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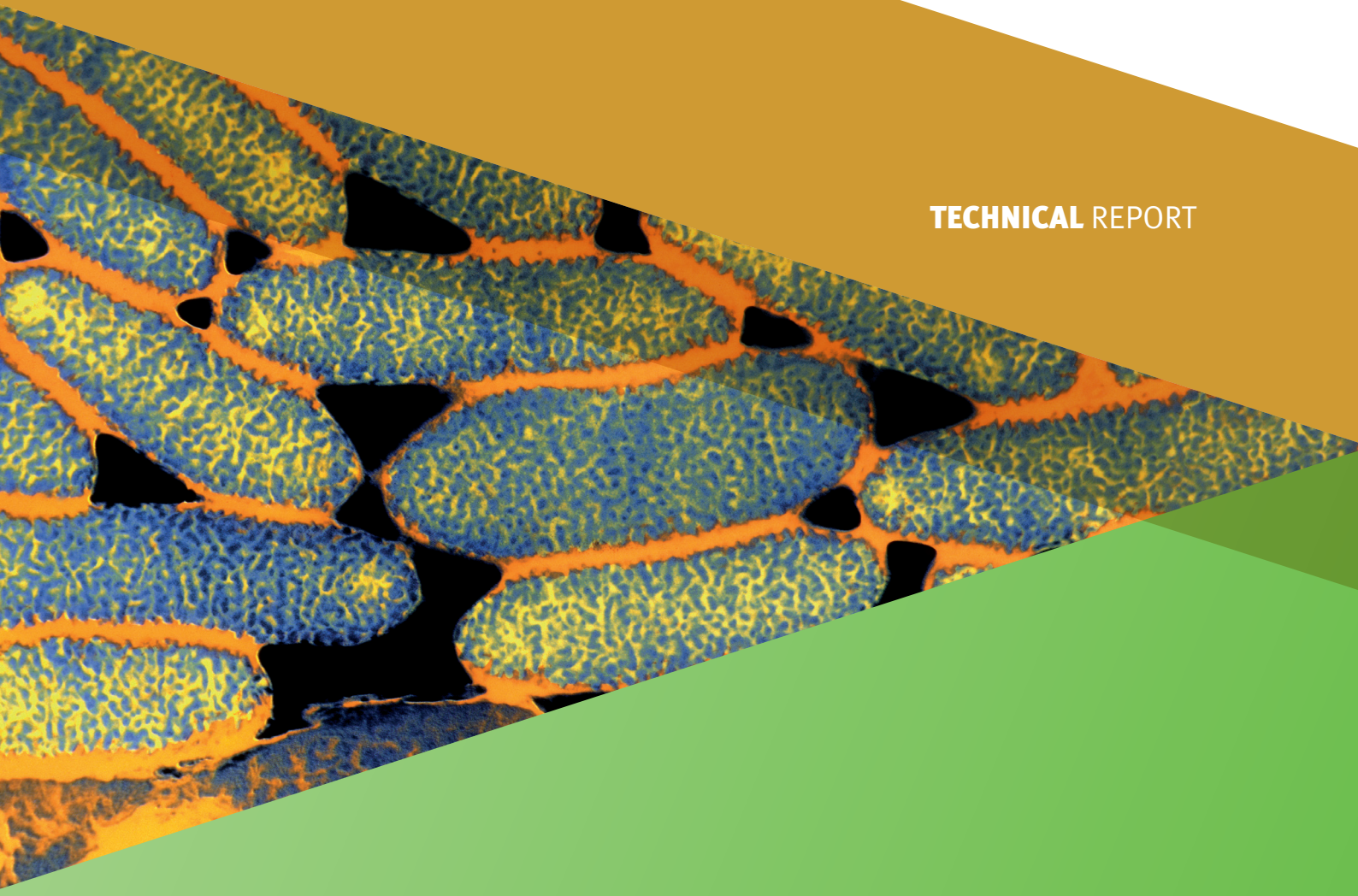

TECHNICAL REPORT

External quality assessment (EQA) schemes to support European surveillance of Legionnaires' disease 2019–2020 - Western Balkans and Turkey

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External quality assessment (EQA) schemes to support European surveillance of Legionnaires' disease 2019–2020

West Balkans and Turkey

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC Framework contract ECDC/2019/024 – *Legionella* EQA, specific contract CPN/2019/OCS/10918, coordinated by Lara Payne Hallstrom and produced by the Food and Environmental Proficiency Testing Unit and United Kingdom National External Quality Assessment Service on behalf of Public Health England.

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Acknowledgements

We would like to thank all staff within EQA services (FEPTU and UK NEQAS) who helped support the delivery of this scheme, staff in the reference laboratory who provided the characterised strains and did the quality control testing, all laboratories that participated in the scheme and national ECDC correspondents (in the Western Balkans and Turkey) who nominated participating laboratories.

Suggested citation: European Centre for Disease Prevention and Control. External quality assessment (EQA) schemes to support European surveillance of Legionnaires' disease 2019-2020 – Western Balkans and Turkey. Stockholm: ECDC; 2021.

Stockholm, June 2021

ISBN: 978-92-9498-543-9
doi: 10.2900/72046
Catalogue number: TQ-02-21-869-EN-N

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Abbreviations

BCYE	Buffered charcoal yeast extract
BAL	Broncho-alveolar lavage
CFU	Colony forming units
COVID-19	Disease caused by coronavirus SARS-CoV-2
DFA	Direct fluorescent antibody
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-Linked Immunosorbent Assay
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
GU/L	Genome units per litre
GVPC	Glycine Vancomycin Polymyxin B Cycloheximide
ID	Identification
LUA	*Legionella pneumophila* urinary antigen
MALDI-TOF	Matrix Assisted Laser Desorption Ionisation Time-of-Flight
PCR	Polymerase chain reaction
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England
MLST	Multilocus Sequence Typing
MVLA	Multiple-Locus Variable-Number Tandem-Repeat Analysis
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
UK	United Kingdom
UK NEQAS	United Kingdom National External Quality Assessment Service
WGS	Whole genome sequencing

Executive summary

In 2019, ECDC implemented the start of an EQA scheme for the European Legionnaires' Disease Surveillance network, for the detection, isolation, identification and enumeration of *Legionella* spp. This was organised under a framework contract with the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) and the United Kingdom National External Quality Assessment Service (UK NEQAS).

This is the first EQA exercise for laboratories participating in surveillance from the ELDSNet network that has been organised by ECDC since 2015 and the EQA format and arrangements have changed. The current EQA scheme uses an outbreak scenario with a package of clinical and environmental samples for the participating laboratories to process, depending on their technical capacity and protocols.

The purpose of the 2019–2020 EQA exercise was to determine the accuracy of *Legionella* testing and results reported by individual laboratories in order to enable comparison of results between laboratories and within countries across Europe. This report presents an analysis of participants' results for the 2019 EQA exercise for the EU enlargement countries.

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

Only one round was completed during 2019–2020, due to the impact of COVID-19.

In summary, there was one delivery of EQA samples which was sent on 4 November 2019. This EQA distribution was sent to a maximum of two laboratories per country for a total of seven EU enlargement countries invited to take part via their national ECDC correspondent in the Western Balkans and Turkey.

This distribution consisted of a total of 20 simulated samples; 10 representing clinical material and 10 representing environmental samples. Strains of *Legionella* was provided by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) and these strains were fully characterised using conventional methods and an analytical profile index system.

Laboratories only needed to report if the sample/specimen contained a *Legionella* spp., and then provide identification, enumeration, serogroup and sequence type.

Individualised reports were generated for each laboratory that included the results for their individual examinations and the overall results submitted by all laboratories for this exercise. This report analyses the EQA performance of laboratories in the seven EU enlargement countries in relation to the detection/isolation, identification, enumeration and quantification of *Legionella* spp. and the further characterisation of *L. pneumophila.* Detection and characterisation involved serogrouping and sequence-based typing of both clinical and environmental samples, where applicable. The report is split into two parts - clinical and environmental analysis - as the aim of this first new EQA exercise was to assess the baseline testing.

A separate survey was also organised on methods/kit information and the frequency of testing performed for each method/kit by the laboratories.

For this EQA the scenario was a simulation of an outbreak associated with a spa facility. The selected outbreak strain, *Legionella pneumophila*, was serogroup (Sg) 1, sequence type (ST) 47.

Laboratories were given the opportunity to examine samples they would routinely test in their laboratory. For the clinical element, five laboratories examined the sputum samples and six examined the urine samples. For the environmental element, five of the laboratories examined the water samples and the swab samples. Where the results reported were not in accordance with the intended exercise, laboratories were advised by contractors to investigate in order to determine the cause.

A total of seven clinical laboratories were sent the clinical distribution in the following countries: Albania, Bosnia and Herzegovina, Kosovo, North Macedonia, Montenegro, Serbia and Turkey. Six laboratories returned results for this EQA distribution.

For the clinical laboratories, five identified the sample and the serogroup, (two did not return results) and no laboratories reported the sequence type.

For the identification of *Legionella pneumophila* serogroup 1 in simulated sputum samples, there was excellent concordance with the intended results, with 100% of participants reporting the correct result (specimens 5706 and 5710). For specimen 5712 containing a *Legionella pneumophilia* serogroup 3, a lower concordance of 80% was achieved for identification. Overall determination of Sg was excellent for serogroup 1, however as with identification, specimen 5712 had a lower concordance, with only 66.7% participants reporting the correct result. One specimen (5714) contained *Legionella longbeachae,* and only one of four participants reported the correct identification. A further 2/4 reported either *Legionella* species or not *Legionella pneumophila*, both of which were

considered correct. Overall, five participants reported on the identification, five reporting on Sg and none reporting on the ST. One specimen (5708) contained commensals only, no *Legionella* spp. was present. All participants reported the correct negative result.

From the methods survey, the majority of laboratories (6/7) reported isolation and identification using culture-based methods. A total of 4/7 then went on to perform molecular methods for the detection of *Legionella* spp.. No laboratories reported the use of whole genome sequencing.

The performance for urinary antigen testing was very good, with an overall mean concordance of 97% of participating laboratories returning a correct result. Overall, performance in identification, serogroup and urinary antigen test detection was very good.

With regard to environmental laboratories, a total of seven laboratories from six EU enlargement countries were sent this EQA (two in Albania and one in Bosnia and Herzegovina, Montenegro, North Macedonia, Serbia and Turkey). Five laboratories in the EU enlargement countries returned a result for this EQA exercise. Albania did not return any examination results; however, one laboratory did provide method information for the methods survey.

For *Legionella* isolation, the overall performance was considered to be below average. Six of the ten simulated samples contained a *Legionella* spp.. The overall performance for correctly identifying the *Legionella* spp. when a sample contained this organism was 66%. Seven of the ten samples were simulated water samples which allowed laboratories to report an enumeration result. The overall performance for the enumeration results reported within the expected range was 68%. The overall performance for reporting a correct serogroup was very good with 83%. For sequence type the overall performance of reporting a correct sequence type was not assessed as only one laboratory reported a result of which four out of five sample results were incorrect.

For the environmental laboratories, 5 laboratories reported a result for isolation, 4 for identification and serogroup and enumeration count and one laboratory undertook sequence type testing. With regard to molecular methods, two laboratories analysed the samples for *L. pneumophila* and one looked at those for *Legionella* spp.. The routine application of molecular methods for water and environmental samples is still being developed in laboratories due to the fact there are currently no guidelines for interpretation of molecular results (GU/L). Therefore, culture remains the preferred method.

