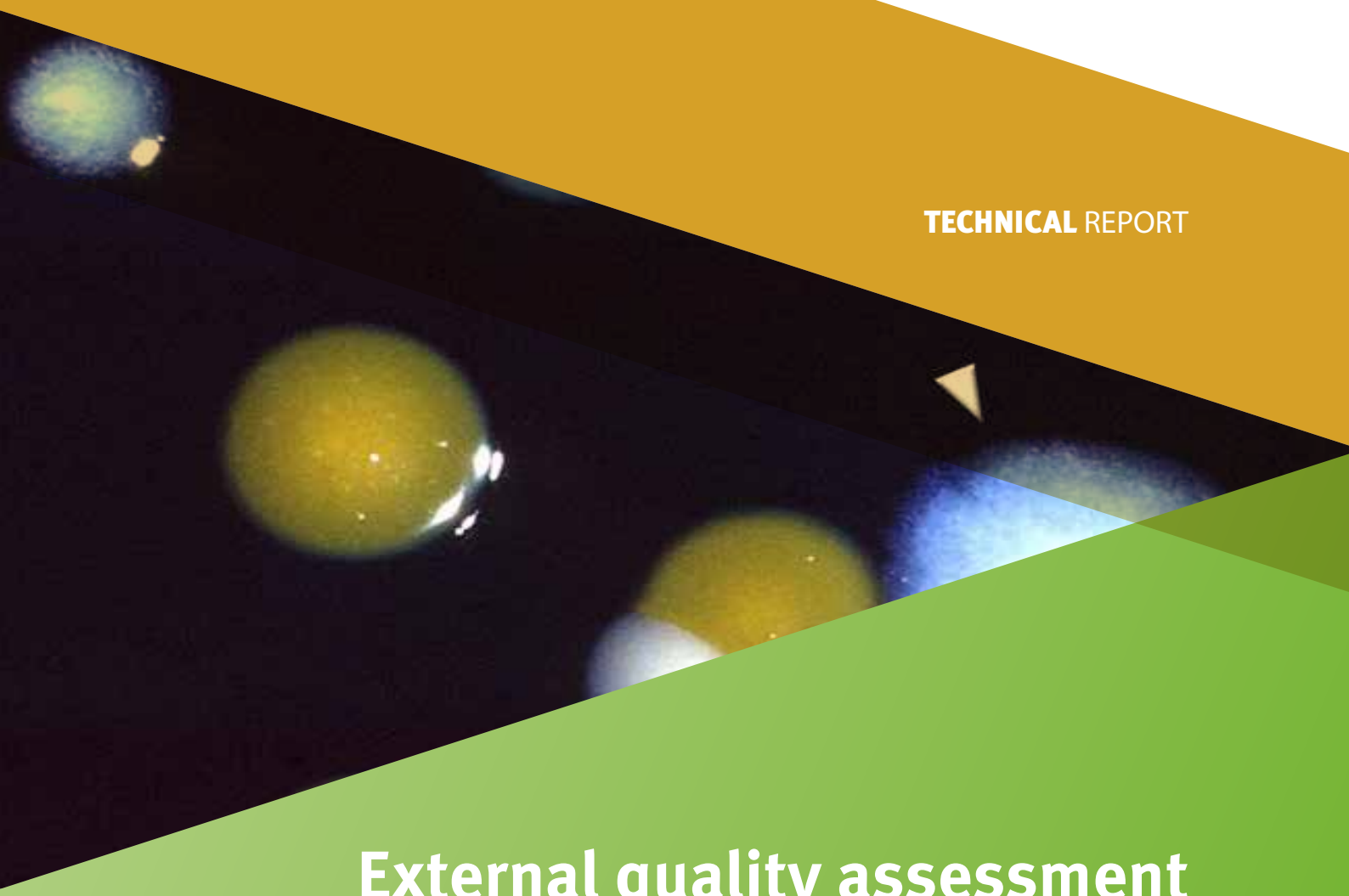

TECHNICAL REPORT

External quality assessment schemes to support European Surveillance of Legionnaires' disease in EU/EEA countries, 2021-2022

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ECDC TECHNICAL REPORT

External quality assessment schemes to support European surveillance of Legionnaires' disease in EU/EEA countries, 2021-2022

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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 (Public Health England)
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
RT-PCR	Real-time polymerase chain reaction
RVPBRU	Respiratory and Vaccine Preventable Bacteria Reference Unit, Public Health England
Sg	Serogroup
SBT	Sequence Base Typing
SNP	Single Nucleotide Polymorphism
ST	Sequence Type
TALD	Travel-associated Legionnaires' disease
UAT	Urinary antigen
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* spp.

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

Two rounds of EQA were completed during 2021–2022, with distributions sent on 15 November 2021 and 4 April 2022. The purpose of the two 2021–2022 EQA exercises 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 participants' results for the 2021-2022 EQA exercises for the European Union/European Economic area (EU/EEA) countries.

For each round, up to two nominated laboratories per EU/EEA country were permitted to participate (to cover clinical and/or environmental testing).

The rounds comprised of distributions 5159 (clinical) and 5160 (environmental) in November 2021 and distributions 5370 (clinical) and 5371 (environmental) in April 2022. A total of 28 EU/EEA countries were invited to take part in these EQA distributions via their national focal points (NFP) for Legionnaires' disease, with a maximum of two laboratories per country proposed based on their involvement in the management of public health incidents associated with *Legionella* in their country.

Each distribution comprised a total of 20 simulated samples: 10 representing clinical material and 10 representing environmental samples. Strains of *Legionella* were provided by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) and these strains were fully characterised using conventional and molecular methods.

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

November's distribution represented an outbreak associated with a hotel. The outbreak strain of *Legionella pneumophila* Sg 1, ST2110 used was isolated from patients and a hotel cooling tower. This sequence type of *L. pneumophila* is rare and has only been identified three times since 2015 in the UK. It has the following allelic profile: 6,10,2,10,13,4,9 and is a single locus variant of ST501, ST969, ST1608 and ST2681.

April's distribution represented an outbreak associated with a chemical plant. The outbreak strain of *Legionella pneumophila* Sg 1, ST2454 was isolated from clinical specimens and environmental samples. This sequence type of *L. pneumophila* is rare and has only been identified six times since 2017 in the UK. It has the following allelic profile: 6,10,2,10,13,4,9 and is a single locus variant of ST1850, ST1608, and ST2681.

This annual report is split into two sections, each covering the two distributions sent. It also includes results from a survey undertaken with all participating laboratories on methods/kit information and frequency of testing performed for each method/kit by the laboratories.

For the clinical element, 25 laboratories returned a result for the November 2021 distribution and 23 laboratories for the April 2022 distribution.

For the environmental element, 20 of the laboratories examined either the water and swab samples in November's distribution and 20 laboratories for either sample types in April's distribution.

Where results reported were not in accordance with the intended exercise, laboratories were advised by the organisers to investigate to determine the root cause.

The November 2021 distribution was associated with an outbreak in a hotel. The outbreak strain *L. pneumophila* Sg1 ST2110, was present in patients one, two, and three. Patient two also contained a non-*L. pneumophila* which was added to determine laboratories' capacity to correctly identify a non-*pneumophila* Legionellae dual pathogen and the reporting procedures i.e. would laboratories report to species level or as a non-*Legionella pneumophila*? Patient four and patient six both contained *L. pneumophila* not associated with the outbreak (Sg1 ST2681 and Sg3 ST728). Patient five contained clinical details associated with Legionnaires' disease but was negative for *Legionella* urinary antigen.

The April 2022 distribution was associated with an outbreak related to a chemical plant. The outbreak strain was *L. pneumophila* Sg1 ST2454, which was present in patients two, three and four. As per the November distribution, patient two contained a second pathogen, a non- *L. pneumophila*. Patient one was negative for *Legionella* by culture, however tested positive by urinary antigen. Patient five was positive for *Legionella* urinary antigen and patient six contained a *L. pneumophila* Sg3 ST 2630.

Similar findings were obtained in both distributions, and a lower concordance was achieved when sputum specimens contained more than one strain of *Legionella*.

Overall, concordance was very good (97%) across both clinical distributions for correct identification of *L. pneumophila.* However, concordance with intended results was reduced when a specimen contained more than one strain of *Legionella*. Concordance was excellent (99.4%) for both distributions for reporting the serogroup, with most laboratories performing serogrouping. In both distributions, a reduced number of laboratories reported ST results. For the November distribution (5159), a total of 13/15 laboratories reported ST for patient two, followed by 12/14 for patient four and 12/13 for patient six. For the April distribution (5370) between 10 and 14 laboratories out of a total of 21 who examined the sputum specimens reported an ST result.

In total, 23 environmental laboratories were sent this EQA, 23 for distribution in November and 20 laboratories returned results. For April's distribution 22 laboratories were sent the EQA, of which 20 returned a result. The environmental laboratories represented a total of 23 EU/EEA countries through participation in either of the two distributions.

For November's distribution 5160, 20 environmental laboratories reported a result for isolation and identification, up to 20 reported a serogroup, up to 20 reported an enumeration count and up to 11 a ST. For molecular methods, up to 11 laboratories analysed the samples for *L. pneumophila* and up to 11 for *Legionella* spp. The overall isolation performance for culture was very good, with 98.6% over the eight samples (two were excluded), with 20 laboratories reporting results. Performance with reporting a correct identification, i.e. a *Legionella* spp. present in the sample, was also high at 91.5%.

For April's distribution 5371, 20 laboratories reported a result for isolation and identification, up to 19 reported a serogroup, up to 19 reported an enumeration count and up to eight a ST. For molecular methods, up to 11 laboratories analysed the samples for *L. pneumophila* and up to 12 laboratories for *Legionella* spp. The overall isolation performance for culture was very good with 89.2% over the 10 samples, with 20 laboratories reporting a result. Performance with reporting a correct identification, when a *Legionella* spp. was in the sample, was also high at 92.4%.

One laboratory reported an incorrect isolation result 4/10 times for distribution 5160 and 3/10 times for distribution 5371. Another laboratory reported an incorrect isolation result once in distribution 5160, but for distribution 5371 they reported an incorrect isolation result on seven occasions.

Background organisms were included that were relevant to the sample type in order 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.

The overall performance for molecular methods was not calculated due to low numbers of laboratories analysing the sample by this method.

Based on the laboratory methods survey among participating laboratories to the environmental EQA samples, routine application of molecular methods for water and environmental samples is not fully implemented. The findings from this EQA exercise suggest that culture remains the preferred method.

In conclusion, for the 2021-2022 scheme, laboratories have demonstrated that they can undertake testing to an acceptable level of at least 80% concordance with intended results and this is relevant for both clinical and environmental laboratories. These data provide a limited assurance of EU/EEA laboratories' ability to undertake effective public health investigations for *L. pneumophila*. Further EQA rounds will provide more data on performance and the robustness of testing.

Overall, the performance of laboratories participating in the 2021-2022 EQA was very good. There were no significant issues arising for species identification, serogroup, enumeration, or sequence type.

The performance of 26 laboratories participating from the 28 EU/EEA countries in these exercises was very good for culture-based/detection methods used by both the clinical (95.3%) and environmental laboratories (92.0%).

From the data collated in this EQA, it was ascertained that for clinical specimens the majority of laboratories identified the pathogen and serogroup, but a significant number did not report the ST (Tables 6 and 7). It was also noted that the number of participants reporting isolation and identification of the non-*Legionella pneumophila* when present as a dual pathogen in the distributions was reduced. For environmental samples, the ISO 11731:2017 requires that suspect colonies are identified to at least *L. pneumophila*. Laboratories reported *L. pneumophila* correctly and went further, reporting a serogroup.

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 man-made 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 1 is the most common cause of outbreaks. The strains of serogroup 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 serogroup 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 two reasons:

- it is underdiagnosed by clinicians, who may not test patients for Legionnaires' disease before empirically prescribing antibiotics likely to cover *Legionella* spp.;
- some health 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), coordinated by ECDC. ECDC also coordinates the collation of annual surveillance data on Legionnaires' disease in the EU/EEA with Member States. The resulting surveillance data are available through the European Surveillance Atlas on ECDC's website. A second ELDSNet surveillance system focuses on Travel-Associated Legionnaires' disease (TALD) cases.

The aim of ELDSNet 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 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].

EQA benefits

The importance 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 water samples for presence and detection of *Legionella* spp.

The benefits of participating in this EQA are:

- to provide laboratories with an insight into their performance;
- to help improve local standards;
- to reveal unsuspected areas of difficulty;
- to provide an educational stimulus for improvement;
- to check the efficacy of internal quality control procedures;
- to demonstrate a commitment to quality to colleagues and customers;
- to provide a method performance evaluation;
- to provide independent evidence of performance for accreditation bodies; and
- to enable the participants 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 2021–2022

The purpose of the EQA exercises was to determine the accuracy of *Legionella* testing and results reported by individual laboratories, to allow comparison of results between laboratories and within countries across Europe. This report presents an analysis of participants' results for the 2021-2022 EQA exercises in EU/EEA countries. The results provided ECDC with information on the laboratories' capabilities to accurately perform *Legionella* testing. This helped to provide confidence in data submitted for surveillance; 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 2021–2022 EQAs were:

- to continue understanding the baseline level of testing undertaken in laboratories in response to routine outbreak scenarios, for both clinical and environmental samples;
- to continue assessing if there were any general performance issues over the two EQAs sent to determine specific issues relating to the different species, levels and background organisms included;
- to provide individual technical support to laboratories as a follow up to the exercises, if requested by the countries.

