *L. longbeachae*, a result of *Legionella* spp. not *L. pneumophila* was also considered correct.

Patient 6

Specimen 8424 – the specimen was negative for *L. pneumophila* urinary antigen. Performance was very good, with 25/27 (92.3%) of participating laboratories reporting the correct result. Two laboratories reported that the specimen was positive for urinary antigen.

Environmental distribution 5581 results

Ten simulated environmental samples were sent to 24 laboratories in 24 EU/EEA countries. All 24 laboratories returned a result.

Sample numbers 8405–8409 – these samples were taken as part of one outbreak investigation.

Sample numbers 8406–8414 – these samples were taken as part of routine monitoring.

Sample number 8407 – this sample was a swab sample.

Analysis of the participating laboratories' performance is presented separately for culture-based methods (Table 5) and molecular methods (Table 6). Culture-based method analysis included results reported for isolation, identification, enumeration, serogroup and sequence type results. Overall performance (i.e. a pooled percentage of concordant results) is given for culture-based method results by sample number and by type of examination. Overall performance by sample was calculated using the mean value across a maximum of the five examinations. For molecular methods, the overall performance was not calculated, as the number of data sets returned for analysis was too low to provide robust performance data.

Table 7 shows the reported enumeration results. The median was used as the assigned value and the expected range was calculated using the 5th and 95th percentiles.

Table 5. Examinations performed and concordance achieved for environmental distribution 5581, cultured samples

Sample number	Contents	Isolation		Identification		Enumeration		Serogroup		Sequence type		Overall performance by sample
		N	%	N	%	N	%[a]	N	%	N	%	%
8405	*L. pneumophila* Sg 1, ST2681	21/24	87.5	21/21	100	18/21	85.7	20/20	100	11/11	100	94.6
8406	*L. pneumophila* Sg 5, ST728	17/24	70.8	17/17	100	15/17	84.2	16/17	94.1	11/11	100	89.8
8407	*L. pneumophila* Sg 1, ST2681	22/23	95.7	22/22	100	NA	NA	21/21	100	13/13	100	98.9
8408	No *Legionella*	22/24	91.7	NA	NA	NA	NA	NA	NA	NA	NA	91.7
8409	*L. pneumophila* Sg 1, ST2681	24/24	100	24/24	100	20/24	83.3	23/23	100	13/13	100	96.7
8410	*L. sainthelensi*[b]	16/24	66.7	16/16	100	16/18	88.8	NA	NA	NA	NA	85.2
8411	*L. pneumophila* Sg 1, ST47	22/24	91.7	22/22	100	20/22	90.9	21/21	100	11/11	100	96.5
8412	No *Legionella*	24/24	100	NA	NA	NA	NA	NA	NA	NA	NA	100
8413[c]	*L. pneumophila* Sg 15, ST336	19/24	79.1	19/19	100	17/19	89.6	17/17	100	8/9	88. 8	91.5
8414	*L. pneumophila* Sg 6, ST68 *L. bozemanii*[b]	23/23	100	20/23[d]	87.0	20/22	90.9	21/21	100	9/10	90.0	93.6
Overall performance by examination		NA	88.3	NA	98.4	NA	87.6	NA	99.2	NA	97.0	NA

[a] *Reported censored values that fell within the expected range were considered correct.*
[b] *The reporting of* Legionella *species not* pneumophila *and* Legionella *species have been analysed as being correct*
[c] *This sample also contains extremely low levels of* L. pneumophila *serogroup 6 ST68. The reporting of this additional species has been considered correct and included in the performance analysis.*
[d] *Twenty laboratories returned the result of* Legionella pneumophila *and* L.bozemanii. *For the remaining participating laboratories, one identified only* Legionella pneumophila, *one identified only* L.bozemanii *and one reported 'Other'.*

Table 6. Identification and concordance achieved for environmental distribution 5581, molecular methods

Sample number	Contents	*Legionella pneumophila*		*Legionella* spp.	
		Intended results	Reported results	Intended results	Reported results
8405	*L. pneumophila* Sg 1, ST2681	Detected	13/15	Detected	10/14
8406	*L. pneumophila* Sg 5, ST728	Detected	11/15	Detected	8/14
8407	*L. pneumophila* Sg 1, ST2681	Detected	12/13	Detected	9/12
8408	No *Legionella*	Not detected	14/15	Not detected	12/14
8409	*L. pneumophila* Sg 1, ST2681	Detected	15/15	Detected	12/14
8410	*L. sainthelensi*	Not detected	14/15	Detected	10/14
8411	*L. pneumophila* Sg 1, ST47	Detected	13/15	Detected	10/14
8412	No *Legionella*	Not detected	14/15	Not detected	13/14
8413	*L. pneumophila* Sg 15, ST336	Detected	11/15	Detected	9/14
8414	*L. pneumophila* Sg 6, ST68 *L. bozemanii*	Detected	14/15	Detected	14/14

[a] *It was unclear if laboratories were including* Legionella pneumophila *in their results for whether or not* Legionella *spp. were present. The request form for this distribution specified that* L. pneumophila *should be included in the reporting of* Legionella *spp., as most kits cannot differentiate this species from the others.*

Table 7. Data on enumeration results, where reported

Sample number	Number of results	Identification	Median reported result (cfu/L)	Intended range (cfu/L)	Number of outlying counts
8405	21	*L. pneumophila*	$1.2x10^3$	$1.0x10^2$–$18.6x10^4$	3 (1 low, 2 high)
8406	17	*L. pneumophila*	$1.0x10^2$	19–$5.0x10^2$	1 (1 low)
8409	24	*L. pneumophila*	$1.4x10^3$	$1.2x10^2$–$7.8x10^3$	4 (2 low, 2 high)
8410	16	*L. sainthelensi*	$5.0x10^2$	17–$1.9x10^3$	2 (1 low, 1 high)
8411	22	*L. pneumophila*	$1.0x10^3$	$1.6x10^2$–$9.1x10^3$	2 (1 low, 1 high)
8413	19	*L. pneumophila*	$1.3x10^3$	$1.0x10^2$–$1.0x10^4$	2 (1 low, 1 high)
8414	22	*L. pneumophila* *L. bozemanii*	$9.5x10^4$	$1.1x10^3$–$6.0x10^5$	2 (1 low, 1 high)

cfu: colony-forming units.

Sample 8405 – Performance was very good, with 21/24 (87.5%) participating laboratories reporting the correct isolation result, 21/21 (100%) reporting the correct identification, 18/21 (85.7%) reporting a count within the intended range, 20/20 (100%) reporting the correct serogroup and 11/11 (100%) reporting the correct sequence type. The overall performance for examinations by culture was 94.6%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 13 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 10 reported a correct result.

Sample 8406 – Performance was below average, with 17/24 (70.8%) participating laboratories reporting the correct isolation result, 17/17 (100%) reporting the correct identification, 16/19 (84.2%) reporting a count within the intended range, 16/17 (94.1%) reporting the correct serogroup and 11/11 (100%) reporting the correct sequence type. The overall performance for examinations by culture was 89.8%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 11 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and eight reported a correct result.