With regard to molecular methods, two laboratories analysed the samples for *L. pneumophila* concurrently with culture methods. The overall performance with detection/absence of *L. pneumophila* for the 10 samples was low as one laboratory reported incorrect results. For molecular detection/absence of *Legionella* spp. only one laboratory undertook this method of examination and 5/10 sample results were incorrect.

The performance of laboratories in this exercise within the EU enlargement countries was very good for culture-based/detection methods used by clinical laboratories (96.2%). For environmental laboratories, the performance was much lower at 66%, which was below average. Environmental laboratories struggled to achieve the correct results for isolation, reporting counts in the expected range for water samples and for ST.

Laboratories have demonstrated that they can undertake testing to an acceptable level of at least 80% concordance with expected results for clinical laboratories. Results for the environmental laboratories were below the average of 70%. This data provides limited assurance of the ability of laboratories in enlargement countries to undertake effective public health investigations for *Legionella pneumophila.* Further EQA rounds will provide more data on performance and the robustness of testing.

In replies to an ECDC questionnaire, laboratories indicated that this EQA exercise was very useful and overall there was very positive feedback on the new ECDC EQA *Legionella* scheme.

Background

Legionnaires' disease (LD) 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 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 routinely be able to differentiate between *L. pneumophila* and other *Legionella* spp. and to 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. LD is described as a severe pneumonia that may be accompanied by systemic symptoms and may lead to a fatal outcome. Cases of LD are mainly reported among persons in older age groups (>50 years), especially males. Other known risk factors for LD 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 the identification of the source of infection often requires international collaboration.

LD in Europe is thought to be under-reported for two main reasons:

- it is underdiagnosed by clinicians who may not test patients for LD before empirically prescribing antibiotics likely to cover *Legionella* spp.;
- some health professionals may fail to notify cases to health authorities. Furthermore, under-ascertainment and differences in laboratory practice may also partly explain the variations in notification rates observed.

Legionnaires' disease surveillance has been carried out at European level since 1987, firstly through a dedicated surveillance network funded by the European Commission and then from April 2010, through ELDSNet (European Legionnaires' Disease Surveillance Network) 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 is available through the European Surveillance Atlas on ECDC's website. A second ELDSNet surveillance system focuses on Travel Associated Legionnaires' Disease (TALD) cases and the Western Balkan countries and Turkey have been able to participate in this system.

The aim of ELDSNet is to detect and communicate on clusters and outbreaks of TALD. The network supports the 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;
- 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, via 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.

EQA exercise 2019−2020

In 2019, ECDC organised an EQA exercise for the detection/isolation, identification, enumeration and quantification of *Legionella* spp. and further characterisation of *L. pneumophila* through serogrouping and sequence-based typing from both clinical and environmental samples, where applicable. The EQA was organised in collaboration with the Food and Environmental Proficiency Testing Unit (FEPTU) of Public Health England (PHE) and the United Kingdom National External Quality Assessment Service (UK NEQAS).

The purpose of this EQA exercise 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 2019 EQA exercise for the EU enlargement

countries. The results provided ECDC with information on the laboratories' capabilities for accurately performing *Legionella* testing. This helped to provide confidence in data submitted in relation to the TALD surveillance system; 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 2019−2020 EQA were:

- to provide a baseline understanding of the level of testing undertaken in laboratories in response to routine outbreak scenarios, for both clinical and environmental samples;
- to determine where there were any general performance issues;
- to provide individual technical support to laboratories as a follow-up to the exercise, if requested by the countries.

Study design and methods

Organisation of EQA

This EQA was organised by FEPTU and UK NEQAS for Microbiology in collaboration with RVPBRU, PHE and ECDC as part of an ECDC Framework contract (ECDC/2019/024). The EQA exercise was for laboratories nominated through national ECDC correspondents (in the Western Balkans and Turkey), and up to two nominated laboratories per country (to cover clinical and/or environmental samples) could participate per round. Two rounds were foreseen for 2019−2020, however due to the COVID-19 situation, only one was implemented.

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 user name and passwords generated for each one. This allowed the laboratory to return results and view individualised reports through a secure web portal and it meant that the results were anonymised for ECDC.

Both FEPTU and UK NEQAS are UKAS accredited EQA providers to the ISO/IEC 17043:2010 (Conformity assessment - General requirements for proficiency testing) and all these principles and practices were applied to the ECDC EQA scheme.

- The EQA distribution was sent on 4 November 2019 to a maximum of two laboratories per country for a total of seven EU enlargement countries.
- ECDC invited national ECDC correspondents (in the Western Balkans and Turkey) to propose up to two laboratories to take part in this EQA exercise - one that undertakes clinical examination of specimens and one that examines environmental samples. One laboratory could also be nominated to participate in both the clinical and environmental part, if they usually processed both types of sample. 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, directly informing them of the EQA arrangements and objectives of the exercise. The letter also provided an opportunity for the laboratories to confirm their interest in participating and to ensure that their details in the system were correct.
- The 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 PHE *Legionella* experts.
- PHE undertook testing of the samples 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 (serogrouping (Sg) and sequence-based typing (SBT)) were also undertaken.
- PHE also ran a separate survey on methods/kit information and frequency of testing performed for each method/kit by the laboratories.
- The distribution exercise simulated an outbreak that was associated with a spa facility. The outbreak *Legionella pneumophila* strain chosen was serogroup (Sg) 1, sequence type (ST) 47.

A total of ten laboratories participated from seven EU enlargement countries (Table 1) with six countries participating for environmental samples and seven for clinical samples. Each laboratory was provided with a unique laboratory identification. Of those taking part, 3/10 laboratories tested both clinical specimens and environmental EQA samples (North Macedonia, Montenegro, Serbia).

Table 1. Countries within the EU enlargement area that participated in the clinical and environmental EQA

Country	Clinical EQA samples	Environmental EQA samples	Number of participating laboratories per country
Albania	Yes	Yes	2
Bosnia and Herzegovina	Yes	Yes	2
Kosovo	Yes	No	1
North Macedonia	Yes	Yes	1
Montenegro	Yes	Yes	1
Serbia	Yes	Yes	1
Turkey	Yes	Yes	2

An EQA protocol was drawn up and sent with the samples which were dispatched in approved United Nations containers. This protocol included information on the sample/specimen details, instructions on how to process samples/specimens, information on results to be provided, a copy of a method questionnaire (information to be

returned electronically) and safety information. All information was also provided electronically to all participants and was available on the UK NEQAS web portal.

A dedicated web page was available on the UK NEQAS website for participants to enter and submit their results. Participants could access instructions for using the secure web portal and download the protocol describing the process for examining the specimens. Detailed instructions were included on how to access the secure website via a unique user ID 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, there was also a copy of the web reply form available for participants to download to enable manual recording of test results prior to submission online. For this first exercise participants were allowed six weeks (42 days) from the date of dispatch of both clinical and environmental samples to examine the EQA specimens/samples and return all their results. The long time period allowed for this exercise reflects the time required to isolate the *Legionella* spp. on culture media (minimum 10 days), to undertake the relevant confirmatory testing and to obtain a result for specialist tests, such as ST, at a reference laboratory.

Six weeks after the date of dispatch (4 November 2019), the web platform was closed for results submission and the intended results were published on the secure website on 20 December 2019 where they could be accessed by participating laboratories. Participants were notified by email that the intended results were available for viewing. Individualised reports were made available ten weeks after the closing date on 6 March 2020.

From 2–23 April 2020, ECDC conducted a short online survey to obtain feedback on the EQA exercise and provide an opportunity for the laboratories to suggest improvements for the next distribution. A summary of this feedback can be found in Section 4 Discussion.

Certificates of participation were sent electronically to the laboratories on 21 April 2020. A hard copy of the certificate was available on request.

EQA exercise scenario and sample design

The strains selected for the November 2019 exercise were chosen in consultation with PHE *Legionella* experts in clinical and environmental microbiology. Sample/specimen design was also developed in collaboration with the PHE, UK NEQAS and ECDC experts and approved by ECDC.

Five outbreak environmental samples were supplied to represent spa pool water, a swab of the biofilm from the spa pool pipeline and water from three cooling towers in the same vicinity as the spa. In addition, five routine monitoring samples were supplied: water and swabs from hot and cold water systems and water from a spa pool.

The clinical samples were from six patients with suspected *Legionella* symptoms (sputum and or urine samples).

The strain of *Legionella pneumophila* serogroup 1, ST47 used for this EQA exercise is considered to be a leading cause of Legionnaires' disease in north-western Europe, however it is rarely isolated from the environment. The *Legionella* strains were provided, tested and fully characterised (before and after sample/specimen preparation) by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU).

Legionella pneumophila serogroup 3, ST2630 was also included, as this is a unique ST with a single documented isolation from a community-acquired clinical case in the UK. Legionnaires' disease attributed to *L. pneumophila* serogroup 3 is less common than detections of serogroup 1 infection. Many commercially available urinary antigen kits for *Legionella pneumophila* are not designed and validated for the detection of non-serogroup 1 type *L. pneumophila* antigens.

Strains of *Legionella* were provided by RVPBRU as fully characterised isolates; commensal/background flora was taken from a bank of organisms held by the EQA organisers and these strains were fully characterised using conventional methods and an analytical profile index system. All isolates are clinical isolates from patients with pneumonia. In 2018, 80% of respiratory samples from patients with *Legionella* were positive for *Legionella pneumophila* serogroup 1, of which ST47 is one of the more commonly isolated strains among patients from the UK. Non-serogroup 1 infections are detected at a much lower frequency. *L bozemanii* is rarely isolated from patients in the UK.

Samples/specimens were prepared and quality-controlled by the EQA organisers and the panels were dispatched as distribution 4680 (clinical) and 4681 (environmental).

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

Additional data was collected and analysed through a questionnaire on methodology used, the annual number of tests done using this method, materials and EQA participation. This information was captured electronically and findings are shown in the annex of this report.

Individual feedback was provided to the participating laboratories on their results in an environmental EQA report. This included recommendations (where necessary), actions to take and method performance (if applicable.) The PHE *Legionella* expert for clinical and water microbiology provided comments on these reports based on performance and strains used.

The individualised laboratory reports detailed a laboratory's reported results for each examination requested and the intended results for each sample (including the simulated microbiological contents). This included the identification of the *Legionella* species, serogroup, serotype, enumeration results, where applicable. The report also provided an overall performance for each examination based on all the laboratories reported results.

Clinical

A total of seven sets of specimens were distributed to seven participating countries. Ten clinical samples were prepared in each set (five simulated sputum samples and five liquid urine samples.) An overview of samples is provided in Table 2.

Participants were asked to provide an organism identification, serogroup and sequence type (simulated sputum samples) and LUA result (urine specimens). Simulated sputum samples were prepared in a lyophilised format. The freeze-dried sample matrix was composed of inositol serum broth with variable concentrations of the pathogen *Legionella pneumophila* or other species. To simulate the specimen close to 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 serogroups and species of *Legionella* to be used were approved by the commissioned experts at PHE. Participants' results were analysed and considered 'concordant' if the reported categorisation agreed with the PHE reference laboratory (RVPBRU) interpretation. In addition, participants were asked to complete a questionnaire to provide further information on methods used, both in general and for this EQA exercise. Additional data was collected and analysed via a questionnaire on methodology used, the annual number of tests done for this method, materials and EQA participation. This was included in each distribution report.