2. Study design and methods

Organisation of the EQAs

The two EQAs were organised by FEPTU and UK NEQAS for Microbiology in collaboration RVPBRU, 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 (NFP) for Legionnaires' disease within ELDSNet and up to two nominated laboratories per EU/EEA country (to cover clinical and/or environmental samples) could participate per round. Two rounds were dispatched as part of this contract.

The laboratories chosen were those involved in the management of public health incidents in their country and/or undertaking expert reference testing for specialised examinations. A unique laboratory identification was created and username and passwords generated for each one. This allowed the laboratory to return results and view individual reports through a secure web portal. Anonymised results are also provided to ECDC.

Both FEPTU and UK NEQAS 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 EQA scheme:

- The EQA distributions were sent on 15 November 2021 and 4 April 2022 to two laboratories per country.
- Prior to each exercise, ECDC National Focal Points for Legionnaires' disease were asked to propose up to two laboratories per country to take part in the EQA exercise; 28 EU/EEA countries were contacted. One laboratory that undertakes clinical examination of specimens and one that examines environmental samples was required. One laboratory could also be nominated to participate in both clinical and environmental examination, if they usually processed both types of samples. Participating laboratories needed to be contributing to national surveillance data or environmental findings that are shared through ELDSNet surveillance activities.
- The EQA organiser sent a letter of invitation to the nominated laboratories informing them of the EQA arrangements 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.
- Each distribution comprised a total of 20 simulated samples: 10 representing clinical material and 10 representing environmental samples. Sample/specimen design and format was agreed in advance with ECDC and UKHSA *Legionella* experts.
- UKHSA undertook testing of the samples/specimens in accordance with published methods, to replicate where possible testing methods that would be used by the participants. Detection, identification, enumeration, confirmation and further characterisation tests (sero-grouping) (Sg) and sequence-based typing (SBT)) were also undertaken.
- UKHSA also ran a separate survey on methods/kit information and frequency of testing performed for each method/kit by the laboratories.
- November's distribution simulated an outbreak associated with a hotel. The outbreak *Legionella pneumophila* strain chosen was Sg 1 and ST2110.
- April's distribution simulated an outbreak associated with a chemical plant. The outbreak *Legionella pneumophila* strain chosen was Sg 1 and ST2454.

In November 2021, a total of 23 environmental laboratories from 23 EU/EEA countries were sent this distribution, of which 20 returned a result and three did not examine any samples. For clinical samples, 26 clinical laboratories from 26 EU/EEA countries took part, with 25 returning a result (Table 1).

In the April 2022 EQA, a total of 22 environmental laboratories from 22 EU/EEA countries were sent a sample, of which 20 returned a result, one laboratory did not examine any of the samples and one did not return a result. For clinical samples, 24 laboratories from 26 participating EU/EEA countries took part (Table 1).

Each laboratory was provided with a unique laboratory identification. Of those taking part, 19/28 participating countries tested both the clinical specimens and environmental samples in the November distribution, and 20/26 in the April distribution. Hungary and Denmark only took part in November's distribution for environmental samples.

17/23 laboratories from a total of 23 countries took part in both the environmental distributions as part of this contract year 2021-2022.

25/28 laboratories from a total of 28 countries took part in both distributions for the clinical distributions under 2021-2022.

Table 1. Countries within the EU/EEA that participated in the clinical and environmental EQA

	November 2021 distribution			April 2022 distribution		
Country	Clinical EQA samples – 5159	Environmental EQA samples – 5160	Number of participating laboratories per country	Clinical EQA samples – 5370	Environmental EQA samples – 5371	Number of participating laboratories per country
Austria	Yes	-	1	Yes	-	1
Belgium	Yes	Not examined	1	Not examined	Not examined	0
Bulgaria	Yes	Yes	2	Yes	Yes	2
Croatia	Yes	Yes	2	Yes	Yes	2
Cyprus	Yes	-	1	Yes	Yes	2
Czechia	Yes	Yes	2	Yes	Yes	2
Denmark	Yes	Yes	2	-	-	0
Estonia	Yes	Yes	2	Yes	Yes	2
Finland	Yes	Yes	2	Yes	Yes	2
France	Yes	Yes	2	Yes	Yes	2
Germany	Yes	Yes	2	Not examined	Not examined	1
Greece	Yes	Yes	2	Yes	Yes	2
Hungary	Yes	Yes	2	-	-	0
Iceland	Yes	Not examined	1	Yes	Yes	2
Ireland	Yes	Yes	2	Yes	Yes	2
Italy	Yes	Yes	2	Yes	Yes	2
Latvia	Yes	Yes	2	Yes	Yes	2
Lithuania	Yes	-	1	Yes	-	1
Malta	Yes	-	1	Not examined	-	0
Netherlands	Yes	Yes	2	Yes	Yes	2
Norway	Yes	Yes	2	Yes	Yes	2
Poland	-	Yes	1	Yes	Yes	2
Portugal	Yes	Yes	2	Yes	Yes	2
Romania	Not examined	Not examined	0	Yes	Yes	2
Slovak Republic	Yes	Yes	2	Yes	Yes	2
Slovenia	Yes	Yes	2	Yes	Yes	2
Spain	Yes	-	1	Yes	-	1
Sweden	Yes	Yes	2	Yes	Yes	2

The panel of EQA samples/specimens were dispatched within approved United Nations containers, including an EQA protocol. This protocol contained information on the sample/specimen details, instructions on how to process samples/specimens' safety data information, and instructions on how to enter the results online. The information was also available electronically to all participants and 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 describing the process for specimen examination via the web page. Detailed instructions were included on how to access the secure website via a unique user identification and password provided for each participant. The deadline for final submission of results was stated on the paperwork detailing the sample/specimen information. For convenience, a copy of the web reply form was available for laboratories to download to facilitate the manual recording of test results prior to submission online. Laboratories were allowed six weeks (42 days) from the date of dispatch of both clinical specimens and environmental samples to examine the EQA specimens/samples and return all their results. The length of time allowed for this exercise was due 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 take to provide a result for specialist tests, such as SBT.

Six weeks after dispatch dates, the web platform was closed for results submission and the intended results were published on the UK NEQAS secure website. Laboratories were notified by email that the intended results were available for viewing. Individual reports were made available in March 2022 for November 2021 distribution and in July 2022 for the April 2022 distribution.

After the close of April's distribution, an additional method questionnaire was sent electronically to the laboratories that took part in both distributions. A summary of the findings is found in Annex 1.

Between 28 June and 31 July 2021, ECDC conducted a short online survey to obtain feedback on the EQA exercise and enable the laboratories to suggest improvement for the next distribution. A summary of this feedback is available on request.

Certificates of participation were sent electronically to the laboratories on 24 January 2022 for November's distribution and 30 June 2022 for April's distribution. A hard copy of the certificate was available on request.

EQA exercise scenario and sample design

The strains selected for both exercises were chosen in consultation with UKHSA *Legionella* experts in clinical and environmental microbiology. Sample/specimen design was developed in collaboration with the UKHSA UK NEQAS and ECDC experts and approved by ECDC.

All packages with samples were dispatched at ambient temperature, in accordance with the latest International Air Transport Association regulations, using an approved airfreight company.

The individual laboratory EQA reports detailed a laboratory's reported results for each examination requested and the microbiological contents for each sample/specimen. This included the identification of the *Legionella* species, Sg, ST and enumeration results, where applicable. The report also provided an overall performance for each examination based on all the laboratories reported results.

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.

15 November 2021 distribution

Five environmental samples were supplied to represent an outbreak associated with a cooling tower. Samples provided were water from a cooling tower in a hotel, tap within a shower, hot water system, water from spa pool balance tank and a swab from a biofilm of pipework of wash hand basin. In addition, five routine monitoring samples were supplied: swabs from a water outlet, water samples from hot and cold water systems, from a car screen wash, from a hospital hot water storage tank and a cooling tower.

Simulated clinical samples were taken from six patients with suspected symptoms of Legionnaires' disease (sputum and or urine specimens).

The outbreak strain of *L. pneumophila* Sg 1, ST2110 used in this EQA exercise has been isolated from patients. This strain is rare and has only been identified three times in the UK since 2015.

Other strains included in these distributions were:

- *L. pneumophila* Sg 1, ST109, this sequence type of *L. pneumophila* has been isolated from patients and domestic water systems and isolated from the environment in several countries across Europe and Canada [3].
- *L. pneumophila* Sg 6, ST2923, this sequence type of *L. pneumophila* is unique and has only been identified once from an environmental sample in UK.
- *L. pneumophila* Sg 1, ST1, this sequence type of *L. pneumophila* is the most common ST with 1,742 of 14,342 entries in the UKHSA sequence based typing (SBT) database. This sequence type of *Legionella pneumophila* has been isolated from clinical and environmental samples and associated with community acquired, travel associated and nosocomial outbreak investigations.
- *Legionella anisa,* this species was first isolated from water during a nosocomial outbreak in the United States between March 1980 and June 1981 [4]. *L. anisa* is one of the most frequent species of *Legionella* other than *Legionella pneumophila* in the environment, and may be hospital acquired in rare cases [5]. A distinguishing characteristic is the ability of colonies to exhibit blue-white autofluorescence when viewed under ultraviolet light. Thus, *L. anisa*, along with several other species of *Legionella*, is sometimes referred to as 'blue-white' *Legionella*.
- *Legionella bozemanii* was first isolated from lung tissue in 1959. The isolate came from a healthy scuba diver in Florida with fatal bronchopneumonia [6]. This species consists of two serogroups, both of which have been reported to cause LD [7,8]. A distinguishing characteristic of this species is the ability of colonies to exhibit blue-white autofluorescence when viewed under ultraviolet light.
- *Legionella micdadei w*as first formally proposed as a new *Legionella* species by Hébert *et al.* in 1980 and the strain is associated with human cases of pneumonia when originally isolated, and *L. micdadei* has since been reported as the aetiological agent of multiple cases of legionellosis including severe pneumonia. *L. micdadei* does not exhibit autofluorescence when viewed under ultraviolet light [9].
- *Legionella longbeachae* was included for the clinical distribution, the most common *Legionella* species after *L. pneumophila* to be isolated from humans. *L. longbeachae* was first isolated in 1982 from a patient with pneumonia in Long Beach, California, United States. There are two serogroups of *L. longbeachae*. This species has been associated with horticultural growth medium and is found in patients that undertake gardening and has been detected in tree bark [10].

Samples/specimens were prepared and quality-controlled by the EQA organisers and the panels were dispatched as distributions 5159 (clinical) and 5160 (environmental).

4 April 2022 distribution

Five environmental samples were supplied to represent an outbreak associated with a chemical plant. These include water samples provided from a cooling tower return, from a disabled toilet hand wash basin, from a wash hand basin cold outlet in the ladies WC and from the shower emergency decontamination unit, one swab from the showerhead in the emergency decontamination unit was included. In addition, five routine monitoring water samples were supplied; these were from a spa pool balance tank, ladies changing room wash hand basin, one from the cold tap and one from the hot tap, and from the ladies and men's changing room showers.

Clinical specimens were taken from six patients with suspected symptoms indicating Legionnaires' disease (sputum, BAL and or urine samples).

The outbreak strain of *L. pneumophila* Sg 1, ST2454 used for this EQA exercise has been isolated from clinical and environmental samples. This strain is rare and has only been identified six times in the UK since 2017.

Other strains included in these distributions samples were:

- *L. pneumophila* Serogroup 1, ST1, this sequence type of *L. pneumophila* is the most common ST with 1,742 of 14,342 entries in the sequence based typing (SBT) database. This sequence type of *L. pneumophila* has been isolated from clinical and environmental samples and associated with community-acquired, travel-associated and nosocomial outbreak investigations.
- *L. pneumophila* Serogroup 3, sequence Type 2630, this sequence type of *L. pneumophila* has only been identified once from a clinical sample, in 2018 in the UK.
- *L. pneumophila* Serogroup 1 ST20, this sequence type of *L. pneumophila* has been isolated from many countries such as Austria, Belgium, Bulgaria, Czech Republic, France, Germany, Italy, Japan, Portugal, Spain, Switzerland, and the UK. This sequence type of *L. pneumophila* has been isolated from clinical and environmental samples.
- *L. pneumophila* Serogroup 8, ST1324, although serogroup 8 is the most common serogroup associated with this ST, other serogroups (Sg3, Sg4 and Sg5) have also been identified as ST1324. This sequence type of *L. pneumophila* has been isolated from clinical and environmental samples since 2006.
- *Legionella rubrilucens* was first isolated from tap water in California by G. W. Gorman in 1980 and formally described by Brenner *et al*. in 1985. This species is a red auto-fluorescing, gram-negative, motile rod that requires L-cysteine for growth on BCYE agar at 36± 1°C in 2.5% CO_2 [11].
- *Legionella moravica* was first isolated from water obtained from an air conditioning cooling tower in Czechia [12]. To date, no clinical cases of legionellosis attributed to this species have been reported in humans. *L. moravica* is a non-autofluorescing, gram-negative rod which is positive for beta-lactamase and catalase, and weakly oxidase positive and grows on BCYE agar at 35°C.
- *Legionella dumoffii* was first described in 1980 following the isolation of atypical *Legionella*-like organisms (ALLO): NY 23^T, from a water cooling tower, and Tex-KL, isolated from the lung of a deceased patient in New York. *L. dumoffii* has been reported to have caused several outbreaks and community-acquired cases of Legionnaires' disease [13].