Sample 8407 – Performance was very good, with 22/23 (95.7%) participating laboratories reporting the correct isolation result, 22/22 (100%) reporting the correct identification, 21/21 (100%) reporting the correct serogroup and 13/13 (100%) of the laboratories reporting the correct sequence type. The overall performance for examinations by culture was 98.9%. Thirteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 12 reported the correct result. Twelve laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 8408 – Performance was very good, with 22/24 (91.7%) participating laboratories reporting the correct isolation result. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 14 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 12 reported a correct result.

Sample 8409 – Performance was excellent, with 24/24 (100%) participating laboratories reporting the correct isolation result, 24/24 (100%) reporting the correct identification, 20/24 (83.3%) reporting a count within the intended range, 23/23 (100%) reporting the correct serogroup and 13/13 (100%) reporting the correct sequence type. The overall performance for examinations by culture was 96.7%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 12 reported a correct result.

Sample 8410 – Performance was below average, with 16/24 (66.7%) participating laboratories reporting the correct isolation result, 16/16 (100%) reporting the correct identification, and 16/18 (88.8%) reporting a count within the intended range. The overall performance for examinations by culture was 85.1%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 14 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 10 reported a correct result.

Sample 8411 – Performance was very good, with 22/24 (91.7%) participating laboratories reporting the correct isolation result, 22/22 (100%) reporting the correct identification, 20/22 (90.9%) reporting a count within the intended range, 21/21 (100%) reporting the correct serogroup and 11/11 (100%) reporting the correct sequence type. The overall performance for examinations by culture was 96.5%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 13 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 10 reported a correct result.

Sample 8412: Performance was excellent, with 24/24 (100%) participating laboratories reporting the correct isolation result. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 14 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 13 reported a correct result.

Sample 8413 – Performance was below average, with 19/24 (79.1%) participating laboratories reporting the correct isolation result, 19/19 (100%) reporting the correct identification, 17/19 (89.6%) reporting a count within the intended range, 17/17 (100%) reporting the correct serogroup and 8/9 (88.8%) reporting the correct sequence type. The overall performance for examinations by culture was 91.5%. This sample also contained extremely low levels of *L. pneumophila* Sg 6 ST68. The reporting of this additional species was considered correct and was included in the overall performance analysis. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 11 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 8414 – Performance was excellent, with 23/23 (100%) participating laboratories reporting the correct isolation result. However, for the identification, only 20/23 (87.0%) reported the presence of both *L. pneumophila* and *L. bozemanii* and 20/22 (90.9%) reported a count within the intended range. The 21 laboratories that reported the presence of *L. pneumophila* all reported the correct serogroup and 9/10 (90.0%) reported the correct sequence type. The overall performance for examinations by culture was 93.6%. Fifteen laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 14 reported the correct result. Fourteen laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and all reported a correct result.

The number of days between receipt of the distribution and reporting of results for all the exercises was analysed and is shown in Figure 1.

4 Discussion

General

Environmental and clinical laboratories play a vital role in protecting the public's health by helping to ensure public health incidents are effectively detected and managed through the provision of quality results for specimens or samples analysed. Laboratories may also need to report detected cases of Legionnaires' disease to their national surveillance systems if this is a requirement in their country.

An EQA provides laboratories with an independent external assessment of their performance. Regular participation in proficiency testing schemes is an important part of laboratory quality procedures and helps to ensure that the results of their tests are accurate and reliable. Participation also ensures high quality surveillance data.

The distributions for the 2022–2023 EQA exercise represented an outbreak associated with a dental practice. The outbreak strain of *L. pneumophila* Sg 1, ST2681 was isolated from clinical specimens and environmental samples. This species of *L. pneumophila* is rare and has only been identified five times in the UK since 2018.

Participating laboratories' overall performance for clinical specimens was very good. There were no significant issues arising for species identification, serogroup, or sequence type. It was, however, noted that the number of laboratories reporting isolation and identification of the non-*L. pneumophila* when present as a dual pathogen in one specimen was lower. Most of the laboratories that returned results for the methods survey stated that only one pick was used for the confirmation testing. This could be the reason for the lower reporting demonstrated during the presence of a dual pathogen. There was also a significantly lower participant concordance when an Sg 15 was included. Again, from the methods survey, we can see that the large majority of participants only tested for Sg1–14, which could explain this finding.

Overall performance for environmental samples was good. In general, there were no significant issues arising for species identification, serogroup, enumeration or sequence type. It was, however, noted for some samples that the number of participants reporting a correct identification of the non-*L. pneumophila* was lower. In addition, when the level of *Legionella* spp. in a sample was low, some laboratories failed to isolate the organism, which could be because the level was below the detection limit for the method they used.

No issues were encountered with the preparation of the simulated specimens or samples. Homogeneity, stability, and viability were consistent throughout all the stages of the preparation and distribution. To maintain these parameters, proven technology for preserving organisms/levels of organisms were used, such as lyophilised or LENTICULE® discs. These preservation techniques indicated that the stability of the organisms would be maintained during transit to the EU/EEA countries. This was important, given that transit time would most likely be longer than that for local or national distribution of specimens or samples to designated laboratories.

In this EQA exercise, the majority of laboratories for clinical specimens identified the pathogen and serogroup, but a significant number did not report the sequence type (Table 3). For environmental samples, the ISO 11731:2017 requires that suspected colonies are identified as at least *L. pneumophila*. Laboratories reported *L. pneumophila* correctly and went further, also reporting the serogroup.

Clinical discussion

The clinical aspect of this EQA was a qualitative exercise designed to assess simulated sputum, BAL and urine specimens. Distribution 5582 contained four paired (sputum/urine or BAL/urine) simulated specimens (8415,8416; 8417,8418; 8419,8420; 8421,8423) and two single specimens (BAL and urine). The paired specimens were designed to mimic an outbreak.

The panel of sputum and BAL specimens was used to ascertain the absence or presence of *L. pneumophila.* Following isolation of the respiratory pathogen, full identification to species level was requested, with accompanying serogroup and sequence type. Examination to detect the urinary antigen for *L. pneumophila* was requested in the simulated urine specimens.

Simulated sputum, BAL and urine specimens were used because published guidance by the UKHSA identifies these as the three most commonly described specimen types. The methods survey (see the Annex) sent to all laboratories following the closure of the exercise confirmed that the most common specimen types routinely examined by participating laboratories were sputum 88% (15/17), urine 70.6% (12/17) and BAL 100% 17/17.

Fourteen survey respondents indicated they were clinical diagnostics laboratories, and nine of these were also reference laboratories. Of the five laboratories who were not reference laboratories, three stated that they did have access to a reference lab, while one did not. A total of 8/17 laboratories also participated in a national EQA scheme. However, this was only a mandatory exercise for two of them. Buffered charcoal yeast extract (BCYE) with cysteine (n = 9) and GVPC (n = 8) were the most frequently used media for the isolation of *Legionella* spp.