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.

Two simulated specimens with no pathogens were also included in the set of 10 specimens.

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 of results (absence or presence of *Legionella pneumophila* or other species).

The simulated sputum samples were examined using the 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

Ten environmental samples were prepared as LENTICULE discs. This method of preparing samples has been extensively validated and proven to preserve organisms over a long period of time. Samples were tested in the FEPTU laboratory in accordance with the international method ISO 11731:2017 (Water quality - Enumeration of *Legionella*) for water, sludge and swabs 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 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;
- 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 onto Glycine Vancomycin Polymyxin Cycloheximide (GVPC) as neat, following heat and acid treatment. Agar plates were incubated for up to 10 days aerobically at 37°C and read on Days 3, 6 and 10. Any suspected *Legionella* spp. was ascertained by means of confirmatory testing.

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

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

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

Data was displayed graphically. Detected/not detected 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).

Results

The methods questionnaire sent to participants to gather details on processes and methods was analysed as part this EQA exercise and findings can be found in Annex 1. Key results are included in the relevant below.

Intended results for 2019/2020 exercise

Sample contents for the specimens included in the clinical and environmental distributions are described in Tables 2 and 3, including the serogroup and sequence base type when *Legionella pneumophila* was present.

The intended results to be returned by participating laboratories for each specimen are listed in Tables 4–15 for clinical samples and Tables 16–18 for environmental samples.

Table 2. Clinical specimens 5706 – 5715 provided in distribution (4 November 2019)

Specimen number	Patient	Sample	Sample type	Sample contents	Sg	SBT	Details
5706	1	1	Sputum	*Legionella pneumophila* *Streptococcus oralis* *Streptococcus salivarius*	1	47	Patient lives in location of cooling tower, has visited spa
5707		2	Urine	*Legionella pneumophila*	1	47	
5708	2	1	Sputum	*Neisseria sicca* *Streptococcus mitis*			Patient lives in location of cooling tower, has visited spa
5709		2	Urine	*Legionella pneumophila*	1	47	
5710	3	1	Sputum	*Legionella pneumophila* *Streptococcus oralis* *Streptococcus salivarius* *Moraxella catarrhalis*	1	47	Patient lives in location of cooling tower, has visited spa
5711		2	Urine	*Legionella pneumophila*	1	47	
5712	4	1	Sputum	*Legionella pneumophila* *Streptococcus mitis* *Streptococcus salivarius*	3	2630	Asthmatic elderly male, lives at home in local area, rarely goes out, except to the shops and local public inn.
5713		2	Urine	*Legionella pneumophila*	3	2630	
5714	5	1	Sputum	*Legionella longbeachae* *Streptococcus oralis* *Streptococcus salivarius*			Community acquired pneumonia, keen gardener, elderly.
5715	6	1	Urine	No organisms			Patient on ECMO, severe pneumonia.

Table 3. Environmental samples 5716 – 5725 provided in the distribution (4 November 2019)

Specimen number	Sample type	Sample contents	Sg	SBT	Comments
5716	Spa water from site 1	*Legionella pneumophila* *Microbacterium luteolum*	1	47	Samples taken as part of an outbreak investigation.
5717	Biofilm swab from spa water pipeline (site 1)	*Legionella pneumophila* *Staphylococcus saprophyticus*	1	47	
5718	Cooling tower water (site 2)	*Klebsiella pneumoniae* *Staphylococcus haemolyticus* *Enterococcus faecium*			
5719	Cooling tower water (site 3)	*Legionella pneumophila* *Enterococcus faecalis*	1	47	
5720	Cooling tower water (site 4)	*Klebsiella pneumoniae* *Staphylococcus haemolyticus* *Enterococcus faecium*			
5721	Hot and cold water system	*Legionella pneumophila* *Brevundimonas vesicularis* *Aerococcus viridans*	1	48	Samples taken as part of routine quality monitoring of water.
5722	Biofilm swab from hot and cold water system	*Pseudomonas putida* *Staphylococcus epidermidis*			
5723	Hot and cold water system	*Legionella bozemanii* *Acinetobacter junii* *Pseudomonas stutzeri*			
5724	Spa water	*Legionella pneumophila* *Citrobacter braakii*	6	2923	
5725	Biofilm swab from hot and cold water system	*Roseomonas aestuarii* *Pseudomonas aeruginosa*			

Clinical

A total of five laboratories from seven countries reported results for the simulated sputum samples, compared to six for urine samples.

From the methods questionnaire it was determined that two participants had reported themselves as clinical diagnostic laboratories and reference laboratories. Five laboratories reported that they were only clinical diagnostic laboratories. Only one laboratory reported being a reference laboratory.

A total of 3/7 laboratories participated in a national scheme. None of the seven laboratories reported a mandatory scheme.

The most commonly tested sample types reported by the laboratories were sputum, broncho-alveolar lavage, blood and urine. BYCE was the most frequently used media for the isolation of *Legionella* spp., together with Gram stain and real-time PCR as the confirmatory tests. (See results in Annex 1).

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

Specimen 5706: This specimen contained *Legionella pneumophila* serogroup 1: ST47. An excellent concordance with intended results was achieved for all participating laboratories.

Table 4. *Legionella pneumophila* (5706) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories

Intended interpretation	Number of laboratories returning correct result/total number reporting a result	Overall concordance (%)
Legionella pneumophila	5/5	100
Serogroup 1	5/5	100
Sequence type 47	0	-

Specimen 5707: This specimen was positive for *Legionella pneumophila* urinary antigen. An excellent concordance with intended results was achieved for all six participating laboratories returning a result.

Table 5. ***Legionella pneumophila*** **(5707) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories**

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila antigen detected	6/6	100

Specimen 5708: This specimen contained *Neisseria sicca* and *Streptococcus mitis* only, no *Legionella* spp. was present. A good concordance with intended results was achieved.

Table 6. Negative for ***Legionella*** **(5708) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories**

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Negative	5/5	100

Specimen 5709: The specimen was positive for *L. pneumophila* urinary antigen. A good concordance with intended results was achieved.

Table 7. ***Legionella pneumophila*** **(5709) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories**

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila serogroup 1 antigen detected	6/6	100

Specimen 5710: The specimen contained *Legionella pneumophila* serogroup 1: ST47. An excellent concordance with intended results was achieved for all participating laboratories.

Table 8. ***Legionella pneumophila*** **(5710) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories**

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila	5/5	100
Serogroup 1	5/5	100
Sequence Type 47	0	-

Specimen 5711: The specimen was positive for *L. pneumophila* urinary antigen. One laboratory incorrectly reported the results as 'antigen not detected'. A good concordance with intended results was achieved for the majority of participating laboratories.

Table 9. ***Legionella pneumophila*** **(5711) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories**

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila antigen detected	5/6	83.3

Specimen 5712: The specimen contained *Legionella pneumophila* serogroup 3: ST2630. A very good concordance with intended results was achieved for identification all participating laboratories returning a result. Only three out of five laboratories tested for serogroup.

Table 10. *Legionella pneumophila* (5712) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila	4/5	80
Serogroup 3	2/3	66.7
Sequence Type 2630	0	-

Specimen 5713: The specimen was negative for *L. pneumophila* urinary antigen. An excellent concordance with intended results was achieved for all participating laboratories.

Table 11. *L. pneumophila* antigen not detected (5713) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila antigen not detected	6/6	100

Specimen 5714: The specimen contained *Legionella longbeachae.* One laboratory reported the result incorrectly as *Legionella pneumophila* and one laboratory did not test the sample. A good concordance with intended results was achieved for the majority of participating laboratories.

Table 12. *Legionella longbeachae* (5714) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella longbeachae	1/4	75
Legionella species, not *L. pneumophila*	2/4	

Specimen 5715: The specimen was negative for *L. pneumophila* urinary antigen. A very good concordance with intended results was achieved for the majority of participating laboratories.

Table 13. *L. pneumophila* antigen not detected (5715) - intended results reported by the PHE reference laboratory and overall concordance with participating laboratories

Intended interpretation	Number of laboratories returning correct results/total number reporting a result	Overall concordance (%)
Legionella pneumophila antigen not detected	6/6	100

Summary of results by specimen type

Overall determination of Sg was good, with 100% of participating laboratories attaining the intended result with Sg1. However, only 66.7% concordance was achieved when an Sg other than Sg1 (specimen 5712), was tested.

Table 14. Serogroup concordance for simulated sputum specimens containing *Legionella* spp.

Specimen number	Serogroup
5706	100 (n=5)
5710	100 (n=5)
5712	66.7 (n=2)

The performance for urinary antigen testing was very good, with an overall mean concordance (across the five specimen numbers) of 97% of participating laboratories returning a correct result.

Table 15. Urinary antigen result concordance

Specimen number	LUA result *L. pneumophila* antigen	Overall concordance (%)
5707	Detected	100
5709	Detected	100
5711	Detected	83.3
5713	Not detected	100
5715	Not detected	100

Environmental samples

Ten simulated environmental samples were sent to seven laboratories in six EU enlargement countries. Five laboratories returned a result for this EQA exercise, two laboratories did not examine the samples.

Sample numbers: 5716–5720 were samples taken as part of an outbreak investigation.
Sample numbers: 5721–5725 were samples were taken as part of routine monitoring.
Sample numbers: 5717, 5722 and 5725 were swab samples.
Sample numbers: 5716, 5718, 5719, 5720, 5721, 5723 and 5724 were water samples.

Laboratory performance was split into culture-based methods (Table 16) and molecular methods (Table 17). Culture-based method analysis included results reported for isolation, identification, enumeration and serogroup. An overall performance assessment column as a percentage has been captured for culture-based methods results by sample number and by each examination (including isolation, identification, enumeration and serogroup). Overall performance by sample was calculated using the mean value across a maximum of four examinations. ST was excluded from the overall performance calculation as only one laboratory reported a result, for which only 1/5 samples was correct. For culture results the overall performance is shown by examination only. Only two data sets were returned for molecular results and quantification results, therefore these have not been statistically analysed as the data generated would not be robust.

Table 18 shows in more detail the enumeration results reported by laboratories.