Samples/specimens were prepared and quality-controlled by the EQA organisers and the panels were dispatched as distributions 5370 (clinical) and 5371 (environmental).

Clinical

Ten clinical specimens were prepared in each set (Four simulated sputum, one BAL and five liquid urine specimens). An overview of specimens is provided in Tables 2 and 4.

Participants were requested to provide an organism identification, serogroup and sequence type (simulated sputum samples) and *L. pneumophila* urinary antigen (LUA result) (urine specimens). Simulated sputum and BAL specimens were prepared in a lyophilised format. The freeze-dried sample matrix was composed of inositol serum broth with variable concentrations of the pathogen *L. pneumophila* or other species. To simulate the specimen to resemble an authentic clinical material, the freeze-dried vials contained a strain of the pathogen and included commensal flora commonly isolated from lower respiratory tract infections. The serogroup and species of *Legionella* to be used were approved by the commissioned experts at UKHSA. Participants' results were analysed and considered 'concordant' if the reported categorisation agreed with the UKHSA reference laboratory (RVPBRU) interpretation. In addition, participants were asked to complete a questionnaire survey to provide further information on methods used, both in general and for this EQA exercise.

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

Instructions provided to participants included:

- how to reconstitute lyophilised specimens with 1mL 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;
- information on reporting results (absence or presence of *Legionella pneumophila* or other species).

The simulated sputum samples were examined using the national documents SMI ID18 Identification of *Legionella* species 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 (Medical laboratories - Requirements for quality and competence).

Environmental

An overview of samples provided is shown in Tables 3 and 5.

For each distribution, 10 environmental samples were prepared as LENTICULE® discs. This method of preparing samples 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 $<10^2 – 10^5$ colony-forming units/L.

Background organisms were included that were relevant to the sample type in order 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 a distribution if:

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

Samples were quality-controlled as they would have been by the participant. This step involved rehydration and culturing a neat sample onto Glycine Vancomycin Polymyxin Cycloheximide (GVPC), following heat and acid treatment. Agar plates were incubated for up to 10 days aerobically at 37°C and read on Day 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 this is 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 calculated using robust statistics (5 and 95% percentiles).

Data were displayed graphically. Detected/not detected, the serogroup and 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

The methods questionnaire sent to participants to gather details on processes and methods was analysed as part of these EQA exercises and findings can be found in Annex 1. Key results are integrated into the separate sections below.

Intended results for the 2021–2022 exercise

Sample contents for the specimens included in the clinical and environmental distributions are described in Tables 2-5, including the serogroup and sequence base type when *L. pneumophila* was present.

Table 2. Clinical specimens 7104-7113 provided in distribution 5159 (15 November 2021)

Specimen number	Patient	Specimen	Specimen type	Specimen contents	Sg	ST	Details
7104	1	1	Sputum	*Streptococcus oralis* *Streptococcus mitis*	-	-	Muscle aches and headache for 4 days following recent stay at a hotel
7105		2	Urine	*Legionella pneumophila*	-	-	
7106	2	1	Sputum	*Legionella pneumophila* *Legionella longbeacha,* *Streptococcus mitis Moraxella catarrhalis*	1	2110	Shortness of breath after recent holiday
7107		2	Urine	*Legionella pneumophila*	-	-	
7108	3	1	Sputum	*Streptococcus salivarius* *Streptococcus oralis*	-	-	Persistent cough and chest pains following recent stay at a hotel
7109		2	Urine	*Legionella pneumophila*	-	-	
7110	4	1	Sputum	*Legionella pneumophila* *Streptococcus mitis Moraxella catarrhalis*	1	2681	Confusion and headache following recent stay at a hotel
7111		2	Urine	*Legionella pneumophila*	-	-	
7112	5	5	Urine	Negative	-	-	Confusion and diarrhoea after afternoon tea at a hotel 6 days prior
7113	6	6	Sputum	*Legionella pneumophila*	5	728	Muscle pains and cough for 5 days

Table 3. Environmental samples 7114-7123 provided distribution 5160 (15 November 2021). Levels of *Legionella* spp. in the sample is shown as approximate colony forming units (cfu) per litre

Specimen number	Sample type	Sample contents	Sg	ST	Comments
7114	Water from tap within a shower	*Legionella pneumophila* ($4.1x10^4$) *Acinetobacter junii* *Aerococcus viridans*	1	2110	Samples taken as part of one outbreak investigation.
7115	Swab from biofilm of pipework of wash hand basin	*Acinetobacter junii* *Pseudomonas fluorescens*	-	-	
7116	Water from hot water system of the hotel	*Legionella pneumophila* ($3.8x10^2$) *Legionella micdadei* ($5.0x10^4$) *Acinetobacter junii* *Pseudomonas putida*	1	2110	
7117	Water from cooling tower	*Legionella pneumophila* ($1.8x10^4$) *Citrobacter braakii* *Brevundimonas vesicularis* *Staphylococcus saprophyticus*	1	2110	
7118	Water sampled from spa pool balance tank of the hotel	*Legionella pneumophila* ($1.4x10^4$) *Microbacterium luteolum*	5	728	
7119	Swab from water outlet biofilm	*Legionella pneumophila* ($9.6x10^3$) *Roseomonas aestuarii* *Pseudomonas aeruginosa*	1	109	Samples taken as part of routine quality monitoring of water.
7120	Hospital hot water storage tank	*Legionella bozemanii* ($3.3x10^3$) *Staphylococcus saprophyticus*	-	-	
7121	Water from cooling tower	*Legionella pneumophila* ($8.0x10^3$) *Microbacterium luteolum*	1	1	
7122	Water from car screen wash reservoir	*Legionella anisa* ($3.3x10^2$) *Citrobacter braakii*	-	-	
7123	Water from hot and cold system from a hair salon	*Legionella pneumophila* ($1.0x10^2$) *Klebsiella pneumoniae* *Staphylococcus haemolyticus* *Enterococcus faecium*	6	2923	

Table 4. Clinical specimens 7764-7773 provided in distribution 5370 (4 April 2022)

Specimen number	Patient	Specimen	Specimen type	Specimen contents	Sg	ST	Details
7764	1	1	Sputum	*Streptococcus oralis* *Streptococcus mitis*	-	-	High temperature, headache and muscle pains in a 22-year-old male
7765		2	Urine	*Legionella pneumophila*	-	-	
7766	2	1	Sputum	*Legionella pneumophila* *Legionella dumoffii* *Streptococcus mitis* *Streptococcus oralis*	1	2454	Diarrhoea and confusion in a 47-year-old male working as a cleaner at a chemical plant
7767		2	Urine	*Legionella pneumophila*	-	-	
7768	3	1	Sputum	*Legionella pneumophila* *Moraxella catarrhalis* *Streptococcus mitis*	1	2454	Cough and muscle pains in a 51-year-old male
7769		2	Urine	*Legionella pneumophila*	-	-	
7770	4	1	BAL	*Legionella pneumophila*	1	2454	Community acquired pneumonia in a 45-year-old female who works at a chemical plant
7771		2	Urine	*Legionella pneumophila*	-	-	
7772	5	5	Urine	Negative	-	-	Diarrhoea and headache in a 38-year-old female engineer
7773	6	6	Sputum	*Legionella pneumophila* *Streptococcus mitis* *Streptococcus oralis*	3	2630	Fever and chills in a 27-year-old nurse

Table 5. Environmental samples 7774-7783 provided distribution 5114 (4 April 2022). Levels of *Legionella* spp. in the sample is shown as approximate cfu per litre

Specimen number	Sample type	Sample contents	Sg	ST	Comments
7774	Water from cooling tower return	*Legionella pneumophila* ($6.0x10^3$) *Citrobacter braakii* *Brevundimonas vesicularis*	1	2454	Samples taken as part of one outbreak investigation.
7775	Water from disable toilet hand wash basin	*Legionella pneumophila* ($8.3x10^3$) *Legionella moravica* ($1.1x10^4$) *Acinetobacter junii*	1	2454	
7776	Water from wash hand basin cold outlet in the Ladies WC	*Acinetobacter junii* *Pseudomonas putida*	-	-	
7777	Swab from the showerhead in the emergency decontamination unit	*Legionella pneumophila* ($8.8x10^3$) *Brevundimonas vesicularis* *Staphylococcus saprophyticus*	1	2454	
7778	Water sampled from the shower emergency decontamination unit	*Legionella pneumophila* ($1.1x10^3$) *Microbacterium luteolum* *Acinetobacter junii* *Aerococcus viridans*	3	2630	
7779	Water from a spa pool balance tank	*Legionella pneumophila* ($3.5x10^4$) *Roseomonas aestuarii* *Staphylococcus saprophyticus*	1	20	Samples taken as part of routine quality monitoring of water.
7780	Ladies changing room wash hand basin cold tap	*Pseudomonas aeruginosa* *Microbacterium luteolum*	-	-	
7781	Ladies changing room wash hand basin hot tap	*Legionella pneumophila* ($7.5x10^2$) *Klebsiella pneumoniae* *Acinetobacter junii*	8	1324	
7782	Ladies changing room shower	*Legionella rubrilucens* ($1.9x10^3$) *Citrobacter braakii,* *Pseudomonas fluorescens*	-	-	
7783	Water from hot and cold system from a hair salon	*Legionella pneumophila* ($1.5x10^4$) *Brevundimonas vesicularis* *Staphylococcus haemolyticus* *Enterococcus faecium*	1	1	

Scoring applied to the examinations

All distributions were scored for the main examinations, either with a score of zero if not correct or two if correct. The allocation of scores is a means of drawing attention to differences between a participant's result and what has been designated as the intended result or the 'assigned value'. Scores help laboratories to identify whether there is a problem with their testing.

Clinical scoring applied: 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 applied: 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.
- For reporting an enumeration value within the expected range. This was calculated by using the participants' median was used as the assigned value and then the expected range calculated using 5 and 95 percentiles – however, there were not enough data sets to score this examination.

15 November 2021

Clinical distribution 5159

A total of 25 of participating laboratories from 26 countries reported results for the simulated sputum samples compared with 24 laboratories who reported results for the urine samples.

Participants were only requested to report information on *Legionella* spp. and not report on the background flora included to simulate a specimen.

Table 6. Examinations performed and concordance achieved for distribution 5159

Sample number	Contents	Identification		Serogroup		Sequence type		Urinary antigen		Overall % performance by sample
		N	%	N	%	N	%	N	%	
7104	*Streptococcus oralis Streptococcus mitis*	24/25	-	-	-	-	-	-	-	96
7105	*Legionella pneumophila*	-	-	-	-	-	-	21/24	87.5	87.5
7106	*Legionella pneumophila* Sg 1 ST2110, *Legionella longbeachae Streptococcus mitis Moraxella catarrhalis*	24/25	96	21/21	100	13/15	86.7	-	-	94.2
7107	*Legionella pneumophila*	-	-	-	-	-	-	24/24	100	100
7108	*Streptococcus salivarius Streptococcus oralis*	23/25	92	-	-	-	-	-	-	92
7109	*Legionella pneumophila*	-	-	-	-	-	-	23/24	95.8	95.8
7110	*Legionella pneumophila* Sg 1 ST2681 *Streptococcus mitis Moraxella catarrhalis*	24/25	96	21/21	100	12/14	85.7	-	-	93.9
7111	*Legionella pneumophila*	-	-	-	-	-	-	24/24	100	100
7112	Negative	-	-	-	-	-	-	24/24	100	100
7113	*Legionella pneumophila* Sg5 ST728	24/25	96	21/21	100	12/13	92.3	-	-	96.1
			95		100		88.2		96.7	95.55

Patient 1:
Specimen 7104: Excellent concordance, with intended results with 96% (24/25) of participants correctly reporting a negative result.

Specimen 7105: Satisfactory performance, with 87.5% (21/24) concordance with intended results from participants returning a result. Three laboratories incorrectly reported a negative result for this specimen.

Patient 2:
Specimen 7106: An excellent performance, with 96% (24/25) of participants reporting the correct result for identification, 100% (21/21) with correct serogroup and 86.7% (13/15) with correct ST. Two laboratories reported 'other' and listed ST69 and ST 6-10-2-10-13-4-9.

Only 28% (7/25) laboratories successfully identified and reported *Legionella longbeachae* which was also present within this specimen.

Specimen 7107: The specimen was positive for *L. pneumophila* urinary antigen. An excellent performance, with 100% (24/24) of participants reporting the correct result.