Identification

- Overall concordance was excellent (96.2%) for correct identification of *L. pneumophila.* This is consistent with previous EQA exercise results.
- Concordance was consistently high for the identification of *L. pneumophila* when specimens contained two species of *Legionella*.
- Concordance was consistently lower for the identification of non-*L. pneumophila.*

Serogroup

- Overall concordance was excellent for all Sg 1 strains.
- A lower number of laboratories performed serogrouping, compared with identification.
- There was significantly reduced concordance (31.8%) when the specimen included an Sg 15 *L. pneumophila.*

Sequence type

- Overall concordance was lower (85%) when sequence type was reported. This is consistent with previous EQA exercises.
- A lower number of laboratories reported the sequence type.

Urinary antigen testing

- Overall concordance was excellent (98.5%) for reporting urinary antigen.
- A lower concordance was obtained when a negative specimen was included.

The source of infection can be identified by comparing clinical and environmental *L. pneumophila* isolates using various typing methods. A variety of rapid identification and sensitivity methods have been developed for isolates from clinical specimens. These include molecular techniques such as polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), multiple-locus variable-number tandem-repeat analysis (MVLA), single nucleotide polymorphism (SNP) assays, whole genome sequencing (WGS) and matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry. Although these applications enable subtyping of unrelated strains, the accuracy, precision and reproducibility are not comparable.

Environmental discussion

The environmental aspect of this EQA was a qualitative and quantitative exercise designed to assess simulated environmental water and swabs. The environmental samples were used to ascertain the presence or absence of *Legionella* spp. and, upon isolation/detection of the organism, a full identification to species level. There was also an option to report enumeration with accompanying serogroup and sequence type for water samples.

Legionella spp. are found in cooling towers, hot and cold water systems, air conditioners, spa equipment, fountains, humidifiers, showers, misting devices, decorative fountains and water features, dentistry tools and thermostatic mixing valves. The main mode of transmission is through inhalation of airborne droplets contaminated with *Legionella* spp.

The detection of *Legionella* by culture is the gold standard method for detecting *Legionella* colonies on BCYE/GVPC agar plates. This is a labour-intensive approach that takes 10 days to complete. Recovery of *Legionella* bacteria by culture can be challenging, as *Legionella* colonies on BCYE agar media can be overgrown or inhibited by competing microbial flora, masking the presence of *Legionella* colonies. Therefore, acid and heat treatment of samples is the key to reducing the amount of background flora [3].

The polymerase chain reaction (PCR) method is a molecular technique that only takes a few hours to complete and can be a useful method to screen environmental and water samples. The disadvantage of this method is that dirt and debris can have an impact on the test outcome. Molecular testing is not widely used to test water and environmental samples for *Legionella* and only a few commercial laboratories offer this service routinely. Moreover, the detection of DNA from dead *Legionella* cells has limited public health significance.

Of the 12 laboratories that responded to the methods survey (see the Annex), 10 stated that they examined the water samples for *Legionella* bacteria using ISO 11731:2017 (Water quality – Enumeration of *Legionella*). Most of the laboratories that returned information about their method responded that they filtered the water sample and would carry out culturing on untreated, acid and heat-treated samples.

For this exercise, up to 24 results were reported. Not all laboratories examined all the samples or undertook all the examinations when the sample contained *L. pneumophila.*

Isolation

- The overall concordance was very good (88.3%) for correct isolation results. One laboratory reported an incorrect isolation result six times, one laboratory four times, seven laboratories twice and four laboratories once.
- The concordance for samples 8406, 8410 and 8413 was low. The reason for this could be that the low level of *Legionella* spp. in the sample was below the lower detection limit for the methods used by the laboratories. For these samples, one laboratory reported an incorrect isolation result three times, five laboratories twice and seven laboratories once.
- Sample 8410 contained *L. sainthelensi.* Concordance for this sample was very low (66.7%). The statistically calculated range was from 17–1.9x10^3 cfu/L and participating laboratories' results had a median of 5.0x10^2 cfu/L. The reasons for this could be multifactorial, such as levels of the *Legionella* spp. in the sample being below the detection limit of the method used or an inability to recognise this strain in the sample.
- Sample 8414 contained two species of *Legionella* and 20/23 (87.0%) laboratories isolated both *L. pneumophila* and *L. bozemanii*.
- The overall concordance for the nine water samples was 87.5%.
- The overall concordance for the eight samples containing a *Legionella* spp. was 86.4%.
- The overall concordance for the two samples not containing a *Legionella* spp. was 95.6%.
- The overall concordance for the five outbreak investigation samples was 89.1%.
- The overall concordance for the five routine monitoring samples was 87.5%.
- The most common isolation media used was GVPC and/or BCYE. There was variation among laboratories in the use of other culture media and acid and/or heat treatment.

Identification

- For the seven samples that contained a *Legionella* spp., the overall concordance was very good (98.4%) for correct identification results.
- The concordance for the correct identification of *Legionella* spp. (where only one species was in the sample and an isolated result was reported) for the seven samples was 100%.
- The concordance for the correct identification of *Legionella* spp. (where two species were in the sample) was lower (87.0%).

Enumeration

- The number of data sets returned for this exercise varied from 16–24 for each sample. This is below the number required to produce robust performance data. (Statistical calculation based on 10–19 results should be interpreted with caution, as they may be overly influenced by outlying results. When there are fewer than 10 reported results, the statistics are not considered robust enough.)
- Enumeration results can only be provided for positive water samples that contain more than one *Legionella* spp. Seven samples in this distribution met that criterion. Concordance was consistent by sample, which ranged from 83.3–90.9% of results reported in the intended range. The overall concordance for this distribution was very good (87.6%).
- Further analysis showed that one laboratory reported an outlying count three times, two laboratories reported an outlying count twice and nine laboratories reported an outlying count once.
- The number of outlying results by sample in this distribution was not influenced by the level of *Legionella* spp. in the sample.

Serogroup

- For the seven samples that contained a *Legionella* spp., the overall concordance for reporting a correct serogroup was 99.2%.
- For the four samples that contained a *L. pneumophila* Sg 1, concordance was excellent (100%).
- For the three samples that contained a *L. pneumophila* non-Sg 1, concordance was 98.0%.
- Sample 8413 contained *L. pneumophila* Sg 15; however, three laboratories reported Sg 6. As this sample contained a low level of *L. pneumophila* Sg 6, these results have been considered correct.

Sequence type

This sample also contains extremely low levels of *L. pneumophila* Sg 6 ST68. The reporting of this additional species has been considered correct and was included in the performance analysis.

- For the seven samples that contained a *Legionella* spp., the overall concordance for reporting a correct sequence type was 97.0%.
- The overall concordance for the four outbreak investigation samples was 100%.
- The overall concordance for the three routine monitoring samples was 92.9%.
- The overall concordance for the three samples that contained the outbreak strain ST2681 was 100%.
- The methods used for sequence type analysis was Sanger sequencing and/or WGS.