Table 16. Examinations done on cultured samples

Sample number	Contents	Isolation		Identification		Enumeration		Serogroup		Sequence type		Overall % performance by sample
		N	%	N	%	N	%	N	%	N	%	
5716	*L. pneumophila* sg 1, ST47	4/5	80	4/4	100	2/4	50	3/3	100	0/1	0	83
5717	*L. pneumophila* sg 1, ST47	4/5	80	4/4	100			4/4	100	0/1	0	93
5718	No Legionella	2/5	40									Not calculated
5719	*L. pneumophila* sg 1, ST47	3/5	60	3/3	100	2/3	67	3/3	100	0/1	0	82
5720	No Legionella	2/5	40									Not calculated
5721	*L. pneumophila* sg 1, ST48	3/5	60	3/3	100	3/3	100	3/3	100	0/1	0	90
5722	No Legionella	5/5	100									Not calculated
5723	*Legionella bozemanii*	4/5	80	2/3	67	1/4	25	-	-			57
5724	*L. pneumophila* sg 6, ST2923	2/5	40	2/2	100	2/2	100	0/1	0	1/1	100	60
5725	No Legionella	4/5	80									Not calculated
Overall performance by examination			**66**		**94**		**68**		**83**		**20**	

Table 17. Data on molecular methods, number of laboratories returning the correct result/total number who performed test

Sample number	Identification	Intended results for *Legionella pneumophila*	Molecular results *Legionella pneumophila* (%)	Quantification *Legionella pneumophila*	Intended results for *Legionella* spp.	Molecular results *Legionella* spp. (%)	Quantification *Legionella* spp.
5716	*Legionella pneumophila*	Detected	2/2 (100)	2 (1490, 41544)	Detected	0/1 (0)	-
5717	*Legionella pneumophila*	Detected	2/2(100)	2 (970, 38320)	Detected	0/1 (0)	-
5718	No Legionella	Not detected	1/2 (50)	1 (52350)*	Not detected	1/1 (100)	
5719	*Legionella pneumophila*	Detected	2/2 (100)	2 (7200, 34958)	Detected	0/1 (0)	-
5720	No Legionella	Not detected	1/2 (50)	1 (56157)*	Not detected	1/1 (100)	
5721	*Legionella pneumophila*	Detected	2/2 (100)	2 (50543, 16900)	Detected	0/1 (0)	-
5722	No Legionella	Not detected	1/2 (50)	1 (54007)*	Not detected	1/1(100)	
5723	*Legionella bozemanii*	Detected	1/2 (50)		Detected	1/1 (100)	2 (5130, 50909)
5724	*Legionella pneumophila*	Detected	1/2 (50)	1 (49643)	Detected	0/1 (0)	-
5725	No Legionella	Not detected	1/2 (50)		Not detected	1/1 (100)	

** This is a false positive result reported for the sample.*

Table 18. Data on enumeration results

Sample number	Identification	Participants median (cfu/L)	Expected Range (cfu/L)	Number of results	Number of outlying counts
5716	*Legionella pneumophila*	$3.1x10^4$	$3.3x10^3 – 6.9x10^4$	4	2 (1 low, 1 high)
5717	*Legionella pneumophila*	-	-	1	-
5718	No Legionella			3	
5719	*Legionella pneumophila*	$2.2x10^4$	$1.1x10^3 – 8.7x10^4$	3	2 low
5720	No Legionella	-	-	3	-
5721	*Legionella pneumophila*	$1.1x10^3$	$98 – 3.9x10^3$	3	All in range
5722	No *Legionella*				
5723	*Legionella bozemanii*	$5.5x10^3$	$87 – 2.8x10^4$	4	1 low
5724	*Legionella pneumophila*	$1.3x10^3$	$1.1x10^2 – 9.8x10^3$	2	All in range
5725	No *Legionella*				

Sample 5716. This sample contained water from a spa (site 1) with a *Legionella pneumophila* serogroup 1 sequence type ST47 at levels of approximately 10^5 colony forming units (cfu) per litre. The background flora was *Microbacterium luteolum*.

Performance was very good, with 4/5 of participants reporting the correct isolation result, 4/4 for identification, 2/4 of the laboratories reporting a count within the expected range and 3/3 reporting the correct serogroup. One laboratory did the examination for sequence type and reported an incorrect result. The overall performance for examinations by culture was 83%. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and both reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported an incorrect result.

Sample 5717. This sample was a swab from a biofilm of a spa water pipeline (site 1) which contained a *Legionella pneumophila* serogroup 1 sequence type ST47 at levels of approximately 10^5 colony-forming units per litre. The background flora was *Staphylococcus saprophyticus*.

Performance was very good, with 4/5 of participants reporting the correct isolation result, 4/4 for identification, and 4/4 reporting the correct serogroup. One laboratory did the examination for sequence type and reported an incorrect result. The overall performance for examinations by culture was 93%. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only, both reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported an incorrect result.

Sample 5718. This sample was water taken from a cooling tower (site 2) which contained no *Legionella* spp.. Background flora were *Klebsiella pneumoniae*, *Staphylococcus haemolyticus* and *Enterococcus faecium.* Performance was below average, with 2/5 of the laboratories reporting the correct isolation result of no Legionella detected (Table 16). Two laboratories reported that the sample contained *L. pneumophila* serogroup 1. The overall performance for *Legionella* examinations by culture for the sample has not been calculated. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only, and one reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported a correct result. Three laboratories provided enumeration results for *Legionella*.

Sample 5719. This sample was water taken from a cooling tower (site 3) which contained a *Legionella pneumophila* serogroup 1 sequence type ST47 at levels of approximately 10^4 colony-forming units per litre. Background flora was *Enterococcus faecium*.

Performance was below average, with 3/5 of participants reporting the correct isolation result, 3/3 for identification, 2/3 of the laboratories reporting a count within the expected range and 3/3 reporting the correct serogroup. One laboratory did the examination for sequence type and reported an incorrect result. The overall performance for legionella examinations by culture was 82%. Two laboratories examined the sample using a molecular kit which only detects *L. pneumophila* and both reported the correct result. In addition, one of these laboratories examined the sample using a molecular kit that detects *Legionella* spp. and reported an incorrect result.

Sample 5720. This sample was water taken from a cooling tower (site 4) which contained no *Legionella* spp.. Background flora were *Klebsiella pneumoniae*, *Staphylococcus haemolyticus* and *Enterococcus faecium.*

Performance was below average, with 2/5 of the laboratories reporting the correct isolation result, one laboratory incorrectly reported that *L. pneumophila* serogroup 1 was present and two laboratories incorrectly reported the isolation of a *Legionella* spp.. The overall performance for *Legionella* examinations by culture for this sample has not been calculated. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and one reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported a correct result.

Sample 5721. This sample was a water taken from a cooling tower which contained a *Legionella pneumophila* serogroup 1 sequence type ST48 at levels of approximately 10^2 colony-forming units per litre. Background flora were *Brevundimonas vesicularis* and *Aerococcus viridans*.

Performance was good with 3/5 of participants reporting the correct isolation result, 3/3 for identification, 3/3 of the laboratories reporting a count within the expected range and 3/3 reporting the correct serogroup. One laboratory did the examination for sequence type and reported an incorrect result. The overall performance for *Legionella* examinations by culture was 90%. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and both reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported an incorrect result.

Sample 5722. This sample was a swab of a biofilm from a hot and cold water system which contained no *Legionella* spp.. Background flora were *Pseudomonas putida* and *Staphylococcus epidermidis*.

Performance was excellent, with 5/5 of the laboratories reporting the correct result.

Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and one reported the correct negative result for *Legionella*. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported the correct negative result for *Legionella*.

Sample 5723. This sample was water taken from a hot and cold water system which contained *Legionella bozemanii* at levels of approximately 10^3 colony-forming units per litre. Background flora were *Acinetobacter junii* and *Pseudomonas stutzeri.*

Performance was good, with 4/5 of participants reporting the correct isolation result, 2/3 for identification and 1/4 of the laboratories reporting a count within the expected range. The overall performance for *Legionella* examinations by culture was 57%.

Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and one reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp. and reported an incorrect result.

Sample 5724. This sample was water from a spa which contained a *Legionella pneumophila* serogroup 6 sequence type ST2923 at levels of approximately 10^3 colony-forming units per litre. Background flora was *Citrobacter braakii*.

Performance was below average, with 2/5 of participants reporting the correct isolation result, 2/2 for identification, 2/2 of the laboratories reporting a count within the expected range and 0/1 reporting the correct serogroup. One laboratory did the examination for sequence type and reported an incorrect result.

The overall performance for legionella examinations by culture was 60%. Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only, one reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and reported an incorrect result.

Sample 5725: This sample was a swab of a biofilm from a hot and cold water system which contained no *Legionella* spp.. Background flora included was *Roseomonas aestuarii* and *Pseudomonas aeruginosa.*

Performance was good, with 4/5 of the laboratories reporting the correct isolation result of no *Legionella*.

Two laboratories examined the sample using a molecular kit which detects *L. pneumophila* only and one reported the correct result. In addition, one of these laboratories also examined the sample using a molecular kit that detects *Legionella* spp., and reported a correct result. Overall, it was not possible to compare performance of culture versus molecular methods due to the very low numbers of results received. In addition, performance of quantification results by molecular methods could not be analysed as there were not enough data sets for robust statistical calculation of the expected range.

Figures 1 and 2 below illustrate the turnaround time for reporting results via the on-line secure system to PHE to complete the exercise. The mean number of days for returning results for clinical laboratories was 31. For environmental laboratories, the mean was 35 days.

Figure 1. Turnaround time for reporting results – clinical laboratories

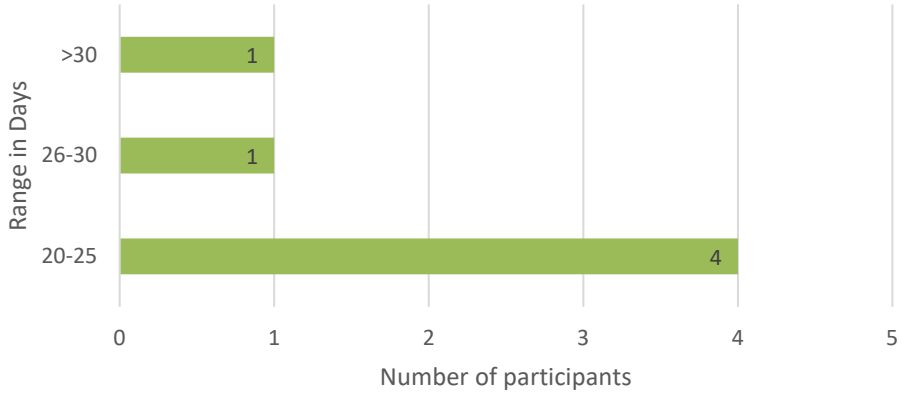
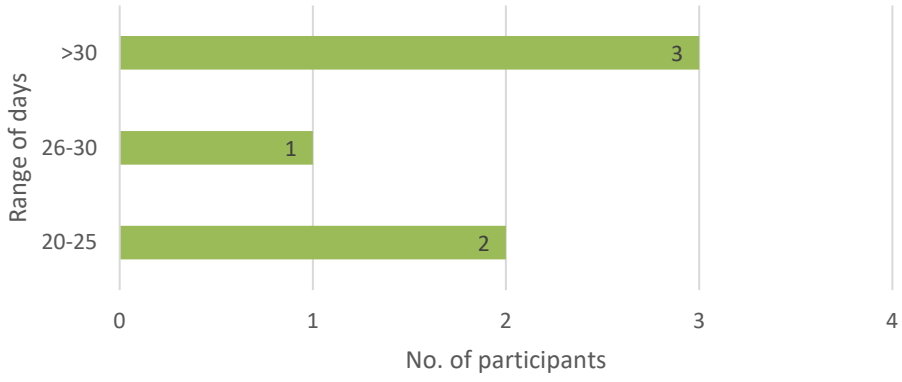


Figure 2. Turnaround times for reporting results - environmental laboratories



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 provision of quality results for samples 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. It also ensures high quality of the surveillance data reported.

Overall, for the clinical sample EQA, the performance of laboratories participating in the 2019−2020 EQA was very good. Concordance with the intended results was 100% for all laboratories reporting the identification and serogroup correctly. However, there was a reduced number of laboratories reporting a result against specimen 5712 (n=2), despite data from the methods questionnaire illustrating a higher number of laboratories having the capacity to test. Furthermore, no laboratories reported the sequence type. In contrast, a very good performance was achieved for urinary antigen testing, with a mean concordance of 96.6%.