Patient 3:
Specimen 7108: Very good concordance, with intended results with 92% (23/25) of participants correctly reporting a negative result. Of the two participants reporting a positive result, one participant stated *L. pneumophila* present, and the other *Legionella* species.

Specimen 7109: The specimen was positive for *L. pneumophila* urinary antigen. An excellent performance, with 95.8% (23/24) of participants reporting the correct result.

Patient 4:
Specimen 7110: An excellent performance, with 96% (24/25) of participants reporting the correct result for identification, a 100% (21/21) with correct serogroup and 85.7% (12/14) with correct ST. Two laboratories reported 'other' and listed ST70, 6-10-2-10-13-14-9.

Specimen 7111: An excellent performance, with 100% (24/24) of participants reporting the correct positive result.

Patient 5:
Specimen 7112: An excellent performance, with 100% (24/24) of participants reporting the correct negative result.

Patient 6:
Specimen 7113: An excellent performance, with 96% (24/25) of participants reporting the correct result for identification, a 100% (21/21) with correct serogroup and 92.3% (12/13) with correct ST. One laboratory reported 'other' and listed ST73.

Environmental distribution 5160

Ten simulated environmental samples were sent to 23 laboratories in 23 EU/EEA countries. 20 laboratories returned a result and three did not examine the samples.

Sample numbers: 7114-7118 were samples taken as part of one outbreak investigation.

Sample numbers: 7119-7123 were samples were taken as part of routine monitoring.

Sample numbers: 7115 and 7119 were swab samples.

Performance of the laboratories on these samples were split into culture-based methods (Table 7) and molecular methods (Table 8). Culture-based method analysis included results reported for isolation, identification, enumeration, serogroup and ST results. An overall performance assessment column as a percentage has been captured for culture-based method results by sample number and by each examination. Overall performance by sample was calculated using the mean value across a maximum of the five examinations. For molecular methods, the overall performance has not been calculated, as the number of data sets returned for analysis is too low to provide robust performance data.

Table 9 shows in more detail the enumeration results reported by the laboratories. The participants' median is used as the assigned value and the expected range is calculated using 5 and 95 percentiles.

Table 7. Examinations done on cultured samples

Sample number	Contents	Isolation		Identification		Enumeration		Serogroup		Sequence type		Overall % performance by sample
		N	%	N	%	N	%±	N	%	N	%	
7114	*L. pneumophila* sg 1, ST2110	20/20	100	20/20	100	19/20	95.0	20/20	100	10/11	90.9	97.2
7115	No *Legionella*	19/19	100	-	-	-	-	-	-	-	-	100
7116	*L. pneumophila* sg 1, ST2110 and *L. micdadei**	20/20	100	7/20 – both 12/20 – LM only 1/20 – LP only	35.0	17/20	85.0	8/8	100	3/4	75.0	79.0
7117	*L. pneumophila* sg 1, ST2110	18/18	100	18/18	100	17/18	94.4	18/18	100	9/10	90.0	96.9
7118	*L. pneumophila* sg 5, ST728	19/19	100	19/19	100	17/19	89.5	19/19	100	10/11	90.9	96.1
7119	*L. pneumophila* sg 1, ST109	17/18	94.4	17/17	100	-	-	17/17	100	8/8	100	98.6
7120	*L. bozemanii* *	20/20	100	20/20	100	18/20	90.0	-	-	-	-	96.7
7121	*L. pneumophila* sg 1, ST1	17/18	94.4	17/17	100	16/18	88.8	16/17	94.1	9/9	100	95.5
7122	*L. anisa* *	13/18	72.2[T]	13/13	100	11/13	84.6					92.3
7123	*L. pneumophila* sg 6, ST2923	9/20	45.0[T]	8/9	88.9	7/9	77.7	8/8	100	5/5	100	91.7
Overall performance by examination			98.6		91.5		88.1		99.2		92.4	

± If a censored value reported falls within the expected range this has been considered as being correct
** The reporting of Legionella species not pneumophila and Legionella species have been analysed as being correct*

T The reason for the low performance is due to the low levels of Legionella sp. in the samples which maybe below the detection limit for methods used by participants. This sample was not scored and therefore the results obtained with isolation have been excluded from the overall percentage performance by sample and by examination.

Table 8. Molecular methods

Sample number	Identification	Intended results for *Legionella pneumophila*	Molecular results *Legionella pneumophila*	Intended results for *Legionella* spp.	Molecular results *Legionella* spp. *
7114	*L. pneumophila*	Detected	11/11	Detected	4/11
7115	No *Legionella*	Not detected	5/5	Not detected	6/6
7116	*L. pneumophila* and *L. micdadei*	Detected	5/11	Detected	11/11
7117	*L. pneumophila*	Detected	11/11	Detected	4/11
7118	*L. pneumophila*	Detected	11/11	Detected	4/11
7119	*L. pneumophila*	Detected	9/9	Detected	3/10
7120	*L. bozemanii*	Not detected	11/11	Detected	10/11
7121	*L. pneumophila*	Detected	10/10	Detected	4/11
7122	*L. anisa*	Not detected	9/9	Detected	8/10
7123	*L. pneumophila*	Detected	8/11	Detected	3/11

**It is unclear if laboratories are including the reporting of Legionella pneumophila as part of the results for Legionella sp. This was made clearer for April's distribution.*

Table 9. Data on enumeration results where reported

Sample number	Identification	Number of results	Participants median (cfu/L)	Intended range (cfu/L)	Number of counts reported outside the intended range or censored values
7114	*L. pneumophila*	20	$1.5x10^4$	$5.0x10^2 – 3.0x10^4$	1 (1 high)
7116	*L. pneumophila* and *L. micdadei*	20	$4.9x10^4$	$2.0x10^2 – 1.1x10^5$	3 (1 low and 2 high)
7117	*L. pneumophila*	18	$8.5x10^3$	$3.5x10^2 – 2.5x10^4$	1 (1 high)
7118	*L. pneumophila*	19	$3.0x10^3$	$1.0x10^2 – 2.3x10^4$	2 (1 low and 1 high)
7120	*L. bozemanii*	20	$4.6x10^3$	$4.6x10^2 – 3.9x10^4$	2 (1 low and 1 high)
7121	*L. pneumophila*	18	$3.9x10^3$	$7.1x10^2 – 2.5x10^4$	2 (1 low , 1 high)
7122	*L. anisa*	13	$7.1x10^2$	$33 – 8.3x10^3$	2 (1 low and 1 high)
7123	*L. pneumophila*	10	$1.4x10^2$	$9 – 1.0x10^3$	2 (1 low and 1 high)

Sample 7114: Performance was excellent, with 20/20 (100%) of participants reporting the correct isolation result, 20/20 (100%) for identification, 19/20 (95.0%) of the laboratories reporting a count within the intended range, 20/20 (100%) reporting the correct serogroup and 10/11 (90.9%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 97.2%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and only 4/11 reported a correct result.

Sample 7115: Performance was excellent, with 19/19 (100%) of the laboratories reporting the correct isolation result.

Five laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. In addition, six laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and all reported a correct result.

Sample 7116: Performance was excellent, with 20/20 (100%) of participants reporting the correct isolation result, however for the identification only 7/20 (35.0) reported the presence of both *L. pneumophila* and *L. micdadei*, 2/20 (10.0%) laboratories reported a *Legionella* sp. and 11/20 (55.0%) reported only the presence of *L. micdadei*. The eight laboratories that reported the presence of a *L. pneumophila* all reported the correct serogroup and 3/4 reported the correct ST. The overall performance for examinations by culture was 79.0%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 5/11 reported the correct result. In addition, 11 of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and all 11/11 reported a correct result.

Sample 7117: Performance was excellent, with 18/18 (100%) of participants reporting the correct isolation result, 18/18 (100%) for identification, 17/18 (94.4%) of the laboratories reporting a count within the intended range, 18/18 (100%) reporting the correct serogroup and 9/10 (90.0%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 96.9%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and only 4/11 reported a correct result.

Sample 7118: Performance was excellent, with 19/19 (100%) of participants reporting the correct isolation result, 19/19 (100%) for identification, 17/19 (89.5%) of the laboratories reporting a count within the intended range, 19/19 (100%) reporting the correct serogroup and 10/11 (90.9%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 96.1%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and only 4/11 reported a correct result.

Sample 7119: Performance was very good, with 17/18 (94.4%) of participants reporting the correct isolation result, 17/17 (100%) for identification, 17/17 (100%) reporting the correct serogroup and 8/8 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 98.6%.

Nine laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Ten laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and only 3/10 reported a correct result.

Sample 7120: : Performance was excellent, with 20/20 (100%) of participants reporting the correct isolation result, 20/20 (100%) for identification and 18/20 (90.0%) of the laboratories reporting a count within the intended range. The overall performance for examinations by culture was 96.7%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and only 10/11 reported a correct result.

Sample 7121: Performance was very good, with 17/18 (94.4%) of participants reporting the correct isolation result, 17/17 (100%) for identification, 16/18 (88.8%) of the laboratories reporting a count within the intended range, 16/17 (94.1%) reporting the correct serogroup and 9/9 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 95.5%.

Ten laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 10/10 reported the correct result. In addition, 11 of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 4/11 reported a correct result.

Sample 7122: Performance was below average, with 13/18 (72.2%) of participants reporting the correct isolation result, 13/13 (100%) for identification and 11/13 (84.6%) of the laboratories reporting a count within the intended range. The overall performance for examinations by culture excluding the isolation results is 92.3%.

Nine laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. In addition, 10 laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 8/10 reported a correct result.

Sample 7123: Performance was below average, with 9/20 (45.0%) of participants reporting the correct isolation result, 8/9 (88.9%) for identification, 7/9 (77.7%) of the laboratories reporting a count within the intended range, 8/8 (100%) reporting the correct serogroup and 5/5 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture excluding the isolation results is 91.7%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and 8/11 reported the correct result. 11 laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and 3/11 reported a correct result.

4 April 2022

Clinical distribution 5370

A total of 21 of participating laboratories from 26 countries reported results for the simulated sputum specimens compared with 21 laboratories who reported results for the urine specimens.

Table 10. Examinations performed and concordance achieved for distribution 5370

Sample number	Contents	Identification		Serogroup		Sequence type		Urinary antigen		Overall % performance by sample
		N	%	N	%	N	%	N	%	
7764	*Streptococcus oralis, Streptococcus mitis*	20/20	100	-	-	-	-	-	-	100
7765	*Legionella pneumophila*	-	-	-	-	-	-	19/21	90.5	90.5
7766	*Legionella pneumophila* Sg1 ST2454 *Legionella dumoffii Streptococcus mitis Streptococcus oralis*	20/21	95.2	16/16	100	8/10	80	-	-	91.7
7767	*Legionella pneumophila*	-	-	-	-	-	-	20/21	95.2	95.2
7768	*Legionella pneumophila* Sg1 ST2454 *Moraxella catarrhalis Streptococcus mitis*	21/21	100	20/20	100	10/12	83.3	-	-	93.4
7769	*Legionella pneumophila*	-	-	-	-	-	-	19/21	90.5	90.5
7770	*Legionella pneumophila* Sg1 ST2454	21/21	100	20/20	100	9/11	81.8	-	-	93.9
7771	*Legionella pneumophila*	-	-	-	-	-	-	21/21	100	100
7772	Negative	-	-	-	-	-	-	21/21	100	100
7773	*Legionella pneumophila* Sg3 ST2630 *Streptococcus mitis Streptococcus oralis*	21/21	100	19/20	95	13/14	92.9	-	-	96
			99		98.8		84.5		95.2	95.1

Patient 1:
Specimen 7764: The specimen was negative for *L. pneumophila.* Excellent concordance with intended results with 100% (20/20) of participants reporting the correct result.

Specimen 7765: The specimen was positive for *L. pneumophila* urinary antigen. A good performance with 90.5% (19/21) concordance with intended results from participants returning a result.

Patient 2:
Specimen 7766: The specimen contained *L. pneumophila* serogroup 1: ST2454 and *Legionella dumoffii.* A good performance with 95.2% (20/21), 100% (16/16), and 80% (8/10) of participants reporting the correct identification, serogroup and ST respectively. The participant who failed to identify *L. pneumophila* serogroup 1: ST2454, identified *L. dumoffii* only. Two laboratories incorrectly reported ST83 and ST813.

Only 14/21 participants successfully identified both *L. pneumophila* serogroup 1: ST2454 and *L. dumoffii.* For the *L. dumoffii* a result of *Legionella* spp. not *L. pneumophila* was also considered correct.

Specimen 7767: The specimen was positive for *L. pneumophila* urinary antigen. A very good performance with 95.2% (20/21) of participants returning a result for this specimen reported the correct result.

Patient 3:
Specimen 7768: The specimen contained *L. pneumophila* serogroup 1: ST2454. An excellent performance with 100% (21/21), 100% (20/20) and 83.3% (10/12) of participants reporting the correct identification, Sg and ST respectively. Two laboratories incorrectly reported ST83 and ST813.

Specimen 7769: The specimen was positive for *L. pneumophila* urinary antigen. A good performance with 90.5% (19/21) of participants returning a result for this specimen reported the correct result.