Molecular methods

- The number of laboratories examining the samples by molecular methods is still low. For this exercise, between 11–15 laboratories examined the samples using a kit that detects *L. pneumophila* and between 8–14 did so using a kit that detects *Legionella* spp. Therefore, performance as a percentage for results reported using molecular methods has not been calculated.
- The overall concordance for identifying *Legionella* spp. using molecular methods was much lower. This may be due to a misunderstanding that the *Legionella* spp. results should also include *L. pneumophila*, even though this was made clear on the instructions included with the distribution.
- One laboratory reported an incorrect result five times for *L. pneumophila* and six times for *Legionella* spp. These results were in consensus with their isolation results, where they reported 'not isolated'.
- One laboratory reported an incorrect result five times for *L. pneumophila* and four times for *Legionella* spp. Four of these reported results were in consensus with their isolation results, where they reported 'not isolated'.
- One laboratory reported an incorrect result two times for *L. pneumophila* and two times for *Legionella* spp. These results were in consensus with their isolation results, where they reported 'not isolated'.
- Two laboratories reported an incorrect result for *Legionella* spp., yet reported the correct result for *L. pneumophila.* This may be due to a misunderstanding that the *Legionella* spp. results should also include *L. pneumophila*, even though this was made clear on the instructions included with the distribution.

The detection and acceptable level of *Legionella* spp. is also an important factor in determining the effectiveness of control measures in an artificial water system. Other types of *Legionella* spp. besides *L. pneumophila* have also been implicated in causing infection, particularly in nosocomial cases. However, the EQA organisers are aware that national guidance documents may only refer to *L. pneumophila* and not necessarily include the requirement to test other species of *Legionella.*

Limitations of this EQA exercise

This EQA exercise was only able to evaluate the analytical and post-analytical stages of the total testing process. The pre-analytical stage of the process was not evaluated. The pre-analytical stages would include the demographics of the patient specimen/sample, correct sample type, volume of the sample, correct tests requested, and suitable container, all of which were pre-determined for this EQA panel.

Up to two laboratories per EU/EEA country could participate, so the breadth of the cohort was limited to those who received a panel and returned results.

A period of eight 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. The number of days from the receipt of the distribution to the reporting of results was recorded (Figure 1), and the mean value was between 45–47 days for clinical specimens and environmental samples. This was a significant increase from mean value identified in the previous EQA exercise (28 days).

A limitation of the system used to report the EQA results is that there is no way to capture the reporting of preliminary results, as some laboratories do.

The clinical specimens sent in lyophilised format did not represent the matrix of an authentic liquid purulent sputum that would normally be received by a diagnostic laboratory. However, the specimens distributed for the detection of urinary antigens were authentic clinical liquid urine, spiked with species antigen, and were provided in plastic tubes to resemble true specimens.

For the environmental samples, once a LENTICULE® disc was rehydrated, it would constitute one litre of water, which would not be representative of the chemical constituents normally found in real samples. For swabs, laboratories were instructed to rehydrate the sample and then absorb the material onto a swab before suspending the swab in a diluent. This was the most practical way to simulate a swab sample, but is not representative of how a swab sample would usually be received in a laboratory for analysis.

For environmental samples, the enumeration results could not be evaluated due to the low number of data sets returned.

5 Conclusions

Laboratories from 29 EU/EEA countries performed well in this year's EQA exercise for culture-based detection methods for isolation of *Legionella* spp. used by clinical (96.2% overall concordance with intended results) and environmental (88.3% overall concordance) laboratories (Tables 4 and 5).

Both clinical and environmental laboratories demonstrated that they can undertake testing to an acceptable level of at least 80% correctness. However, there are a few laboratories that fall short of this threshold. These laboratories should review their methodologies and seek professional expertise to identify the root causes, so that improvements can be made. Although the data provide some assurance of the laboratories' ability to undertake effective public health investigations for *L. pneumophila* and other *Legionella* spp., laboratories need to continue taking part in EQA schemes so they can determine their actual ongoing performance with the varying designs of EQA specimens and samples. If laboratories report accurate data, the information provided to surveillance systems is also accurate.

In this EQA exercise, laboratories examined specimen and sample types that they routinely test. For the clinical specimens, 26 laboratories examined the sputum, BAL and urine specimens. For the environmental samples, 24 laboratories examined both water and swab samples. Where reported results were not concordant with the intended results, laboratories were advised to investigate further to determine the root cause.

For the clinical laboratories, all 26 reported an identification, 23 reported the serogroup and up to 16 reported on sequence type. Isolation in culture remains the gold standard for the diagnosis of infection caused by *Legionella* spp., due to the low sensitivity and specificity associated with serotyping. MALDI-TOF MS is then frequently used to identify isolates to the species level. Most clinical laboratories were able to identify a non-*pneumophila Legionella* to the species level. Differentiation and typing of strains can be achieved using a range of molecular techniques, including sequence-based typing and PCR methods.

For the environmental laboratories, all 24 reported a result for isolation and identification, up to 23 reported a serogroup, up to 24 reported an enumeration count and up to 13 reported a sequence type. For molecular methods, up to 15 laboratories analysed the samples for *L. pneumophila* and up to 14 for *Legionella* spp.

The overall concordance for isolation using culture was 88.3%, with up to 24 laboratories reporting a result. The concordance for isolation for samples 8406, 8410 and 8413 was lower than expected. The reason for this low performance could be due to the low level of *Legionella* spp. in the sample, which may have been below the lower detection limit for the methods used by these laboratories. Sample 8410 contained *L. sainthelensi*; concordance for this sample was very low (66.7%). The statistically calculated range was from 17–1.9x10^3 cfu/L and participating laboratories' results had a median of 5.0x10^2 cfu/L. The reasons for this could be multifactorial, such as levels of the *Legionella* spp. in the sample being below the detection limit of the method used or an inability to recognise this strain in the sample.

The overall performance for molecular methods was not calculated due to the low number of laboratories analysing the specimens and samples by this method. The overall concordance was much lower with the *Legionella* spp. results; this may have been due to a misunderstanding that the *Legionella* spp. results should also include *L. pneumophila*. One laboratory reported an incorrect result five times for *L. pneumophila* and six times for *Legionella* spp. and another laboratory reported an incorrect result five times for *L. pneumophila* and four times for *Legionella* spp.

6 Recommendations

EQA schemes provide an ongoing baseline understanding of the level of testing undertaken in EU/EEA laboratories, identify performance issues and, where possible, provide follow-up support to laboratories/countries that have identified limitations so they can address making improvements to their testing capabilities or capacity-building.

Laboratories are encouraged to take part in EQA schemes provided by accredited EQA service providers throughout the year. Regular participation will allow laboratories to have regular assurance and confidence in the results reported. This is especially important when there are changes in environmental factors. Ongoing performance assessment is designed to identify genuine problems.

Ongoing objectives for laboratories

- To continue understanding the different *Legionella* spp. that may be isolated from clinical specimens and environmental samples.
- To continue understanding the limitations of confirmatory tests done when *Legionella* spp. is isolated from specimens or samples.
- To understand the importance of following standardised methods when managing public health incidents.
- To understand that regular participation in the EQA helps to ensure that their results are accurate.