For environmental laboratories, the performance was much lower at 66%, which is below average. Environmental laboratories struggled to achieve the correct results for isolation, reporting of counts in the expected range for water samples and for ST.

The sequence results were not assessed as only one laboratory undertook this examination and reported an incorrect result for four out of the five samples.

The outbreak strain chosen to simulate samples and specimens was *L. pneumophila* Sg 1, ST47. Two clinical specimens (5712 and 5713) contained *Legionella pneumophila* serogroup 3, ST2630. This is a unique ST, with a single documented isolation from a community-acquired clinical case in the UK. Legionnaires' disease attributed to *L. pneumophila* serogroup 3 is less common than serogroup 1 infection and many of the commercially available urinary antigen kits are not designed or validated for the detection of *L. pneumophila* non-serogroup 1 antigens.

Comparing clinical and environmental isolates using serological and molecular techniques can help identify the source of Legionnaires' disease during potential outbreak investigations. *Legionella* is frequently found in the environment, and examination of clinical isolates can help interpret the findings of an environmental investigation.

There were no issues encountered with the preparation of the simulated specimens/samples. Homogeneity, stability and viability were consistent throughout all the stages of production and distribution. To maintain these parameters, proven technology for preserving organisms/levels of organisms was used, such as lyophilised or LENTICULE® disc. This preservation technique, used to produce simulate EQA samples/specimens, meant that stability of the organisms would probably be guaranteed during transit of the distributions to the seven EU enlargement countries. This was important, given that transit time would probably be longer than that for local or national distribution of samples to designated laboratories.

From the results reported in this EQA it was ascertained that for clinical samples the majority of laboratories identified the pathogen and serogroup, but a significant number did not report the ST (Tables 1, 5 and 7). For environmental samples the ISO 11731:2017 requires that suspect colonies are identified to at least *L. pneumophila*. Laboratories did report *L. pneumophila* correctly and went further, reporting a serogroup. Like the clinical finding, only one laboratory examined the sample for ST.

Clinical discussion

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

Based on published guidance by PHE in the UK, the three most commonly described sample types analysed were urine and lower respiratory tract fluids including sputum and broncho-alveolar lavage (BAL). Using this information, simulated sputum and urine specimens were designed for distribution as part of the EQA exercise. A survey of methods (Annex 1) was sent out simultaneously with the EQA panel and this confirmed the most common specimen types examined routinely by participating laboratories to be sputum 71.4% (5/7), urine 100% (7/7), BAL 57.2% 4/7 and blood samples 57.2% (4/7).

Three paired (sputum/urine) simulated specimens (5707, 5708; 5709, 5710; 5711, 5712) with relevant accompanying clinical details were sent for evaluation. These specimens were designed to mimic an outbreak.

One laboratory did not test the lyophilised sputum specimens and none of the laboratories within the EU enlargement countries performed sequence typing. The non-performance of sequence typing might be a reflection of the level of services provided by the participating laboratories. All participating laboratories were clinical diagnostic (n=7) and two were also regional reference laboratories (RRL), and all RRLs would be expected to report the ST. Further information will be captured in future EQA schemes, to determine the reason why no sequence typing was performed.

Patient 1

Both the simulated sputum (specimen 5706) and the urine sample (specimen 5707) contained *Legionella pneumophila* Sg1 ST47, one of the most prevalent circulating serogroups for *Legionella pneumophila*.

Of the five laboratories reporting an identification and serogroup, 100% attained the correct result for the presence of *Legionella pneumophila* Sg1.

All six laboratories who tested for urinary antigen attained the correct positive result, evidently meeting the desired quality of service in laboratory reporting for clinical cases of Legionnaires' disease.

Patient 2

This reflected a possible scenario in patient screening/or samples taken from a patient presenting with Legionnaires' disease symptoms, but not infected with *Legionella* spp.. Patient 2 sputum specimen 5708 was negative for the presence of the pathogen and 100% of the laboratories correctly reported the absence of the organism.

In contrast, the urine for patient 2 (specimen 5709) was a simulated urine containing a urinary *Legionella* antigen and here too, 100% of laboratories reported the correct result.

To conclude, the overall intended results for this patient could represent the possibility of an inadequate sputum specimen having been received, and only the positive urine result having been taken into consideration for interpretation of Legionnaires' disease. In this case, a request for a repeat sputum or more sensitive specimen (e.g. BAL) would be appropriate.

Patient 3

With the third set of samples, the sputum specimen (5710) contained a high yield of *L. pneumophila* Sg1, ST47 and a very high level of urinary antigen (specimen 5711) for *L. pneumophila* Sg1, ST47. A 100% concordance was achieved from laboratories reporting the identification and serogroup. Of the urinary antigen results, 83.3% reported the correct result, detecting the *Legionella* antigen. Performance was satisfactory (urinary antigen) to excellent (identification/serogroup) in concordance with the intended results for all samples. This demonstrates the competence level of participating laboratories in isolating and identifying the causative agent and detecting the presence of circulating antigen. There was one discrepant result which would prompt further investigation into the possible reason for failure.

Patient 4

One set of simulated samples (5712 and 5713) contained *L. pneumophila* Sg 3, ST2630 and this ST was included as a patient suspected of having had contact with *Legionella*, with indicative accompanying clinical details (asthmatic elderly male, lives at home local area, rarely goes out, except to the shops and local pub). This set of patient samples is not associated with the simulated outbreak, based on the reported Sg and ST and an absence of the antigen for *L. pneumophila* Sg1 being detected in the urine.

An 80% concordance was achieved with 4/5 reporting the correct species and 66.7% (2/3), reporting the correct Sg.

There are a plethora of testing kits available for use in clinical diagnostic laboratories which are designed and validated for the detection of the surface antigen for Sg 1 in urine (serogroup 1 being the predominant circulating antigen). Results for specimen 5713 concluded that the kits used by the participants do not detect the Sg3 circulating antigen and this was confirmed by all 6/6 laboratories reporting the absence of *Legionella* antigen.

With regard to concordance with the intended results, performance was very good to excellent for all four sets of samples, demonstrating the competence level of participating laboratories in isolating and identifying the causative agent and detecting the circulating *Legionella* antigen.

The overall performance between the results reported by the clinical laboratories and those reported by environmental laboratories was very good for the three sets of samples and clearly demonstrated that the fourth set of samples was not associated with the 'outbreak'.

Patient 5

Legionella longbeachae is the second most commonly reported causative agent of Legionnaires' disease. *L. longbeachae* was distributed in a simulated sputum specimen (5714) as an educational objective. This proved a little challenging, with only 75% of participants reporting the correct result of a non-pneumophila *Legionella*. This was derived from one laboratory reporting the presence of *L. longbeachae* and two reporting the absence of *L. pneumophila*. This illustrates the different testing methods adopted by the reporting laboratories. More details on the methodologies used will be obtained and collated in the next exercise.

Patient 6

EQAs often include a negative sample in the assessment. It is just as important for the participating laboratories to be able to provide a true negative result as it is to determine a positive one. Specimen 5715 was a simulated urine, containing no antigen. Participant performance was excellent with 6/6 (100%) of laboratories reporting the absence of circulating *L. pneumophila* antigen.

The various methods used for the identification of the pathogen were indicated by laboratories in the questionnaire and conventional testing (culture) and Gram stain were the most commonly-reported. The most frequently reported confirmatory tests included real-time PCR.

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 (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. In this EQA exercise six out of seven participants reported culturing the simulated sputum samples, five performed a Gram stain and four went on to perform real-time PCR. Other methods used included UV microscopy (1), serology (1), MALDI-TOF (2), semi-automated methods (1), ELISA (2), immunochromatographic tests (1) and monoclonal antibody typing (2).

Most failures with EQA specimens can be a result of inadequacies in the other components of the quality system, including methodologies used.

Environmental discussion

The environmental aspect of this EQA was a qualitative and quantitative exercise designed to assess simulated environmental waters 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/quantification with accompanying Sg and ST, if applicable and as requested.

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 (TMVs). 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 background flora [4].

Polymerase chain reaction (PCR) method is a molecular technique that only takes a few hours to complete and can be useful for the screening of 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. In 2015, the ISO/TS 12869 standard method was published for the detection and quantification of *Legionella* and/or *L. pneumophila* by concentration and genic amplification using real-time polymerase chain reaction (qPCR) in order to standardise this methodology.

A methods survey questionnaire (Annex 1), sent out simultaneously with the EQA panel, confirmed that the most common sample types routinely examined by participating laboratories are water from hot/cold water systems 100% (5/5), cooling towers waters 100% (5/5), water from spas 100% (5/5) and swabs from biofilms 100% (5/5).

The overall performance of the laboratories in the EU enlargement countries was below average for isolation and enumeration examinations and very good for identification and serogroup tests. The sequence results were not

assessed as only one laboratory undertook this examination and reported an incorrect result for four out of the five samples. A total of five laboratories examining water samples for *Legionella* bacteria indicated that they followed ISO 11731:2017 (Water quality - Enumeration of *Legionella*). All laboratories that returned information responded that they filtered the water sample. The majority cultured from the untreated sample or after heat and acid treatment.

- For isolation: the overall performance for isolation of *Legionella* was 66%, with up to five of the laboratories reporting a result. One laboratory consistently reported an incorrect isolation result for samples 5716, 5718, 5719, 5720, 5721 and 5724 (all water samples). Another laboratory reported incorrect result for samples 5717, 5718, 5719, 5720 and 5721 (four water and one swab samples). The most common isolation media used was GVPC and/or BCYE. There was variation between laboratories in the use of other culture media and whether acid and/or heat treatment was applied.
- For identification: six of the ten samples contained a *Legionella* spp.. The overall performance for correctly identifying the *Legionella* spp. was 94%, with up to four of the laboratories reporting a result. Sample 5723 contained a *Legionella bozemanii*, one laboratory incorrectly reported the *Legionella* as *Legionella pneumophila*.
- For enumeration: seven of the ten samples were simulated water samples. Of these, five contained a *Legionella* spp.. The overall performance for counts being reported within the expected range was 68%. Performance was lower with three samples, 5716 (50%), 5719 (67%) and 5723 (25%). This may be due to the low bacterial load and therefore being at the lower end of the detection limit for methods used (10^2-10^3).
- For serogroup: the overall performance for serogroup confirmation was good, with 83% of results being reported correctly. One laboratory reported an incorrect serogroup 14 for sample 5724 when the strain was a serogroup 6. According to the data from the questionnaire, a majority of the laboratories used the 'Oxoid Dry spot' *Legionella* latex test.
- For sequence type: the overall performance of reporting a correct sequence type was very poor, only one laboratory did this examination and returned a correct result for 1/5 of the samples, ST8 was reported for the four incorrect samples.
- For molecular methods: only two laboratories analysed the samples for *L. pneumophila* using molecular methods concurrently with culture methods. The overall performance with detection/absence of *L. pneumophila* for the 10 samples was not assessed. One laboratory reported an incorrect result for samples 5718, 5720, 5722, 5723, 5724 and 5725: samples 5722 and 5725 did not contain *L. pneumophila*. For molecular detection/absence of *Legionella* spp. the overall performance was not determined. Only one laboratory examined the samples using a molecular method for *Legionella* spp.. They reported an incorrect result for samples 5716, 5717, 5719, 5721 and 5724. An analysis of the kits used from the method questionnaire did not indicate that one specific molecular test was commonly being used.
- For quantification: two laboratories reported a quantification result as genomic copies per litre. Due to the low number of data sets returned, it was impossible to analyse the values.