Patient 4:
Specimen 7770: The specimen contained *L. pneumophila* serogroup 1: ST2454. A very good performance was noted with 100% (21/21) of participants returning a correct result, 100% (20/20) reporting the correct Sg and 81.8% (9/11) reporting the correct ST. Two laboratories incorrectly reported ST83 and ST813.

Specimen 7771: The specimen was positive for *L. pneumophila* urinary antigen. An excellent performance with 100% (21/21) of participants reporting the correct result.

Patient 5:
Specimen 7772: The specimen was negative for *L. pneumophila* urinary antigen. An excellent performance with 100% (21/21) of participants reporting the correct result.

Patient 6:
Specimen 7773: The specimen contained *L. pneumophila* serogroup 3: ST2630 with 100% (21/21), 95% (19/20) reporting the correct serogroup and 92.9% (13/14) reporting the correct ST. One laboratory incorrectly reported ST450.

Environmental distribution 5371

Ten simulated environmental samples were sent to 22 laboratories in 22 EU/EEA countries. 20 laboratories returned a result. One laboratory informed the organisers that they would not be able to examine the samples and one laboratory did not return a result.

Sample numbers: 7774-7778 were samples taken as part of one outbreak investigation.

Sample numbers: 7779-7783 were samples were taken as part of routine monitoring.

Sample numbers: 7777 was a swab sample.

Performance of the laboratories on these samples were split into culture-based methods (Table 11) and molecular methods (Table 12). Culture-based method analysis included results reported for isolation, identification, enumeration, serogroup and sequence type results. An overall performance assessment column as a percentage has been captured for culture-based method results by sample number and by each examination. Overall performance by sample was calculated using the mean value across a maximum of the five examinations. For molecular methods the overall performance has not been calculated as the number of data sets returned for analysis is too low to provide robust performance data.

Table 13 shows in more detail the enumeration results reported by the laboratories. The participants' median is used as the assigned value and the expected range is calculated using 5 and 95 percentiles.

Table 11. Examinations done on cultured samples

Sample number	Contents	Isolation		Identification		Enumeration		Serogroup		Sequence type		Overall % performance by sample
		N	%	N	%	N	%±	N	%	N	%	
7774	*L. pneumophila* sg 1, ST2454	16/18	88.8	16/16	100	13/15	86.7	16/16	100	6/7	85.7	92.2
7775	*L. pneumophila* sg 1, ST2454 and *L. moravica**	18/19	94.7	Both 7/18 LM only: 1/18 LP only 8/18 L. sp only: 2/18	38.9	15/17	88.2	15/15	100	6/7	85.7	81.5
7776	No *Legionella*	18/19	94.7	-	-	-	-	-	-	-	-	94.7
7777	*L. pneumophila* sg 1, ST2454	16/19	84.2	16/16	100	-	-	16/16	100	6/7	85.7	92.5
7778	*L. pneumophila* sg 3, ST2630	20/20	100	19/19	100	18/19	94.7	19/19	100	8/8	100	98.9
7779	*L. pneumophila* sg 1, ST20	18/19	94.7	18/18	100	18/18	100	18/18	100	5/6	83.3	95.6
7780	No *Legionella*	18/20	90.0	-	-	-	-	-	-	-	-	90.0
7781	*L. pneumophila* sg 8, ST1324	18/20	90.0	18/18	100	16/18	88.8	17/18	94.4	6/6	100	94.6
7782	*L. rubrilucens**	13/20	65.0	13/13	100	10/12	83.3	-	-	-	-	82.8
7783	*L. pneumophila* sg 1, ST1	18/20	90.0	18/18	100	14/18	77.8	18/18	100	7/7	100	93.6
Overall performance by examination			89.2		92.4		88.5		99.2		91.5	

± If a censored value reported falls within the expected range this has been considered as being correct
** The reporting of Legionella species not pneumophila and Legionella species have been analysed as being correct*

Table 12. Molecular methods

Sample number	Identification	Intended results for *Legionella pneumophila*	Molecular results *Legionella pneumophila*	Intended results for *Legionella* spp.	Molecular results *Legionella* spp.
7774	*L. pneumophila*	Detected	9/10	Detected	7/11
7775	*L. pneumophila* and *L. moravica*	Detected	8/10	Detected	9/11
7776	No *Legionella*	Not detected	7/9	Not detected	9/10
7777	*L. pneumophila*	Detected	9/10	Detected	7/10
7778	*L. pneumophila*	Detected	11/11	Detected	9/12
7779	*L. pneumophila*	Detected	11/11	Detected	9/11
7780	No *Legionella*	Not detected	9/10	Not detected	10/11
7781	*L. pneumophila*	Detected	10/11	Detected	8/11
7782	*L. rubrilucens*	Not detected	10/11	Detected	8/12
7783	*L. pneumophila*	Detected	10/11	Detected	9/12

**It is unclear if laboratories are including the reporting of Legionella pneumophila as part of the results for Legionella sp. This was made clearer for April's distribution that the reporting of Legionella spp. results by molecular should include the L. pneumophila as most kits used cannot differentiate this species.*

Table 13. Data on enumeration results where reported

Sample number	Number of results	Identification	Participants median (cfu/L)	Intended range (cfu/L)	Number of outlying counts
7774	15	*L. pneumophila*	$2.5x10^3$	$1.1x10^2 – 1.7x10^4$	2 (1 low, 1 high)
7775	17	*L. pneumophila* and *L. moravica*	$2.5x10^3$	$2.5x10^2 – 4.1x10^4$	2 (1 low, 1 high)
7778	19	*L. pneumophila*	$8.7x10^2$	$93 – 1.0x10^4$	1 low
7779	18	*L. pneumophila*	$6.0x10^3$	$4.0x10^2 – 5.0x10^4$	0
7781	18	*L. pneumophila*	$5.6x10^2$	$25 – 2.2x10^3$	2 (1 low, 1 high)
7782	12	*L. rubrilucens*	$6.5x10^4$	$9.8x10^2 – 4.1x10^5$	2 (1 low, 1 high)
7783	18	*L. pneumophila*	$4.7x10^3$	$1.1x10^2 – 1.5x10^4$	4 (2 low, 2 high)

Sample 7774: Performance was very good, with 16/18 (88.8%) of participants reporting the correct isolation result, 16/16 (100%) for identification, 13/15 (86.7%) of the laboratories reporting a count within the intended range, 16/16 (100%) reporting the correct serogroup and 6/7 (85.7%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 92.2%.

Ten laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and nine reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and seven reported a correct result.

Sample 7775: Performance was very good, with 18/19 (94.7%) of participants reporting the correct isolation result, however for the identification only 7/18 (38.9%) reported the presence of both *L. pneumophila* and *L. moravica*, 2/18 laboratories reported a *Legionella* sp., 1/18 reported only the presence of *L. moravica* and 8/18 reported only the presence of *L. pneumophila*. The 12 laboratories that reported the presence of a *L. pneumophila* all reported the correct serogroup and 6/7 (85.7%) reported the correct ST. The overall performance for examinations by culture was 81.5%.

Ten laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and eight reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 7776: Performance was very good, with 18/19 (94.7%) of the laboratories reporting the correct isolation result.

Nine laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and seven reported the correct result. Ten laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 7777: Performance was excellent, with 16/19 (84.2%) of the laboratories reporting the correct isolation result, 16/16 (100%) for identification, 16/16 (100%) reporting the correct serogroup and 6/7 (85.7%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 92.5%.

Ten laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and nine reported the correct result. Ten laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and seven reported a correct result.

Sample 7778: Performance was excellent, with 20/20 (100%) of participants reporting the correct isolation result, 19/19 (100%) for identification, 18/19 (94.7%) of the laboratories reporting a count within the intended range, 19/19 (100%) reporting the correct serogroup and 8/87 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 98.4%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 7779: Performance was very good, with 18/19 (94.7%) of participants reporting the correct isolation result, 18/18 (100%) for identification, 18/18 (100%) of the laboratories reporting a count within the intended range, 18/18 (100%) reporting the correct serogroup and 5/6 (83.3%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 95.6%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and all reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

Sample 7780: Performance was very good, with 18/20 (90.0%) of the laboratories reporting the correct isolation result.

Ten laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and nine reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and ten reported a correct result.

Sample 7781: Performance was very good, with 18/20 (90%) of participants reporting the correct isolation result, 18/18 (100%) for identification, 16/18 (88.8%) of the laboratories reporting a count within the intended range, 17/18 (94.4%) reporting the correct serogroup and 6/6 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 94.6%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and ten reported the correct result. Eleven laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and eight reported a correct result.

Sample 7782: Performance was below average, with 13/20 (65.0%) of participants reporting the correct isolation result, 13/13 (100%) for identification, 10/12 (83.3%) of the laboratories reporting a count within the intended range. The overall performance for examinations by culture was 82.8%.

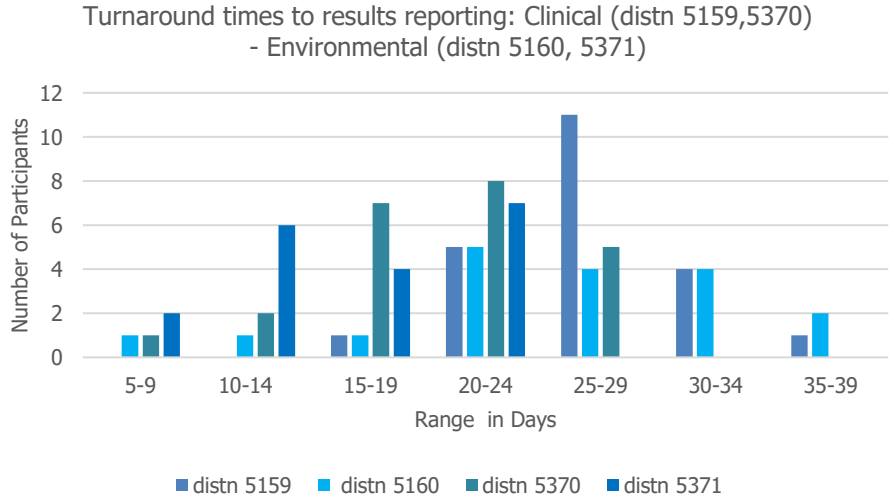
Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and ten reported the correct result. Twelve laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and eight reported a correct result.

Sample 7783: Performance was very good, with 18/20 (90.0%) of participants reporting the correct isolation result, 18/18 (100%) for identification, 14/18 (77.8%) of the laboratories reporting a count within the intended range, 18/18 (100%) reporting the correct serogroup and 7/7 (100%) of the laboratories reporting the correct ST. The overall performance for examinations by culture was 93.6%.

Eleven laboratories examined the sample using a molecular kit that only detects *L. pneumophila* and ten reported the correct result. Twelve laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and nine reported a correct result.

The turnaround time of reporting results for all the exercises was analysed and is shown in Figure 1.

Figure 1. Turnaround times to reporting results via the online secure UK NEQAS system for clinical distributions (5159, 5370) and environmental distributions (5160, 5371)



Distribution 5159: November 2021 clinical, distribution 5160: November 2021 environmental, distribution 5370: April 2022 clinical and distribution 5371: April 2022 environmental

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 samples/specimens analysed. Laboratories may also be required to report detected cases of Legionnaires' disease to their national surveillance systems if this is a requirement in their country.

External quality assessment 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 of the surveillance data reported.

Overall, the performance of laboratories participating in the 2021-2022 EQA for clinical specimens was very good. There were no significant issues arising for species identification, serogroup, enumeration, or sequence type. It was however noted, that the number of participants reporting isolation and identification of the non-*Legionella pneumophila* when present as a dual pathogen in the distributions was reduced. The majority of participants returning results for the methods survey stated 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.

Overall, the performance of laboratories participating in the 2021-2022 EQA 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/specimens, that the number of participants reporting a correct identification of the non-*L. pneumophila* when present as a dual pathogen was reduced. In addition, when levels of the *Legionella* spp. in the samples was low, some laboratories failed to isolate the organism, which could be due to the fact that for some methods used by laboratories, the levels were below the detection limit for the method they used.

November's distribution represented an outbreak associated with a hotel cooling tower. The outbreak strain of *L. pneumophila* Sg 1, ST2110 used was isolated from patients and a domestic water system. This species of *L. pneumophila* is rare and has only been identified three times in the UK since 2015.

April's distribution represented an outbreak associated with a chemical plant. The outbreak strain of *L. pneumophila* Sg 1, ST2454 was isolated from clinical specimens and environmental samples. This species of *L. pneumophila* is also rare and has only been identified six times in the UK since 2017.

No issues were encountered with the preparation of the simulated specimens/samples. Homogeneity, stability, and viability were consistent throughout all the stages of preparation of the specimens/samples 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 used to produce simulated EQA samples/specimens 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 samples to designated laboratories.

From the results data collated in this EQA, it was ascertained that the majority of laboratories for clinical specimens identified the pathogen and Sg, but a significant number did not report the ST (Tables 6 and 7). For environmental samples, the ISO 11731:2017 requires that suspect colonies are identified to at least *L. pneumophila*. Laboratories reported *L. pneumophila* correctly and went further, reporting a serogroup.