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Annex. Methods survey findings

As part of this EQA exercise, a survey on the methods/kit information and frequency of testing performed for each method/kit used to analyse the specimens or samples was sent to all participating laboratories. The laboratories were only asked to provide information on the methods they use in their laboratory. This information was gathered online. The data presented below are for EU/EEA countries.

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 performance of the various molecular kits/processes.

Findings for clinical specimens

The survey was completed by 17 of the participating clinical laboratories in the following EU/EEA countries: Belgium, Bulgaria, Croatia, Czechia, Estonia, Finland, Greece, Hungary, Ireland, Italy, Latvia, Malta, Norway, Portugal, Slovenia, Spain and Sweden.

The total numbers will not always correspond to 18, as some respondents did not respond to every question and some questions allowed for more than one option to be selected.

Overview of survey responses

1. Are you a clinical diagnostic laboratory?
2. Are you a national reference laboratory?

A total of 14 laboratories reported that they were clinical diagnostic laboratories; of these, nine were also national reference laboratories. Four stated that they were national reference laboratories only. Of the five laboratories that were not national reference laboratories, three stated they did have access to a reference laboratory, while one did not.

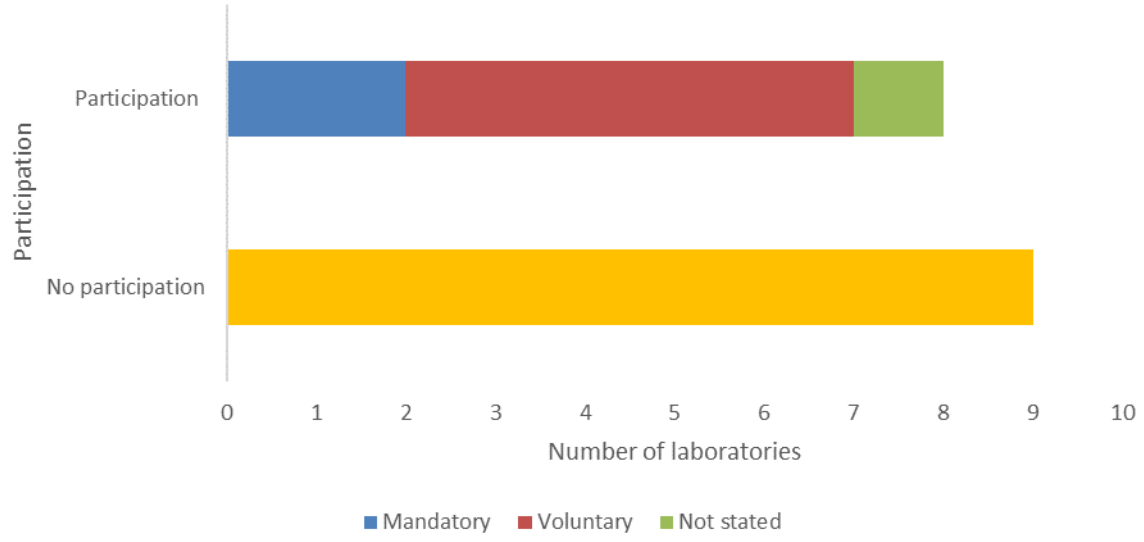
3. Approximately what percentage of all Legionnaires' disease cases in your country in 2022 involved a surveillance notification from your lab?

Responses ranged from 3–100% (The following responses were received: 3%, 20%, 30% (n =2), 33%, 60%, 80%, 99%, 80–90%, 100%).

4. Does your laboratory participate in a national EQA scheme for the detection of *Legionella* species?
4a. Is this a voluntary participation?
4b. Or a mandatory requirement?

Responses are presented in Figure 1A.

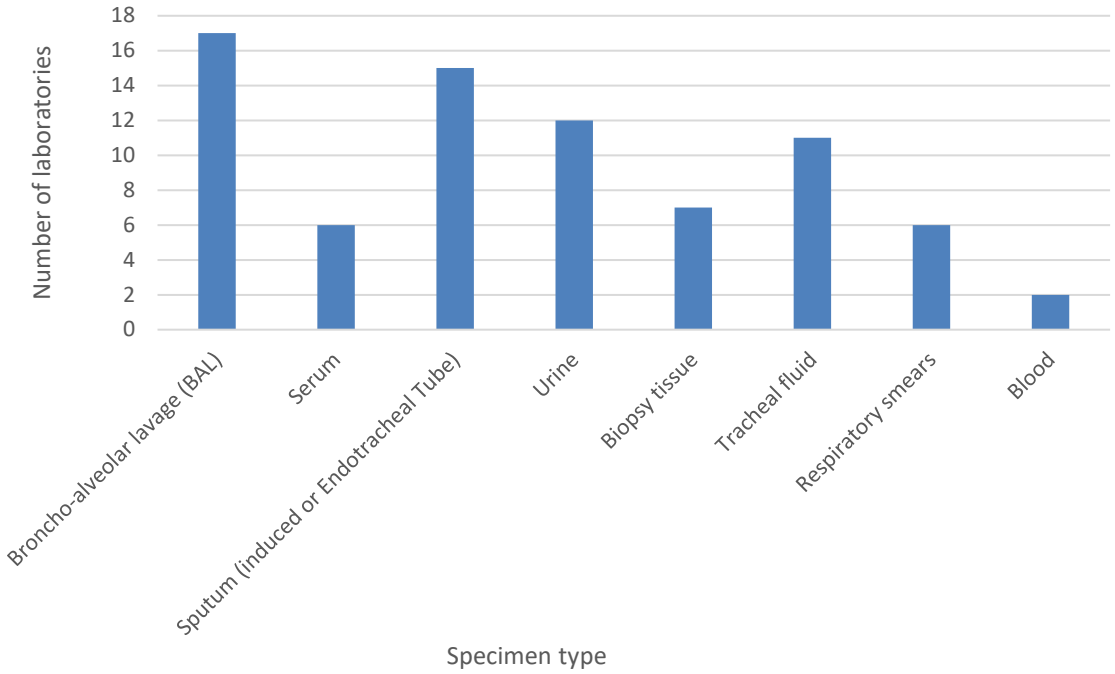
Figure 1A. Number of laboratories participating in a national *Legionella* EQA



5. Which types of clinical specimen does your laboratory receive for *Legionella* infection investigations?

Responses are presented in Figure 2A.