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 as the cause of 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, which have all been pre-determined in this EQA panel.

The EQA scheme was only available to a maximum of two selected laboratories per EU enlargement country, therefore 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 of time was allocated to allow sufficient time for the panel to arrive at the laboratories via air freight. 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 and 2). Interestingly, the mean for returning results was determined to be 30 days for clinical and 35 days for environmental samples. The turnaround time to report results indicates 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). For a service provider, turnaround times of 35 and 40 days respectively would be unacceptable. However, one limitation of the system used to report results is that it does not allow for the capture of preliminary results, as some laboratories do. There is a need to understand whether the six-week period given to report results truly reflects the way in which laboratories work with genuine samples/specimens.

The clinical samples 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 detection of urinary antigens were authentic clinical liquid urine, spiked with species antigen and provided in plastic tubes to resemble a true sample.

For the environmental water samples, once the LENTICULE discs were rehydrated this would constitute one litre of water but would not be representative of the chemical constituents normally 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, however this is not representative of how a swab sample would be received in a laboratory for analysis.

For environmental samples, the molecular quantification results could not be assessed 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).

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 the presence and detection of *Legionella* spp..

The benefits of participating in this EQA are:

- it provides laboratories with an insight into their performance;
- it helps improve local standards;
- it reveals unsuspected areas of difficulty;
- it provides an educational stimulus for improvement;
- it checks the efficacy of internal quality control procedures;
- it demonstrates a commitment to quality to colleagues and customers;
- it provides a method performance evaluation
- it provides independent evidence of performance for accreditation bodies;
- it enables 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 in order to acknowledge compliance with defined objectives and quality standards, such as those detailed in ISO 17025:2017 or ISO 15189:2012. Results of consistently good quality can be expected only when all the components of a quality system are in place.

Participant feedback on this EQA

A short feedback evaluation survey was sent to all participating laboratories by ECDC after the first exercise, with the online survey open from 2–13 April 2020 and then extended to 23 April 2020.

A total of five questions were asked:

Question 1. Regarding any of your analytical test results that did not conform with the intended results, can you specify which corrective action(s), if any, was taken (e.g. review and adjust SOPs, verify reagents)?
Question 2. Are the results of this EQA exercise to be used as documentation for accreditation and/or licensing purposes for the method(s) used in your laboratory?
Question 3. Overall, is this EQA exercise important for your laboratory to assure its diagnostic capability?
Question 4. Were you satisfied with the EQA report of results specific to your laboratory?
Question 5. Do you see a benefit in participating in this EQA scheme compared to other (commercial) EQAs for *Legionella*? Please describe why/why not.

Feedback was provided by 7/11 of the EU enlargement participating laboratories. Among the respondents, 2/7 had participated in both clinical and environmental distributions, 3/7 in only the clinical distribution and 2/7 in the environmental distribution only.

Five EU enlargement country respondents indicated that corrective actions were taken based on the EQA distribution results. The types of corrective actions were that environmental samples are now sent to a specific environmental laboratory; sample preparation and handling procedures had been extended for different matrices;

procedures had been adopted for environmental samples to check for overgrowth (in the event of high background flora) and antigen kits or reagents had been replaced with different types.

In all, 2/7 participating laboratories indicated they would not use the EQA exercise results as evidence for accreditation and/or licencing purposes for the methods used. This was because the scheme was not considered to be from a recognised accredited provider or the participating laboratory itself was not accredited/needed licences for the methods used.

A total of 1/7 laboratories indicated the exercise was important but not essential, 6/7 responded that it was very important. The reasons given for its high importance were:

- use of results as documentation for accreditation;
- lack of other available EQA schemes for the laboratory to participate in (e.g. complex diagnostics for *Legionella*; no other EQA available covering culture/clinical samples; no urine antigen EQA available);
- a means of ensuring the high-quality of testing protocols to a high standard (e.g. good laboratory practice; review of sensitivity and specificity of tests using the lab methods).

Suggested areas of consideration for the next EQA round are:

- a better assessment of quantitative counts for environmental samples;
- inclusion of all data such as sequence types, allele profile, Dresden typing;
- making the environmental report clearer so that it was easier to see where the method results came from.

Responses from EU enlargement laboratories on the performance of this EQA compared with other providers included:

- inclusion of swabs in addition to water sample materials in environmental EQA;
- undertaking of testing given their actual conditions;
- ability to compare results with other laboratories participating to the distribution.

Overall, there was a very positive feedback to this new ECDC EQA *Legionella* scheme.

Although the comments and feedback did not represent all the laboratories that participated in this distribution, it is a good indication that the offer of this EQA scheme to the wider ELDSNet network of EU enlargement countries is beneficial for surveillance and response for Legionnaires` disease in Europe. Moreover, the format of this EQA scheme as an outbreak simulation, consisting of environmental samples and clinical specimens, was considered to offer added value compared to other EQA services available in Europe.

Conclusions

The performance of laboratories from the EU enlargement countries in this exercise was very good for culture-based/detection methods used by the clinical laboratories (91%). For environmental laboratories, the overall performance was much lower (66%), which is below the general expectation for performance of at least 70% for this EQA. The laboratories demonstrated that they could undertake testing to an acceptable level of at least 80% for clinical laboratories. Results were below average for the environmental laboratories. This data provided limited assurance of the laboratories' ability to undertake effective public health investigations for *Legionella pneumophila*. More EQA data is required to determine the actual robustness. If laboratories report accurate data this also ensures that the information provided to surveillance systems is accurate.

Environmental laboratories struggled to achieve the correct results for isolation, reporting of counts in the expected range for water samples and for ST. One laboratory reported incorrect results for isolation for six of the seven water samples, and another laboratory for four of the seven water samples and one of the three swab samples.

Laboratories were provided the opportunity to examine samples they would routinely test in their laboratory. For clinical specimens, up to five laboratories examined the sputum samples and six examined the urine. For environmental samples five examined the water and swab samples. Where results reported were not in accordance with those intended, laboratories were advised by contractors to investigate in order to determine the root cause.

For the clinical laboratories, five reported for identification, five for serogroup and none for sequence type. The majority of laboratories (6/7) reported isolation and identification of *Legionella spp.* using culture-based methods. 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. Differentiation and typing of strains can be achieved using a range of molecular techniques, including SBT and RT-PCR methods. Differentiation and typing of strains can be achieved using a range of molecular techniques, including SBT and RT-PCR methods. A total of 4/7 of participants went on to perform molecular methods for the detection of *Legionella*.

For the environmental laboratories, five laboratories reported a result for isolation, up to four for identification, up to four reported on serogroup, up to four reported an enumeration count and one laboratory undertook sequence type testing. For molecular methods, two laboratories analysed the samples for *L. pneumophila* and one for *Legionel*la spp. It is known that laboratories are still developing the routine application of molecular methods for water and environmental samples due to the fact there are currently no guidelines for the interpretation of molecular results (GU/L). Therefore, culture remains the preferred method.

The data analysed suggests that two environmental laboratories undertaking *Legionella* testing need additional support or training as they experienced difficulty in obtaining the correct result.

The results of a survey carried out by ECDC indicated that laboratories found this EQA exercise to be very useful. Overall, the feedback on this new ECDC EQA *Legionella* scheme was very positive.

Recommendations

This exercise will continue to provide a baseline understanding of the level of testing undertaken in the laboratories, determine any performance issues and, where possible, provide support to laboratories/countries who 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 this EQA exercise and include different *L. pneumophila* serogroups, sequence types (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 identify through further EQA exercises whether there are issues when less commonly encountered species, SG or ST are included. A single EQA distribution cannot confirm this.
- For environmental samples, to include levels of *Legionella* spp. that are at the lower end of the detection limit for culture. In addition, to confirm if molecular assessment is of value since, with low numbers of laboratories currently using this method of examination, performance cannot be assessed.
- To continue to include more than one species of *Legionella* spp. within the simulated sample/specimen set. This will help educate and improve knowledge and experience of organisms which are otherwise not frequently encountered.

Methods

- To gather more 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 routinely perform, but did not report results for, or instances where the organisers did not allow for these examination results to be reported.
- To include the option for laboratories to examine clinical specimens using molecular methods, as the evidence from the methods questionnaire suggests that laboratories do undertake this type of testing.

Scoring

- To determine if performance assessment should be scored and design an appropriate scoring system to make it easier to identify those laboratories that experience significant, ongoing difficulties with their examinations.
- The allocation of scores is provided as a visual management tool to help assess performance.
- Ongoing performance assessment for a laboratory can only be done if the same laboratory takes part in all the EQA distributions.

Forthcoming objectives

- To improve awareness of the different *Legionella* spp. that may be isolated from clinical specimens and environmental samples.
- To improve awareness of the confirmatory tests done and their limitations when confirming *Legionella* spp. isolates in samples.
- To improve 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 performance assessment should be scored and design the appropriate scoring system to make it easier to identify those laboratories that experience significant, ongoing difficulties with their examinations. The allocation of scores is provided as a visual management tool to help assess performance.
- To identify the additional support/training needed by the EU enlargement countries to process environmental samples so that accurate results are reported.
- 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 explore participants' feedback from the evaluation survey in greater depth to improve the exercise (e.g. improving information in the individual EQA reports.)
- To update the IT platform so that results on methods used can be provided.
- To communicate the results of this EQA at future ELDSNET and *Legionella* conference meetings to increase awareness of the importance of EQAs for the quality of *Legionella* detection in laboratories.

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Annex 1. Findings from methods survey questionnaire

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

Methods survey findings for clinical specimens

Distribution 4680, which closed on 16 December 2019, consisted of 10 simulated clinical samples. A questionnaire sent out together with the EQA was completed by seven participating laboratories from EU enlargement countries.

A total of 7/7 (100%) of the responders from EU enlargement countries provided information on their method/processes. The total numbers will not always correspond to seven as some participants did not provide information for all the questions and some questions allowed for more than one option to be selected. Countries within the EU enlargement participating in this EQA include Albania, Bosnia and Herzegovina, Kosovo, Montenegro, North Macedonia, Serbia and Turkey.

General information

A total of 2/7 participants reported that, in addition to being reference laboratories, they were clinical diagnostic laboratories. Five laboratories reported that they were only a clinical diagnostic laboratory.

Laboratories participating in a voluntary national EQA was 42.9% (3/7) of participants.