Clinical discussion

The clinical aspect of this EQA was a qualitative exercise designed to assess simulated sputum and urine specimens. The panel of sputum specimens were used to ascertain the absence or presence of *L. pneumophila* and when, following isolation of the respiratory pathogen, full identification to species level was requested, with accompanying Sg and ST. Examination to detect the urinary antigen for *L. pneumophila* was requested in the simulated urine specimens.

Based on published guidance by UKHSA in the UK, the three most commonly described specimen types analysed were urine and lower respiratory fluids, including sputum and broncho-alveolar lavage (BAL). Using this information, simulated sputum, BAL and urine specimens were designed for distribution as part of the EQA exercise. A survey of methods (Annex 1) was sent to all laboratories following the closure of distribution 5370, and this confirmed the most common specimen types examined routinely by participating laboratories to be sputum 91.7% (11/12), urine 83.3% (10/12) and BAL 91.7% 11/12.

From the methods questionnaire, it was determined that a total of 9/15 participants had indicated they were clinical diagnostics laboratories, as well as reference laboratories. Five participants were clinical diagnostics laboratories only, with three stating they had access to a reference laboratory, while two did not.

A total of 9/12 laboratories participated in a national EQA scheme. However, this was only a mandatory exercise for one of them. This is a very low number, especially as eight laboratories are noted to be reference laboratories and EQA is a requirement for accreditation. BCYE with cysteine (n=8) and GVPC (n=4) were the most frequently used media for the isolation of *Legionella* spp. (See results in Annex 2).

Distribution 5159: Three paired (sputum/urine) simulated specimens (7104;7105, 7106;7107, 7108;7109) with relevant accompanying clinical details were sent for evaluation.

Distribution 5370: Four paired (sputum/urine or BAL/urine) simulated specimens (7764;7765, 7766;7767, 7768;7769, 7770;7771). These paired specimens were designed to mimic an outbreak.

Identification:

- Overall concordance was excellent (97%), across both distributions for correct identification of *L. pneumophila.*
- One laboratory consistently failed to detect the presence of *L. pneumophila* in all sputum specimens in distribution 5159. However, demonstrated remarkable improvement in the subsequent distribution.
- Concordance with intended results was reduced for samples containing two species of Legionella.

Serogroup:

- Overall concordance was excellent (99.4%), for both distributions for reporting the serogroup.
- The large majority of laboratories performed serogrouping across both distributions.

Sequence type:

- A reduced number of laboratories reported against ST for both distributions. Distribution 5159: A total of 13/15 laboratories reported ST for patient 1, followed by 14/25 for patient 4 and 13/15 for patient 6. For distribution 5370 between 10 and 14 laboratories out of a total of 22 who examined the sputum specimens reported a result.
- Overall, the concordance was lower (86.4%), for both distributions on reporting on ST.
- Two laboratories stated the incorrect STs for all outbreak associated specimens in distribution 5370.

Urinary antigen testing:

- Overall concordance was very good (96%), for both distributions for reporting the urinary antigen.
- Previous distributions have demonstrated a lower concordance when paired with a sputum negative for *L. pneumophila.* This has again been highlighted, distribution 5159 specimen 7105 and distribution 5370 specimen 7765.

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 samples. These include molecular techniques such as Real-time Polymerase Chain Reaction (RT 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 these EQA's 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 ST for water samples.

Legionella spp. are found in cooling towers, hot and cold water systems, air conditioners, spa equipment, fountains, humidifiers and 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 buffered charcoal yeast extract (BCYE)/glycine vancomycin polymyxin B cycloheximide (GVPC) agar plates. This is a labour-intensive approach which takes ten 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].

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.

A methods survey questionnaire (Annex 1) was sent out in May 2022 to the all laboratories that took part in distribution 5160 and 5371; only nine responses were received.

The overall performance of the laboratories in the EU/EEA countries was very high. Of the six laboratories providing method data, five stated 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.

16/23 laboratories in total took part in both distributions. These laboratories will be able to assess their performance over time, which EQA is a valuable tool to provide this.

For distribution 5160, the maximum results reported was 20 and for distribution 5371 this was 20. Not all laboratories examined all the samples or undertook all the examinations when the sample contained a *L. pneumophila.*

Isolation:

The isolation results for samples 7122 and 7123 (distribution 5160) have been excluded from the overall performance calculation due to the low performance achieved by laboratories as the levels *of Legionella* sp. in the samples may have been below the detection limit for methods used by participants. These samples were not scored for the isolation results.

- The overall performance for isolation of *Legionella* was very good with performance of 98.6% for distribution 5160 and 89.2% for distribution 5371. Two laboratories reported an incorrect isolation five times in distribution 5371, both did not take part in distribution 5160
- The performance for samples 7122 and 7123 in distribution 5160 was low. The reason for this low performance is due to the low level of *Legionella* spp. in the sample which may have below the lower detection limit for methods used by laboratories.
- Sample 7782 contained a *L. rubrilucens* and the performance was 65%, the reason for this is unknown as the participants median was $6.5x10^4$ cfu per litre.
- Two samples (7116 and 7775) contained two species of legionellae, only 7/20 (35.0%) isolated both species in sample 7116 and 7/18 (38.9) for sample 7775.
- Three samples contained a single species of *Legionella* other than *pneumophila* (7120, 7122 and 7782). 20/20 (100%) of the laboratories isolated the *L. bozemanii* in sample 7120. 7122 contained a *L. anisa* and 13/18 (72.2) isolated this species and for sample 7782 which contained a *L. rubrilucens* performance was 13/20 (65.0%).
- Over the two distributions, the overall performance for six water samples in distribution 5160 was 99.1% and 89.8% for distribution 5371 containing nine water samples.
- Over the two distributions, the overall performance for two swab samples in distribution 5160 was 97.2% and 84.2% % for distribution 5371 containing one swab sample.
- Over the two distributions, the overall performance for 15 samples containing a *Legionella* spp. was 93.1%.
- Over the two distributions, the overall performance for three samples not containing a *Legionella* spp. was 95.0%.
- Over the two distributions, the overall performance for the 10 outbreak investigation samples was 96.2%.
- Over the two distributions, the overall performance for the eight routine monitoring samples was 90.0%.
- 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:

- Over the two distributions, the performance for the correct identification of *Legionella* spp. (where only one species was in the sample) for 15 samples containing this organism was 99.3%.
- Over the two distributions, the performance for the correct identification of *Legionella* spp. (where two species was in the sample) for two samples containing this organism was much lower at 37.0%.
- Twelve samples contained a *L. pneumophila* only and performance was 99.1%. Three samples contained non-*pneumophila* strains, the performance for correct identification for these strains was 100%.

Enumeration:

- The number of data sets returned over the two distributions varied between 7-20 for the samples. Therefore, this is below the number required to produce robust performance data.
  - When statistical calculation is based on 10-19 results, they 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. This equated to a total of 15 samples over the two distributions, performance was consistent by sample which ranged from 100%- 77.7% of results reported in the expected range. The performance by distribution was 88.1 for distribution 5160 and 88.5 for distribution 5371, which is very good.

- Further analysis showed that one laboratory reported a low outlying count once in distribution 4896 and once in distribution 5114. Another laboratory reported a high outlying count three times over the two distribution, once in distribution 4896 and twice in distribution 5114.
- The number of outlying results by samples over the two distribution was not influenced by the level of the *Legionella* spp. in the sample.

Serogroup:

- Over the two distributions, 10 samples contained a *L. pneumophila* serogroup 1, performance very good with 99.4%. Four samples contained non-serogroup 1 and performance was 98.6%.

Sequence type:

- The overall performance over the two distributions for 14 samples in reporting a correct ST was 91.9%.
- The overall performance over the two distributions for eight samples as part of the outbreak investigation was 88.0%.
- The overall performance over the two distributions for six samples as part of the routine monitoring was 97.2%.
- The overall ST performance was 92.4% for distribution 5160, where three samples contained ST2110, one contained ST728, one contained ST109, one contained ST1 and one contained ST2923.
- The overall ST performance was 91.5% for distribution 5371, where three samples contained ST2454, one contained ST2630, one contained ST20, one contained ST1324 and one contained ST1. One laboratory reported an incorrect ST on 8/14 occasions across both distributions.
- Method used for ST analysis was Sanger sequencing and/or WGS.

Molecular methods:

- The number of laboratories examining the samples by molecular methods is still low. For distribution 5160, between 5-11 laboratories examining the samples used a kit that detects *L. pneumophila* and between 6-11 for *Legionella* spp. For distribution 5371, between 9-11 laboratories used a kit that detects *L. pneumophila* and between 10-12 for *Legionella* spp. Therefore, performance as a percentage for results reported using molecular methods has not been calculated.
- The overall performance with distribution 5160 was much lower with the *Legionella* species results, this may have been due to the lack of understanding that the *Legionella* species results should also include *L. pneumophila.* This was clarified for distribution 5371 and the reporting of a correct result for the species improved.
- Despite this clarification three laboratories still reported incorrect results for the *Legionella* species result even though for the *L. pneumophila* a correct result was reported for distribution 5371.
- For distribution 5371 one laboratory reported incorrect results on 5 occasions for the *L. pneumophila* and four times for *Legionella* spp. This laboratory did not take part in distribution 5160.
- For distribution 5160, only one laboratory reported an incorrect result twice for *L. pneumophila*.
- An analysis of the kits from the method questionnaire did not indicate that one specific molecular test was commonly being used.

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 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 sample, correct sample type, volume of sample, correct tests requested, and suitable container, all of which were pre-determined for this EQA panel.

The EQA scheme was only available to a maximum of two selected laboratories per EU/EEA country, so the breadth of the cohort was limited to those who received a panel and returned results.

A period of six weeks was given for laboratories to return results. This period was allocated to allow sufficient time for the panel to arrive at the laboratories via air freight to the various countries. The time allowed for the return of results was not meant to reflect the expected turnaround times for clinical or environmental laboratories when investigating and returning results. Nevertheless, the number of days taken to report results from the receipt date by the laboratory was recorded (Figures 1). The mean value for returning results was determined to be 28 days for both clinical specimens and environmental samples. The mean values illustrate a significant improvement from the first EQA in 2019, in which the mode was 35 days for clinical and 40 days for environmental.

Nevertheless, the turnaround times to report results indicate that the participating laboratories may not have treated the EQA specimens as they would routine samples (having several staff analyse the results prior to reporting online rather than just one member of staff processing and reporting). One limitation to the system used to report the EQA results is there is no facility 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, which 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 provided in plastic tubes to resemble a true specimen.

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

An EQA is of limited value without at least some of the other components of a quality system, such as adequate documentation, training of staff and internal quality control (IQC).

5. Conclusions

Laboratories from 28 EU/EEA participating countries performed well in this year's exercises for culture-based/detection methods used by both the clinical (95.3%) and environmental laboratories (92.0%).

Both clinical and environmental laboratories demonstrated that they could undertake testing to an acceptable level of at least 80% correctness. Although the data provides some assurance of the laboratories' ability to undertake effective public health investigations for *Legionella pneumophila* and other *Legionella* spp., more EQA data are required to determine the actual ongoing performance of the laboratories with the varying design of EQA sample/specimen sent. If laboratories report accurate data, this also ensures that the information provided to surveillance systems is accurate.

Laboratories were provided with the opportunity to examine samples that they would routinely test. For clinical samples, 25/27 laboratories examined the sputum samples in the November distribution and 21/26 laboratories in the April distribution. A total of 24/27 laboratories examined the urine specimens in the November distribution, compared with 21/26 laboratories in the April distribution. This indicates that fewer participating laboratories processed urine samples than sputum. For environmental samples, 20 of the laboratories examined both water and swab samples in November's distribution and 20 laboratories examined both sample types in April's distribution. Where results reported were not in accordance with those intended, laboratories were advised by contractors to investigate further in order to determine the root cause.

For the clinical laboratories in the November distribution 5159, 25 reported an identification, 21 for serogroup and between 13-15 for ST. For April's distribution, 21 reported on the identification, between 16-20 for serogroup and between 10-14 for ST. 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 species level. For clinical laboratories, 90.5% of participants 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 SBT and RT-PCR methods.

For the environmental laboratories in November's distribution 5160, 20 laboratories reported a result for isolation and identification, up to 20 reported a serogroup, up to 19 reported an enumeration count and up to 11 a ST. For molecular methods, up to 11 laboratories analysed the samples for *L. pneumophila* and for *Legionella* spp. The overall isolation performance for culture was 98.6%, with 20 laboratories reporting a result. For April's distribution 5371, 20 laboratories reported a result for isolation and identification, up to 19 reported a serogroup, up to 19 reported an enumeration count and up to seven a ST. For molecular methods, up to 11 laboratories analysed the samples for *L. pneumophila* and for *Legionella* spp. The overall isolation performance for culture was 89.2%, with 20 reporting a result. Two laboratories that did not take part in distribution 5160 reported an incorrect isolation five times in distribution 5371. The performance for isolation for samples 7122 and 7123 in distribution 5160 was low. The reason for this low performance is due to the low level of *Legionella* spp. in the sample which may have below the lower detection limit for methods used by laboratories. Sample 7782 contained a *L. rubrilucens* and the performance for isolation was 65%, the reason for this is unknown as the participants median was $6.5x10^4$ cfu per litre.