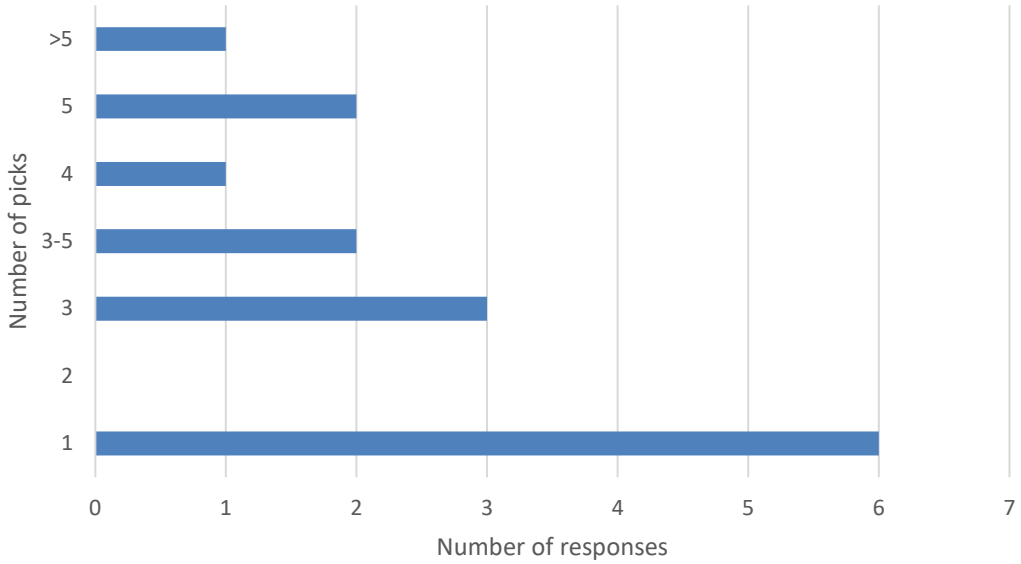
Figure 2A. Number of participants reporting they test against each specimen type



6. When a *Legionella* species has been isolated, how many picks of the colonies do you take for confirmation testing? (n = 15)

Responses are presented in Figure 3A.

Figure 3A. Number of picks taken for confirmatory testing



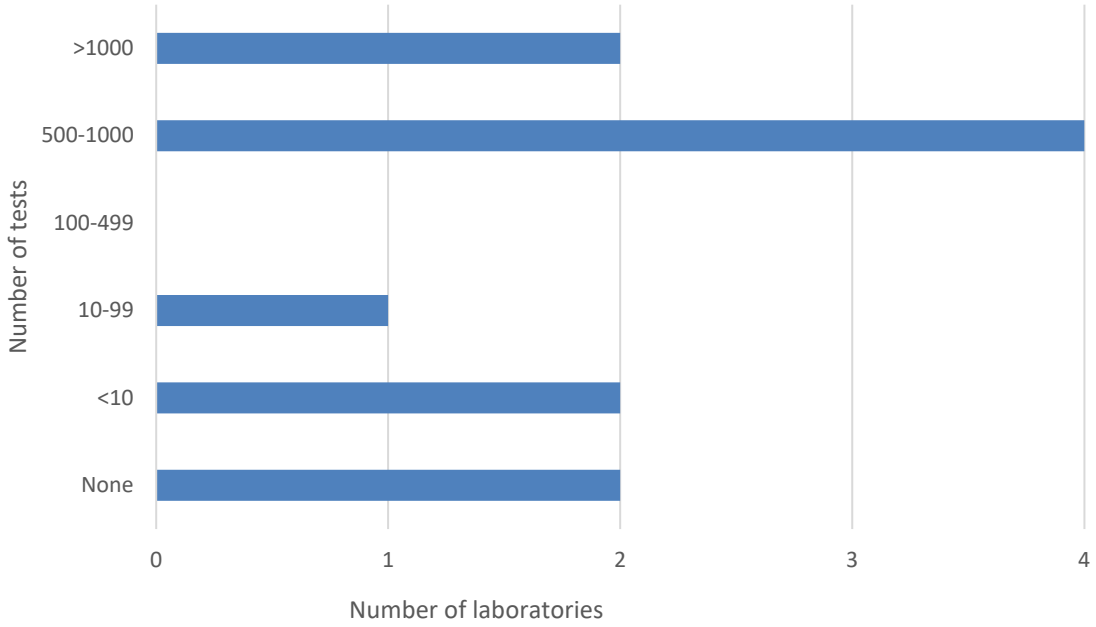
7. Does your laboratory check fluorescence as a primary confirmation test?

Only five of the 12 laboratories that responded to this question stated that fluorescence is performed. Of the laboratories stating that this is not performed, one said they would perform fluorescence as a secondary confirmation test, one said they would perform PCR as confirmation and another said they would perform latex agglutination. Two stated equipment was not available to perform this test.

8. Approximately how many *Legionella* urinary antigen tests are undertaken in your laboratory each year?

Responses are presented in Figure 4A.

Figure 4A. Number of *Legionella* urinary antigen tests undertaken each year



9. Approximately how many *Legionella* spp. clinical tests for culture isolation and identification, serology and PCR are undertaken in your laboratory each year?

Responses are presented in Table 1A.

Table 1A. Number of *Legionella* spp. clinical tests performed

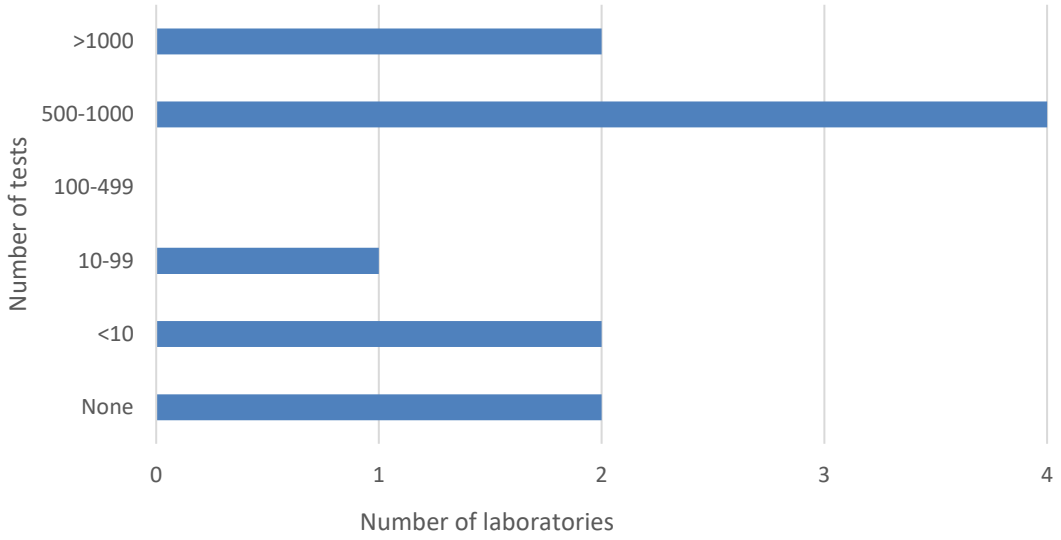
NUMBER OF TESTS PERFORMED	NONE	<10	10–99	100–499	500–1 000
Culture	1	0	10	1	0
PCR tests	2	0	4	1	1
7 alleles ESGLI method	6	0	4	1	0
Direct fluorescent antibody (DFA) staining	9	1	2	0	0
Mip sequencing	7	2	3	0	0
Serotyping	5	1	4	0	0
Monoclonal antibody	7	0	1	0	0
Latex agglutination	1	1	8	1	0
Sequencing (Sanger)	7	5	0	0	0
Sequencing (whole genome sequencing)	7	1	2	2	0

ESGLI: European Study Group for Legionella Infections.