Figure A3. Sample types analysed for *Legionella* by participating laboratories

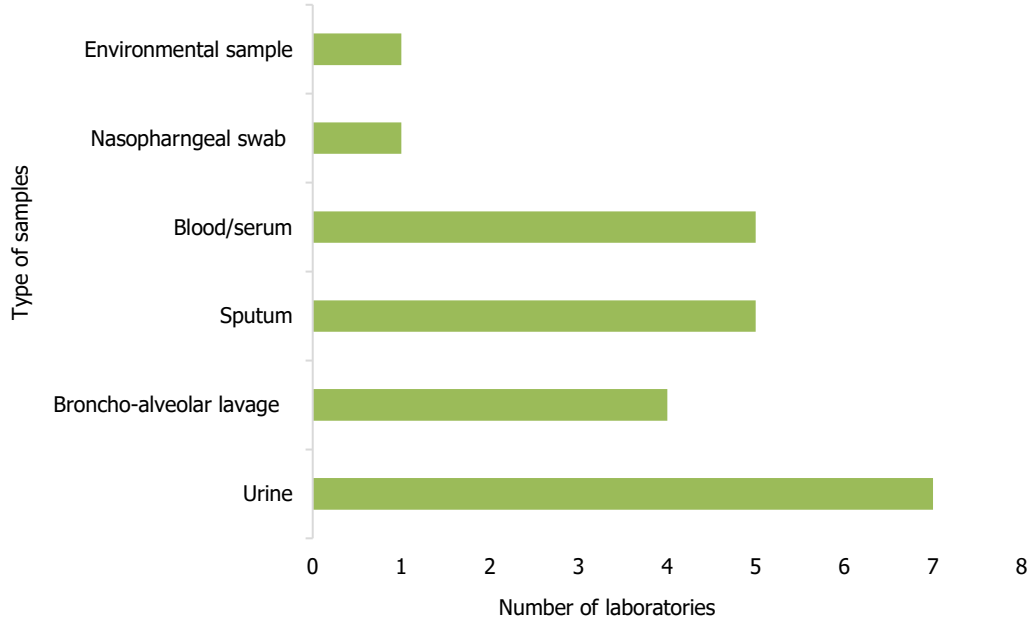
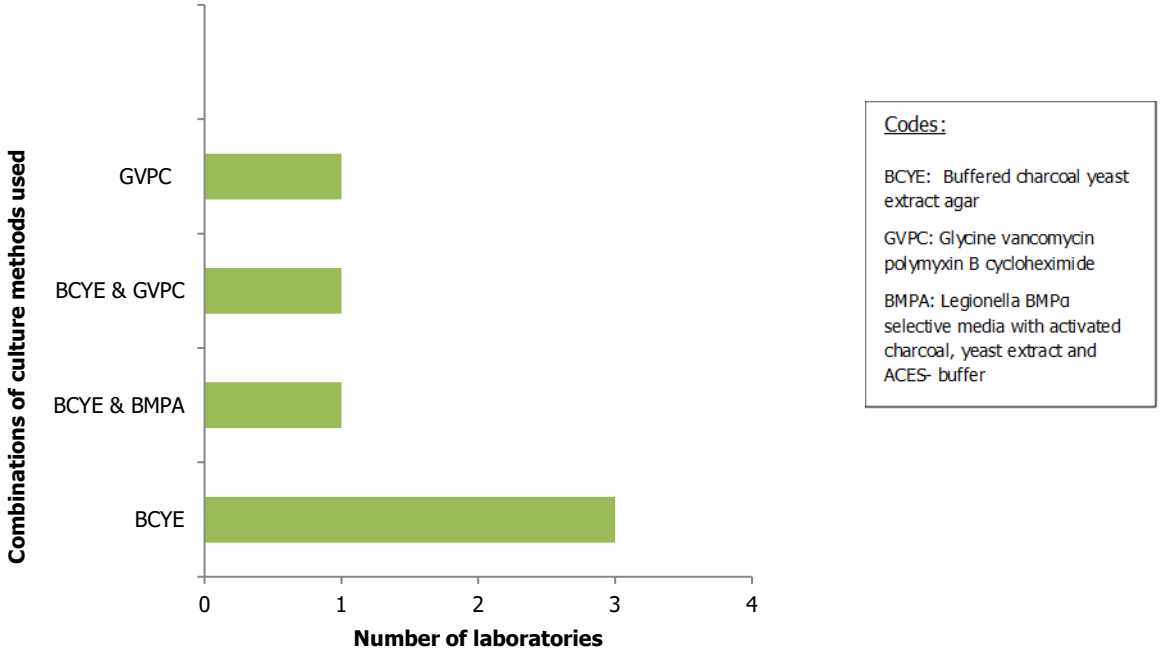


Figure A4. Different types of media used for the detection of *Legionella*



Comments:
One laboratory did not culture the specimens and went on to perform direct molecular testing. Of those listed in the survey, manufacturers included; Biomerieux, Bio-Rad and Biolife.

Incubation period ranged from 3–5 days (n=1), five days (n=1), seven days (n=2), 10 days (n=1) and 14 days (n=1).

Figure A5. Confirmatory tests used for detection of *Legionella*

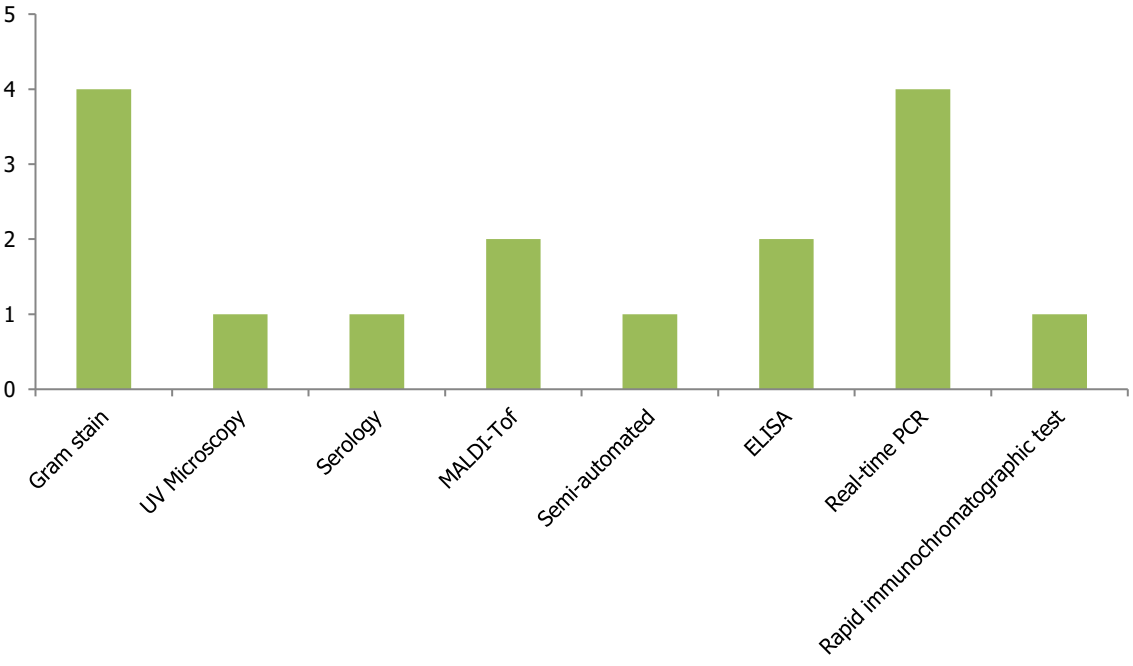
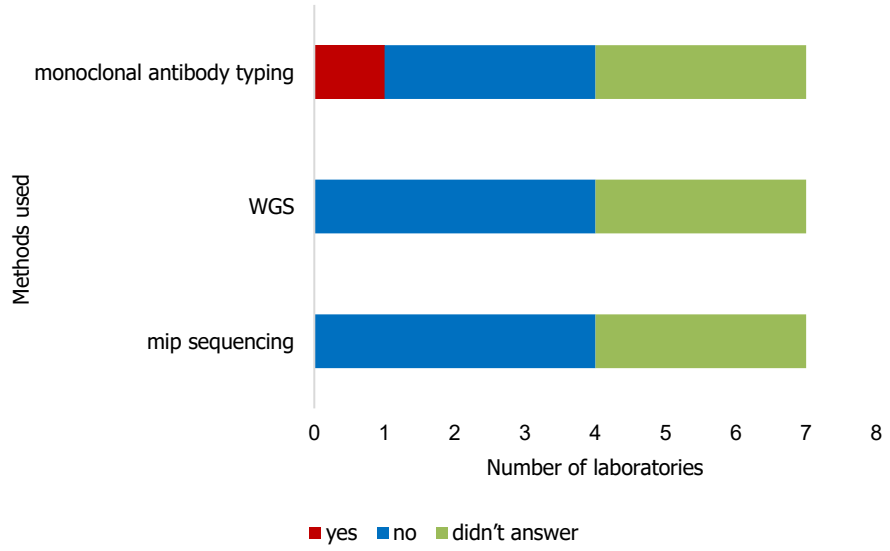


Figure A6. Typing and species identification methods, including phenotypic and genotypic



Methods survey findings for environmental samples

A questionnaire was sent to all participants who participated in distribution 4681. The objective was to gather information on the method/processes used for this proficiency testing exercise.

A total of seven laboratories were sent this distribution in six of the EU enlargement countries, 6/7 returned results on the samples examined. The countries sent the questionnaire were Albania, Bosnia and Herzegovina, Montenegro, North Macedonia, Serbia and Turkey.

A total of 6/7 of the participating laboratories provided information on their method/processes. No replies were provided for one laboratory in Albania. Of the responders, the total numbers will not always correspond to six 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 relation to a method/process used, or attempt to compare performance of the various molecular kits/processes with each other.

All data is presented for the participating laboratories from the EU enlargement countries that responded to the EQA November 2019 exercise.

General information

Figure A7 below shows the approximate number of water and environmental samples examined for *Legionella* spp. in a year (n=4).

Figure A7. Number of samples examined per year for *Legionella*

Figure A8 shows the type of samples examined by the laboratories (n=6).

Figure A8. Type of samples analysed by laboratories for *Legionella* (n=6)

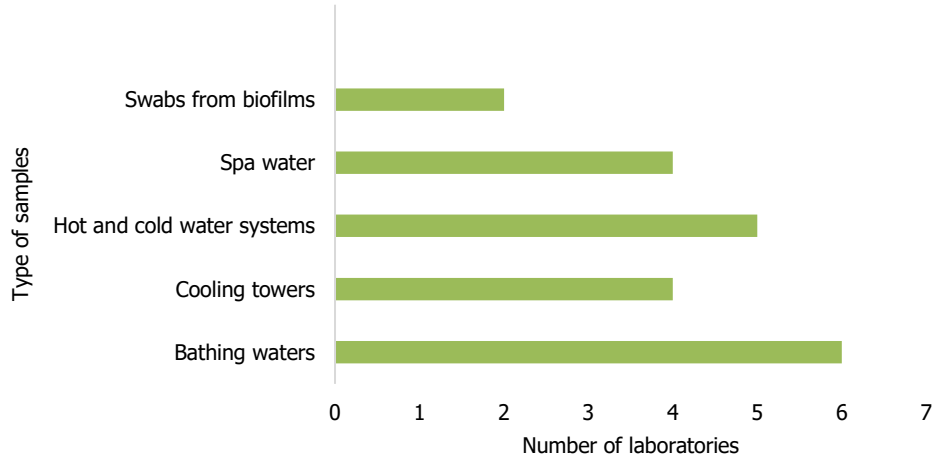
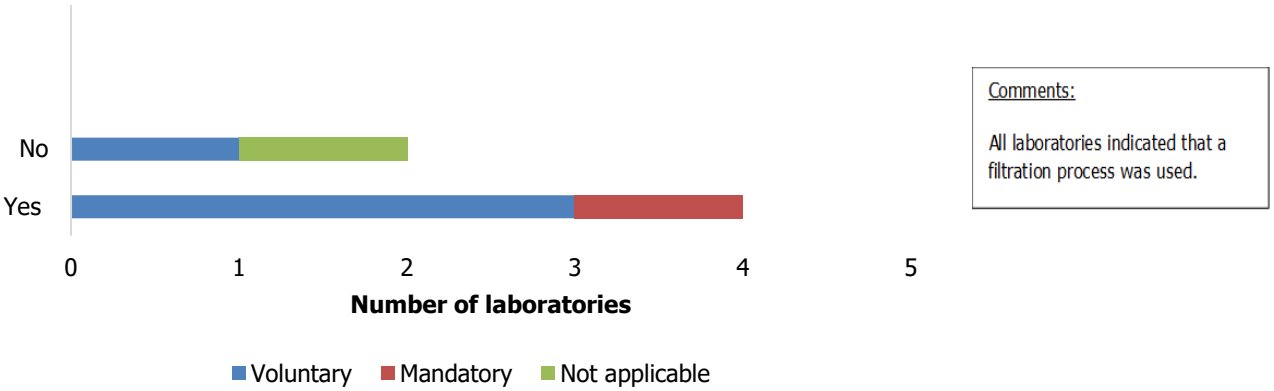


Figure A9 shows whether laboratories participate in their national EQA schemes programme and if these schemes are voluntary or mandatory (n=6).