The overall performance for molecular methods was not calculated due to low numbers of laboratories analysing the sample by this method. The overall performance with distribution 5160 was much lower with the *Legionella* species results, this may have been due to the lack of understanding that the *Legionella* species results should also include *L. pneumophila*. This was clarified for distribution 5371 and the reporting of a correct result for the species improved. Despite this clarification, three laboratories still reported incorrect results for the *Legionella* species result, even though for the *L. pneumophila* a correct result was reported for distribution 5371. For distribution 5371, one laboratory reported incorrect results on five occasions for the *L. pneumophila* and four times for *Legionella* spp. This laboratory did not take part in distribution 5160.

6. Recommendations

These exercises will continue to provide a baseline understanding of the level of testing undertaken in the laboratories, determine performance issues and, where possible, provide support to laboratories/countries that have identified limitations in addressing improvement of their testing capabilities or capacity-building.

Main recommendations for future EQA exercises

Sample/specimen design

- To continue providing these EQA exercises and include different *L. pneumophila* Sg, STs and *Legionella* species. This will allow a better understanding of a laboratory's ability to undertake testing to the level required for successful management of public health incidents.
- To continue to include levels of *Legionella* spp that are at the lower end of the detection limit for culture for environmental samples.
- To continue to include more than one species of *Legionella* spp. within the simulated samples/specimens set. This will help educate and improve knowledge and experience with organisms which are otherwise not frequently encountered.

Methods

- To continue to gather information on the methods used to report results on the samples/specimens – this will be required when returning results.
- To link the method information more closely to the results reported, in order to identify tests that laboratories carry out routinely but did not report a result for, or instances where the organisers did not allow for these examination results to be reported.

Ongoing objectives

- To continue improving the awareness of the different *Legionella* spp. that may be isolated from clinical specimens and environmental samples.
- To continue raising awareness of the confirmatory tests done and their limitations when confirming *Legionella* spp. isolates in samples.
- To continue raising awareness of the importance of following standardised methods when managing public health incidents.
- To encourage regular participation in the EQA by the same laboratories in the countries as it is an important element of their quality procedures and helps to ensure that the results of their tests are accurate. Laboratories should participate regularly throughout the year in order to review performance on an ongoing basis. Ongoing performance assessment is designed to identify genuine problems.
- To determine if participating in EQA exercises can improve understanding of the link between clinical and environmental laboratories within countries when dealing with outbreaks, to make the management of public health incidents more effective.
- To continue exploring participants' feedback from the evaluation survey to develop the exercises (e.g. improving information in the individual EQA reports).
- To continue communicating the results of these EQAs at ELDSNET and *Legionella* conferences to increase the awareness of the importance of EQAs for the quality of *Legionella* detection in laboratories.

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Annex 1. Findings from the method questionnaire

As part of the EQA exercise, a questionnaire was sent out on the methods used to analyse the samples/specimens. This information was gathered online and was sent out to all laboratories that took part in any distribution in May 2022. The data presented below are for all EU/EEA countries.

A. Methods survey findings for clinical specimens

As part of the EQA exercise, a questionnaire was sent out on the methods used to analyse the samples/specimens. The data presented below are for all EU/EEA countries.

A was questionnaire sent out and completed by 12 of the participating laboratories in the 28 EU/EEA countries. Laboratories completing the questionnaire included; Bulgaria, Croatia, Estonia, Finland, Iceland, Ireland, Latvia, Norway, Portugal, Romania, Slovakia and Spain.

The method data shown are for information only. It does not evaluate or associate the data with a failure in the EQA or method/process used and it does not attempt to compare performance of the various molecular kits/processes.

General information on questions asked

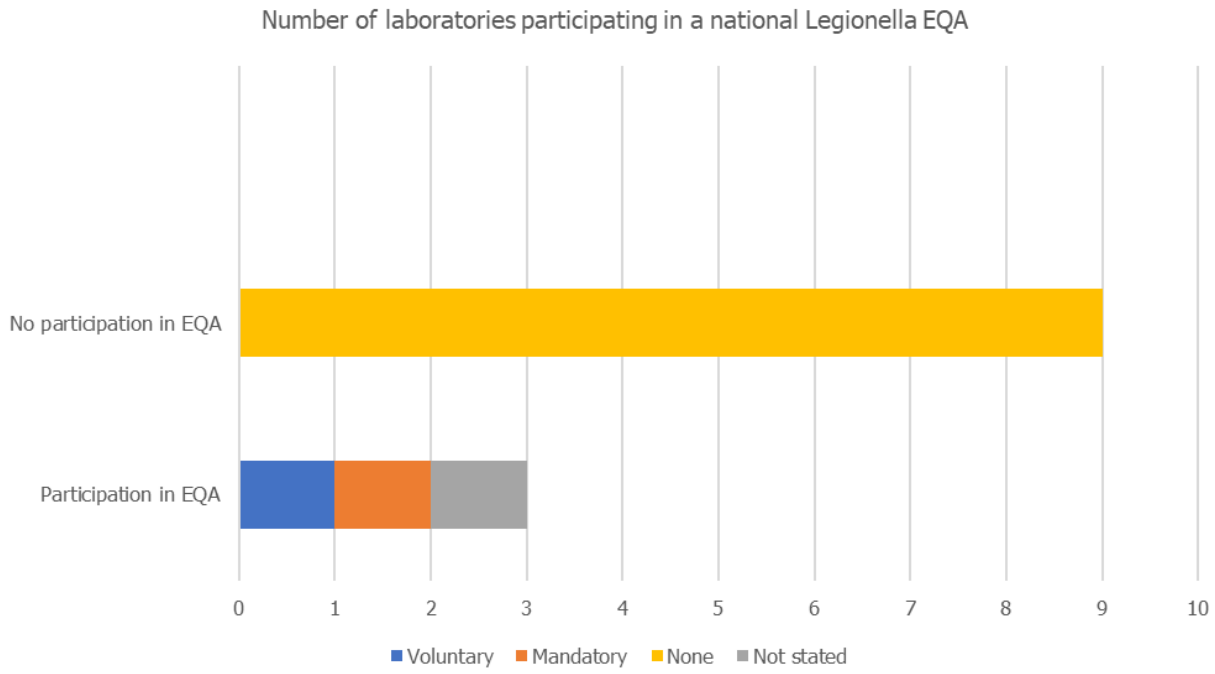
1. Are you a clinical diagnostic laboratory?
2. Are you a National Reference Laboratory?

All 12 reported they were clinical diagnostic laboratories. Of these 12 laboratories, eight were also reference laboratories. Of the four laboratories who were not reference laboratories, two stated they did have access to a reference lab, while two did not.

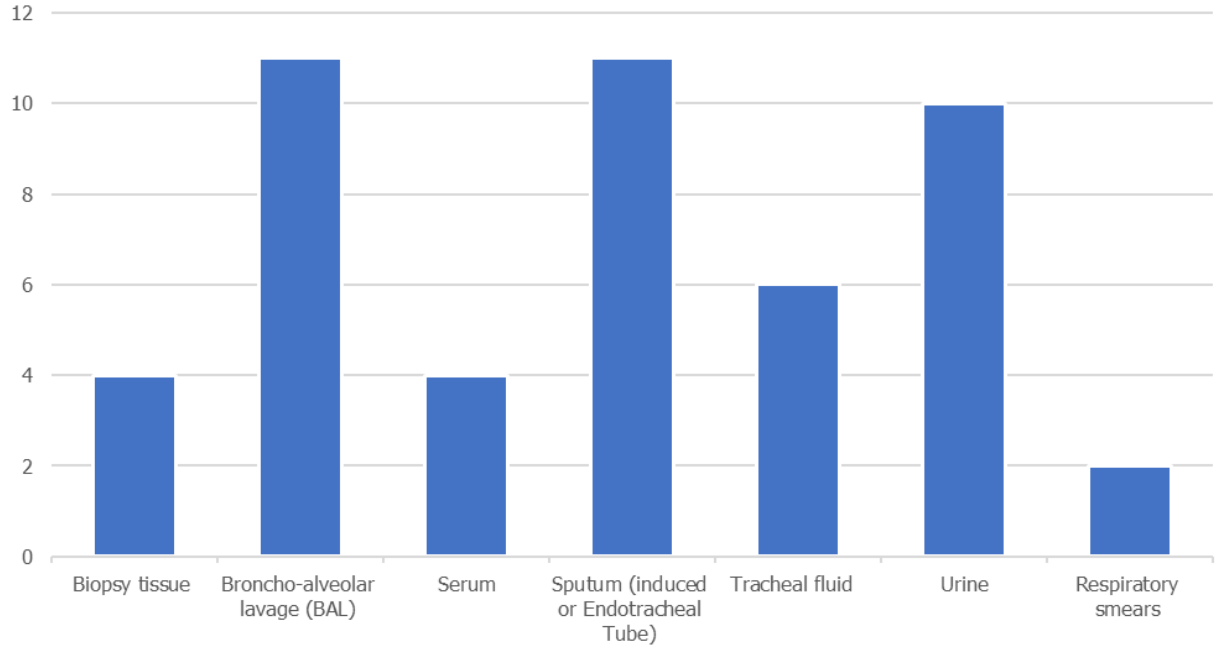
3. Approximately what percentage of all Legionnaires' disease cases in your country in 2020 involved a surveillance notification from your lab?

The percentage of Legionnaires' disease in 2020 which involved surveillance notification by those replying to the methods survey included; all cases, <10%, not available, unknown and none.

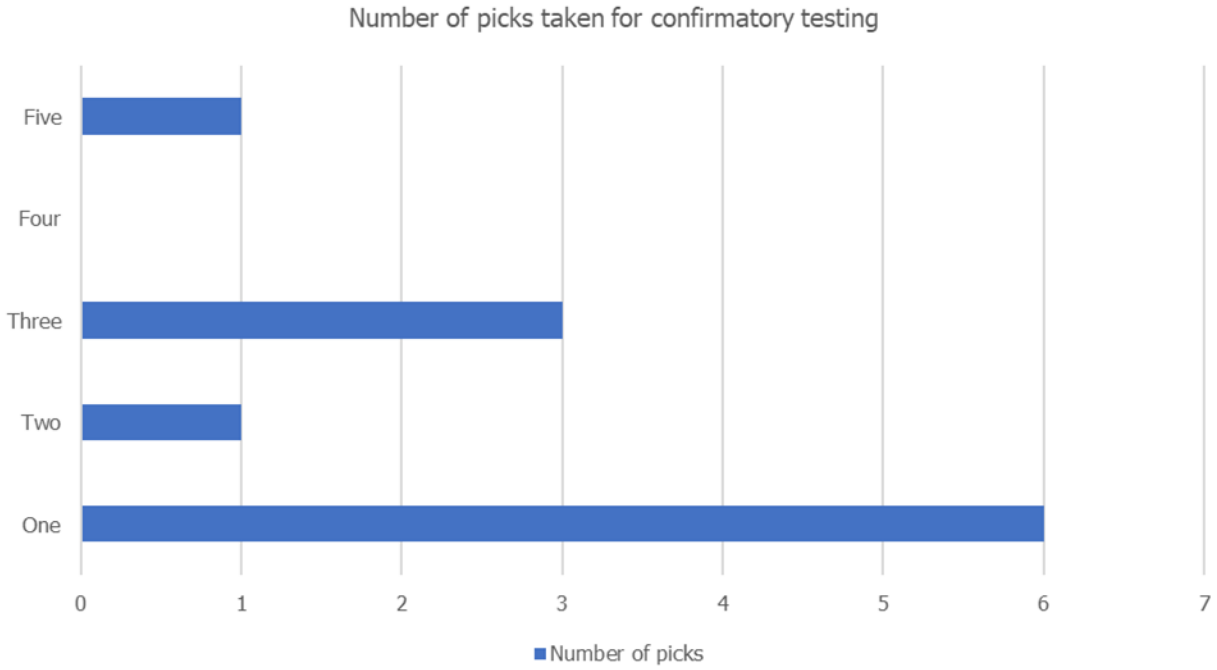
4. Does your laboratory participate in a National EQA scheme for the detection of *Legionella* species?
4a. Is this a voluntary participation?
4b. Or a mandatory requirement?