10. Which of the following patient groups do you receive specimens from routinely for *Legionella pneumophila* testing in your laboratory?

Responses are presented in Figure 5A.

Figure 5A. Patient groups routinely tested for *Legionella pneumophila*



11. Did you outsource any tests used in this distribution to another laboratory?

Most laboratories (n = 10) did not outsource any tests to other laboratories. Of the two that did, outsourced tests included urinary antigen testing and sequencing.

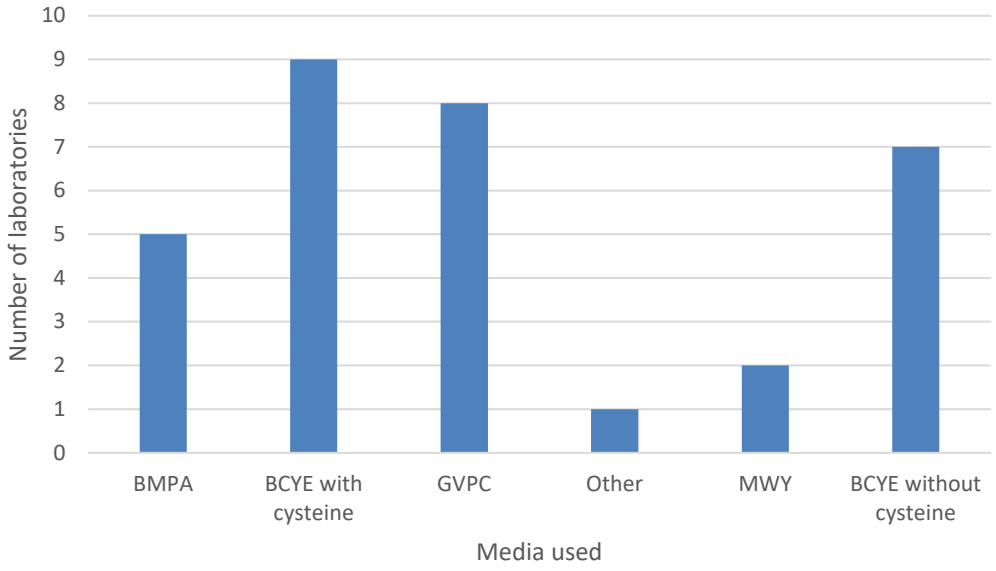
12. What, if any, other methods/elements other than your routine protocol did you apply to this EQA?

No laboratories reported that any additional methods/elements were performed to process the EQA specimens.

13. What media do you use in your laboratory?

Responses are presented in Figure 6A.

Figure 6A. Media used to isolate *Legionella pneumophila*



BCYE: buffered charcoal yeast extract agar; GVPC: glycine vancomycin polymyxin B cycloheximide; MWY: Modified Wadowsky Yee; BMPA: Legionella *BMP selective media with activated charcoal, yeast extract and ACES buffer.*
The media used were reported to be in-house (n = 2) and commercial (n = 7). Three laboratories noted using a combination of commercial and in-house media.

14. Please indicate days plates are checked or read on?

One laboratory stated that they only read plates on day 2. The majority of laboratories read plates from day 2 or 3 and then continuously, either every day or every other day from this point.

15. How long do you incubate your culture plates for?

Incubation ranged from 3–5 days (n = 1), 7–10 days (n = 3), 10 days (n = 7) and 11–14 days (n = 1).

16. At what temperature do you incubate your culture plates for?

All laboratories stated that they incubate between 35–37ºC.

17. At what atmosphere do you incubate your culture plates?

Four participants incubated between 2–5% CO_2, one >5% CO_2, five in O_2, and one stated that they used a different atmosphere, which was not specified.

18. Do you use a moist chamber when culturing specimens for *Legionella*?

Most (n = 11) laboratories stated that they incubated in a moist chamber. One participant stated that they didn't use a moist chamber.

19. If your laboratory performs urinary antigen testing, please state the kit and manufacturer used.

Responses are presented in Table 2A.

Table 2A. Urinary antigen testing kits used

URINARY ANTIGEN TESTING KITS	NUMBER OF LABORATORIES
BinaxNOW Abbott	4
Alere Binax	1
Ridascreen *Legionella* ELISA	1
Bartels ELISA *Legionella* Urinary Antigen	1
Sofia *Legionella* FIA, quidel corporation	3

20. If you use multiplex PCR, which other respiratory pathogens does the assay include?

Responses are presented in Table 3A.

Table 3A. Additional respiratory targets in multiplex PCRs

ADDITIONAL RESPIRATORY TARGETS IN MULTIPLEX PCR	NUMBER OF LABORATORIES
Chlamydophila pneumoniae, Chlamydia pneumoniae	2
Bordetella pertussis/parapertussis	3
Streptococcus pneumoniae	3
Haemophilus influenzae	2
Mycoplasma pneumoniae	3
Chlamydia psittaci	2
Viruses: adenovirus, coronavirus, metapneumovirus, rhinovirus, enterovirus, parainfluenzae, respiratory syncytial virus	1

21. If you use PCR methods, please state the kit and manufacturer used.

Responses are presented in Table 4A.

Table 4A. PCR method kits used

PCR METHODS KITS	NUMBER OF LABORATORIES
Seegene respiratory panel 4, Allplex	2
GeneProof	1
Qiagen QiaStat	1
Biofire	1

22. If you use PCR methods, please state the extraction platform.

Responses are presented in Table 5A.

Table 5A. Extraction platform used for PCR methods

EXTRACTION PLATFORM	NUMBER OF LABORATORIES
EMag	2
Applied Biosystems MagMax	1
GeneAll Ribospin	1
Qiacube	2
NucliSENS	1
MagnaPure	2
Biofire	1

23. If you use PCR methods, please state the amplification platform.

Responses are presented in Table 6A.

Table 6A. Amplification platform used for PCR methods

AMPLIFICATION PLATFORM	NUMBER OF LABORATORIES
Light cycler	2
CFX-96	4
LC480	1
Rotogene	1
T100	1
Biofire	1

24. Do you use an internal quality control (IQC) when performing molecular methods?

Of the nine laboratories that responded to this question, all stated that they used IQC.

25. Which *Legionella pneumophila* serogroups do you have serology tests for?

Responses are presented in Table 7A.

Table 7A. Serology test availability, by *Legionella pneumophila* serogroups

SEROGROUPS	NUMBER OF LABORATORIES
1–4	1
1–6	1
1–7	3
1–14	5
1–15	2

26. Do you perform whole genome sequencing (WGS)?

Five laboratories stated the use of WGS. Three said WGS was used for *Legionella pneumophila* only and two for *Legionella* spp.

27. Which bioinformatic analysis is performed for *Legionella pneumophila*?

Three laboratories stated that they use SNP analysis, two stated cgMLST and one stated 'other' as SBT (legsta, el_gato, ONTmompS).

28. Which number of genes are used for cgMLST?

Two laboratories used 1 521 genes (Moran-Gilad et al., 2012), one used 1 896 genes (Qin et al., 2016) and one used 'other'.

29. Please specify if you use any of the following platforms in your laboratory

Responding laboratories indicated that they use the following: Sanger (1 laboratory), Illumina (4 laboratories), IonTorrent (1 laboratory), Nanopore (1 laboratory) and 'Other, please specify' (zero laboratories).

Findings for environmental samples

Twelve of the 24 (50%) participating environmental laboratories provided information on their methods/processes. Responses were received from: Belgium, Bulgaria, Cyprus, Czechia, Estonia, Finland, Greece, Ireland, Italy, Liechtenstein, Poland and Sweden.

The total numbers will not always correspond to 12, as some respondents did not respond to every question and some questions allowed for more than one option to be selected.

Overview of survey responses

1. Are you a national reference laboratory for environmental and water samples?

From 12 responses, six laboratories stated that they were a national reference laboratory and six that they were not. Of the six that were not a national reference laboratory, five stated that they had access to one if required.

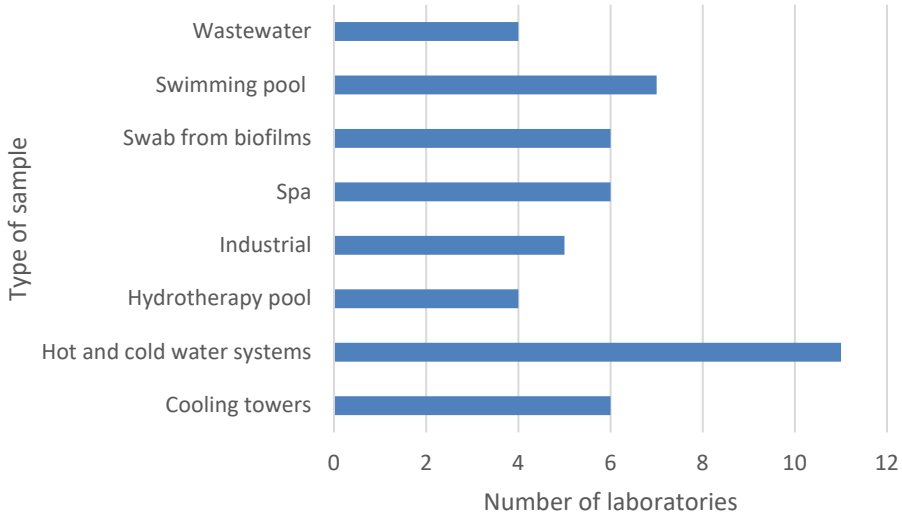
2. Does your laboratory participate in a national EQA scheme for the detection of *Legionella* species?

Of 12 responses, seven stated that they did not take part in national EQA schemes. Of the five that did take part, one stated it was mandatory to do so and two stated it was voluntary.

3. Which type of sample does your laboratory receive for *Legionella* investigations?

Eleven responses were received and are presented in Figure 7A.

Figure 7A. Types of samples received for *Legionella* investigations



4. Approximately how many *Legionella* spp. tests for culture isolation, identification and PCR are undertaken in your laboratory each year?

The number of samples cultured for isolation and identification varied, with three laboratories reporting 10–99, four reporting 100–499, three reporting 500–1 000, one reporting >1 000 and one reporting none (n = 12).

5. Which published method does your laboratory follow for analysing water and swab samples?

Ten laboratories followed the method in ISO 11731:2017 for both water and swab samples. One laboratory only examined water samples and used ISO 11731:2017 (n = 11).

6. Do you perform molecular work direct from the sample?

Four laboratories indicated that they examine samples using molecular methods. Two laboratories reported that they performed between 10–99 tests per year and two reported that they performed 100–499 tests per year.

7. Did you outsource any tests used in this distribution to another laboratory?

Two laboratories outsourced serotyping and sequence typing to another laboratory.

8. Approximately how many *Legionella* spp. tests for serogrouping and sequence typing are undertaken in your laboratory each year?

The number of tests for serogrouping done in each laboratory varied from 0–499 (n = 6). Four laboratories indicated that they examine samples using Sanger sequencing and three laboratories used WGS methods.

9. What volume was used to examine the water sample for filtration method?

Five laboratories analyse 1 L of a sample, one laboratory analyses 500 mL, one laboratory analyses 200 mL, three laboratories analyses 100 mL and one laboratory analyses 50 mL of a sample twice (n = 11).

10. Did you have any difference in protocol for processing routine samples or outbreak samples in the EQA distribution?

Two laboratories indicated that they process routine samples differently than outbreak samples (n = 12). One laboratory stated that the difference was that 100 mL of sample is cultured on GVPC and BCYE.

11. When a *Legionella* species has been isolated, how many picks of the colonies do you take for confirmatory testing?

The number of colonies selected to do confirmation tests on varied from 1–5 (n = 11), with five colony picks being the most common.

12. Does your laboratory check colonies for fluorescence as a primary confirmation test?

Eight laboratories do a fluorescence check on colonies and three do not (n = 11). The reason for not doing a check was mainly due to the method not being available.

13. What media do you use in your laboratory?

The most common media used was BCYE without cysteine (BCYE with antibiotics: buffered charcoal yeast extract agar with selective supplements) (n = 12).

14. Do you use in-house or commercially prepared media?

Media was commercially bought by seven of the laboratories. Two used media made in-house and three used a combination of commercial and in-house media (n = 12).

15. How long do you incubate your culture plates for and is a moist chamber used?

Culture plates were incubated between 7–14 days (n = 12), of which eight laboratories used a moist chamber.

16. Please indicate when plates are checked and read?

Plates were read from day 3 onwards, up to day 14. The frequency of reading the plates varied from daily after 3 days to two or three times during the examination.

17. At what atmosphere do you incubate your culture plates?

The culture plates were incubated aerobically by six laboratories, 2–5% CO_2 by three laboratories, and >5% CO_2 conditions by one laboratory (n = 10).

18. At what temperature do you incubate your culture plates?

Nine laboratories incubated their culture plates at 35–37°C and one laboratory at 28°C (n = 10).

19. Do you perform direct/heat/acid/untreated testing of the samples? If acid is used, please indicate when this is applied.

Of the 10 responses received, a combination of testing may be performed using a neat, heat and acid treatment, this is shown in table 8A

Table 8A. At what stage of the processing is acid applied?

Treatment	When and where the acid treatment is applied	Number of laboratories
Untreated, heat and acid	On concentrated sample	4
Untreated and acid	Directly on membrane	2
Acid only	Directly on membrane	1
	After centrifugation, on concentrated sample and directly on membrane	1
	After filtration and release of bacteria from membrane	2

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