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



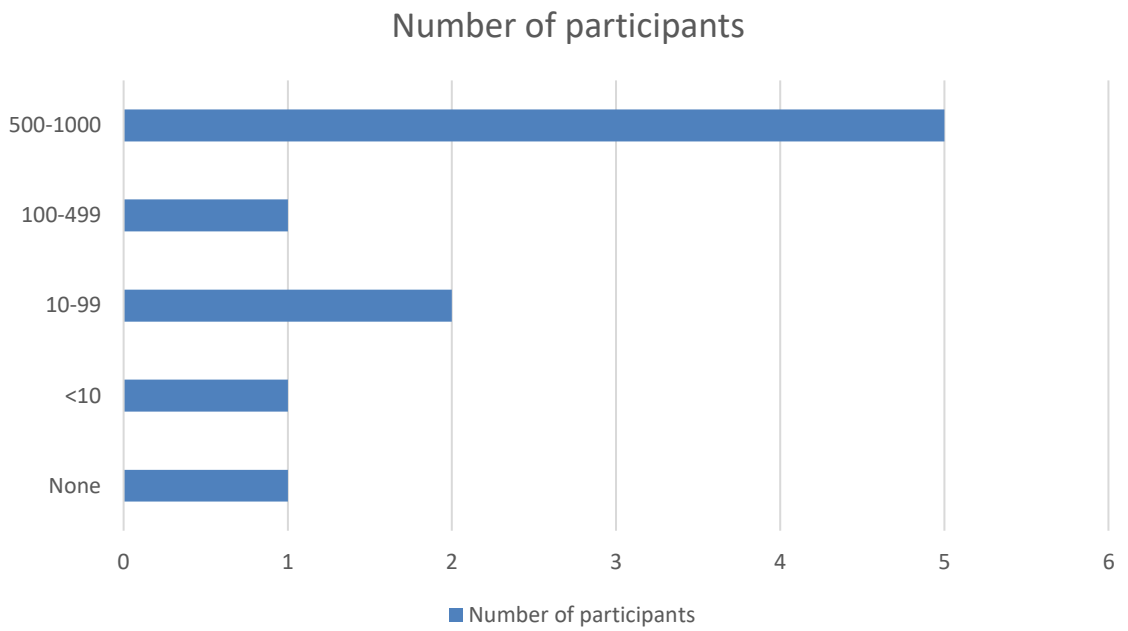
6. When a *Legionella* species has been isolated, how many pick of the colonies do you take for confirmation testing?



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

Only three participants from 12 returning results for the survey stated that fluorescence is performed. One laboratory stated that if morphology appears different, multiple colonies are isolated and identified by mip sequencing.

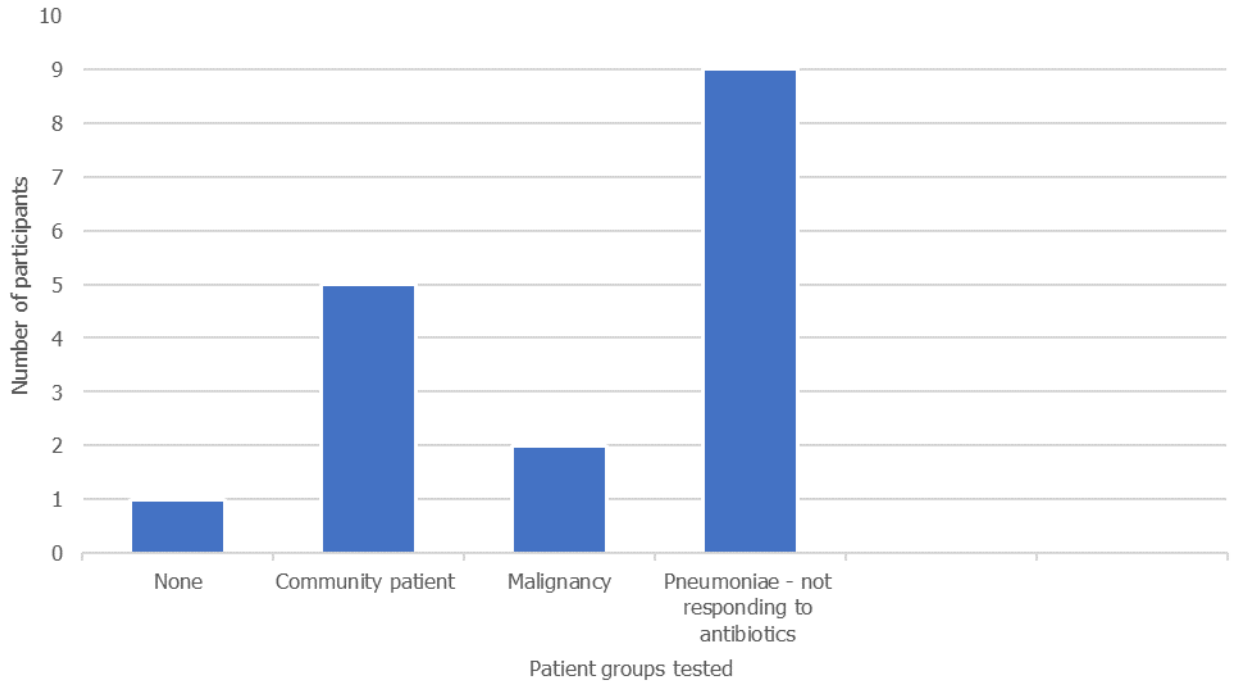
8. Approximately how many *Legionella* urinary antigen tests are undertaken in your laboratory each year?



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

Number of tests performed	None	<10	10-99	100-499	500-1 000
Culture	0	2	6	2	0
PCR tests	3	0	2	5	0
7 alleles ESGLI method	0	0	0	1	0
Direct Fluorescent Antibody (DFA) staining	0	1	0	0	0
Mip sequencing	0	1	0	0	0
Serotyping	0	2	1	0	0
Monoclonal antibody	0	0	0	1	0
Latex agglutination	0	1	2	2	0
Sequencing (sanger & WGS)	0	0	0	2	0

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

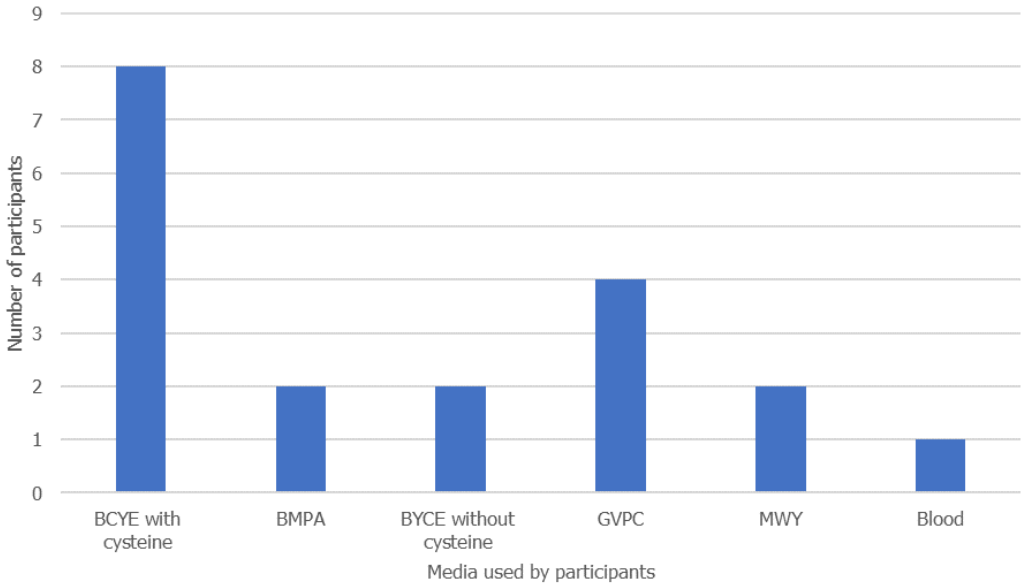


11. Did you outsource any tests used in this distribution to another laboratory?
The large majority (n= 9) did not outsource any tests to other laboratories. Of the two that did, tests outsourced included urinary antigen testing and sequencing.

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

No laboratories reported any additional methods/ elements were performed for the processing of the EQA specimens.

13. What media do you use in your laboratory?



The media used was reported to be in-house n= 1 and commercial n= 6. Two noted using a combination of commercial prepared and in-house media.
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.

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

Incubation ranged from 3-5 days (n=2), 7-10 days (n=5), 11-14 days (n= 2) and 10 days (n=2).

15. Do you use a moist chamber when culturing samples for *Legionella*?

During incubation, the large majority (n=9) laboratories stated they incubated in a moist chamber, two participants did not provide a response and two stated they didn't use a moist chamber.

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

Urinary antigen testing kit	Number of laboratories
BinaxNOW Abbott	5
AccuDiag ELISA	1
BIOGNOST LegioGnost1	1
Trinity Biotech - Bartels ELISA Legionella Urinary Antigen	1
Sofia Legionella FIA, quidel corporation	1

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

Additional respiratory targets in multiplex PCR	Number of participants
Legionella species	2
Chlamydophila pneumoniae, Chlamydia pneumoniae	3
Bordetella pertussis/parapertussis	2
Streptococcus pneumoniae	2
Haemophilus influenzae	2
Mycoplasma pneumoniae	3

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

PCR methods kit	Number of participants
Seegene respiratory panel 4, Allplex	2
AlphaCube Pan Legionella and AlphaCube Legionella.	1

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

A total of 6/7 participants reporting the use of molecular methods stated they did use IQC.

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

Serogroups	Number of participants
1-6	1
1-14	4
1-15	1

21. Do you perform WGS?

Only three laboratories stated the use of WGS. Bioinformatic analysis was listed as in silico *L. pneumophila* sequence based typed, SBT (legsta), SNP (RedDog) and SNP analysis. No responses were obtained for number of genes used. Sequencing platform was noted as Illumina for all three laboratories performing WGS.

Methods survey findings for environmental samples

A questionnaire was sent to all participants who participated in distribution 5160 or 5371. The objective was to gather information on the method/processes used for this EQA exercise. The laboratories were only asked to provide information on the methods they use in their laboratory.

All 8/23 (39%) of the participating laboratories provided information on their methods/processes. Responses were received from Bulgaria, Estonia, Iceland, Norway, Poland, Portugal, Romania and Sweden.

The total numbers will not always correspond to eight as some participants did not provide information on all the questions and some questions allowed for more than one option to be selected.

The method data shown is for information only. It does not evaluate or associate the data with a failure in the EQA or method/process used and it does not attempt to compare performance of the various molecular kits/processes.

General information on questions asked

1. Are you a National Reference Laboratory for environmental and water samples?

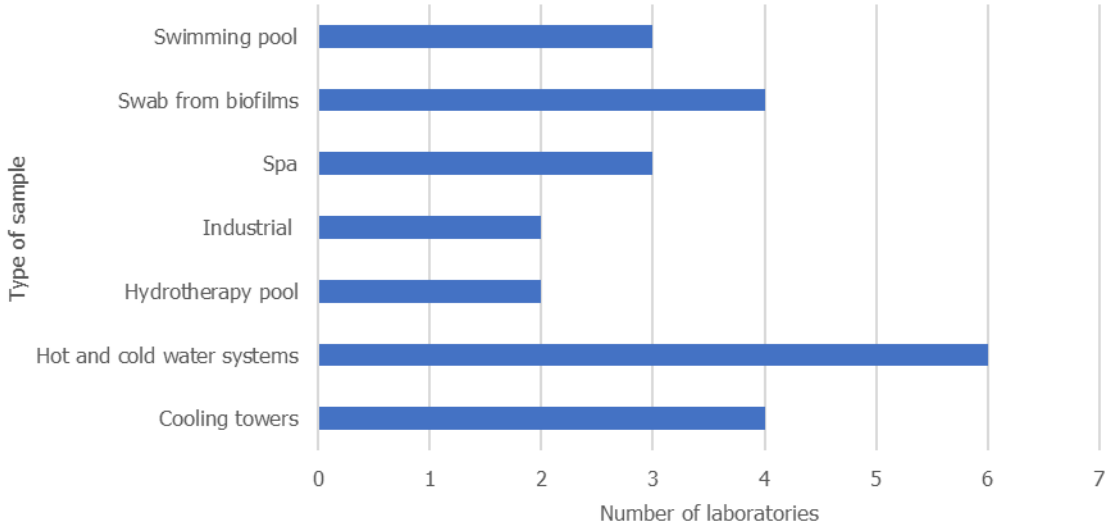
From eight responses, five stated they were a reference laboratory and three were not. The three that were not a reference laboratory, only one stated they had access to one if required.

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

Of the eight responses, seven stated they did not take part in national EQA programmes. Of the one that did take part, it was mandatory to do so.

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

Six responses were received, as shown in the graph below:



Most laboratories followed ISO 11731:2017 for analysing both water and swab samples.

4. Due to the low responses on some of the questions, the below are a summary of the responses received:

- The number of colonies selected to do confirmation tests on varied from 1 to 5 (n=5).
- Three laboratories do a fluorescence check on colonies, four do not (n=7), the reason for not doing a check was due to the fact that an alternative method was used for identification.
- The number of samples culture for isolation and identification varied between 10 - >1000 a year (n=6).
- Media was commercially bought by five of the laboratories and used (n=6)
- The most common media used was BYCE without cysteine (BCYE with antibiotics: Buffered charcoal yeast extract agar with selective supplements)(n-5).
- Culture plates were incubated between 7-10 days (n=6), of which four laboratories use a moist chamber.
- Five laboratories analyse 1 litre of a sample, and one laboratory examines 500mL (n=6).
- Of the six responses received, a combination of testing is performed on neat, heat and acid treated samples, three of these indicated that the acid is applied after filtration and bacterial released from membrane and directly on membrane.
- Only one laboratory indicated they examine samples using molecular methods.
- The number of serotyping done in a laboratory varied between 0 - >100 (n=6)
- Only one laboratory indicated they examine samples using sequencing or WGS methods.
- Only one laboratory outsourced the sequencing testing in this EQA exercise (n=6).
- No laboratory stated they made changes to the protocol they follow for this EQA (n=5).

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