Figure A9. Participation in national EQA schemes



Information on water examination

Figure A10 shows the published methods used to examine environmental and water samples (n=6).

Figure A10. Published methods used to examine swabs and water

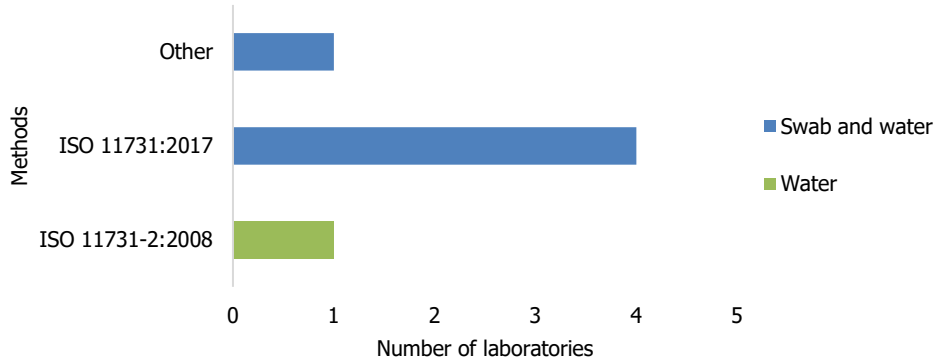


Figure A11 shows the method and volume used to examine the water samples in this exercise (n=6).

Figure A11. Method and volume used to examine water samples

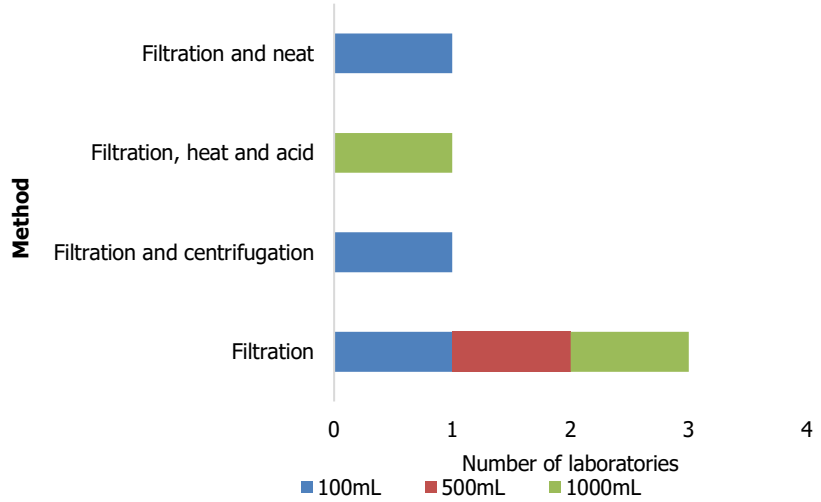
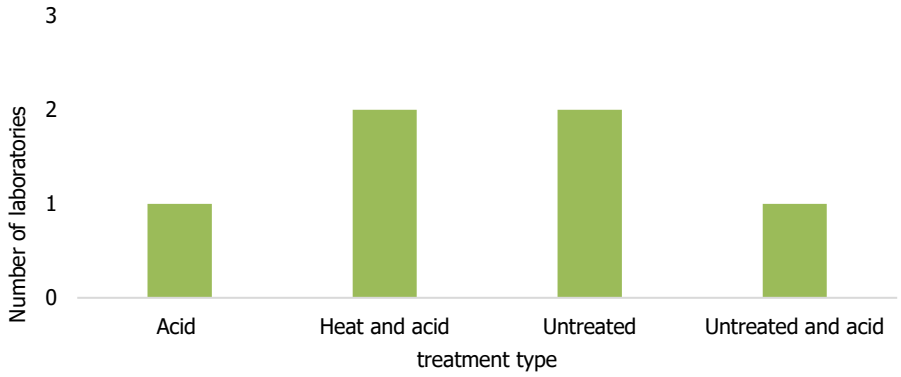


Figure A12 provides detail about how the sample was processed (n=6).

Figure A12. Information on sample processing



For the laboratories that undertook acid treatment as part of the examination, the information in Figure A14 provides details on the stage of the process at which the acid was applied (n=6).

Figure A13. Stage at which acid was applied

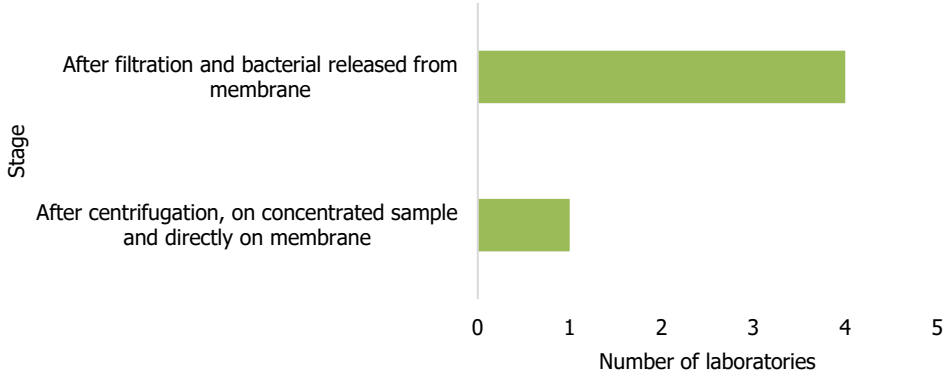


Figure 14 shows the different type of media used (n=6).

Figure A14. Different types of media used

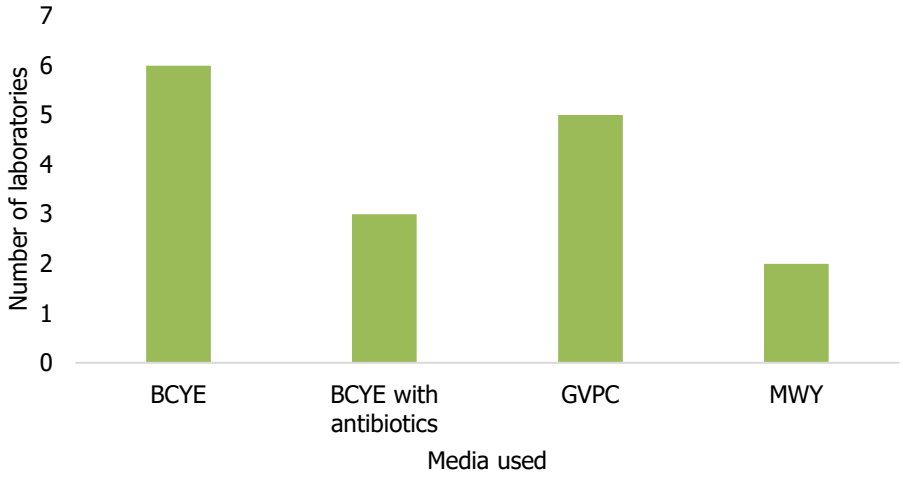


Figure A14 Codes:

BCYE: Buffered charcoal yeast extract agar

BCYE with antibiotics: Buffered charcoal yeast extract agar with selective supplements

GVPC: Glycine vancomycin polymyxin B cycloheximide

MWY: Modified Wadowsky-Yee

Figure A15 shows the combinations of media used and the source of this media (n=6).

Figure A15. All media used and source of media

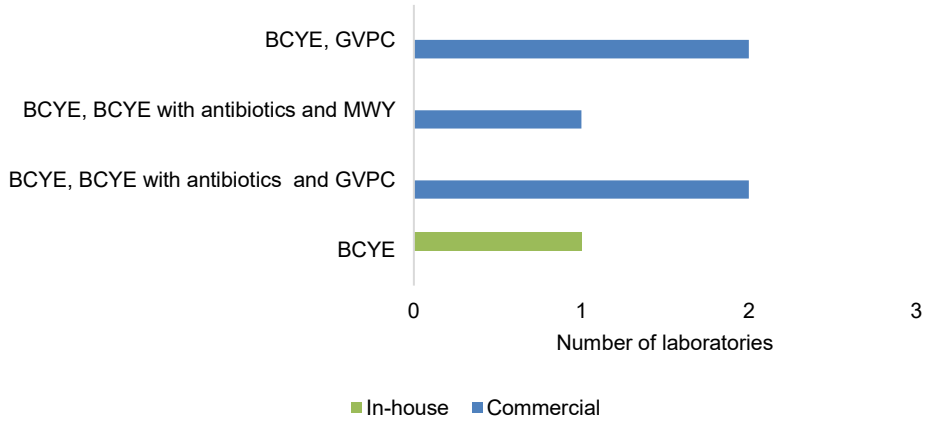


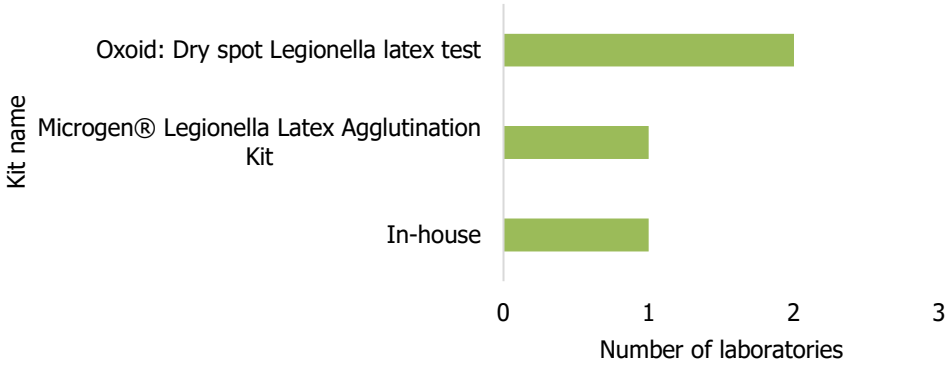
Table A20 provides information on the confirmation tests done on presumptive colonies of *Legionella* (n=6).

Table A20. Confirmation tests undertaken on presumptive colonies of *Legionella*

Confirmation tests done	Number of laboratories
Subculture on BCYE	2
Subculture on BCYE with cysteine	2
Subculture on BCYE, BCYE with cysteine and PCR	2

Figure A16 details the kits used to carry out the serogroup testing, with some laboratories using a combination of kits (n=4).

Figure A16. Kit used for serogroup testing



Information on molecular testing

Only one laboratory stated they would use SBT with Sanger sequencing. Table A21 below shows how samples were processed for molecular examination.

Table A21. Sample processing for molecular examination

Process	Number of laboratories
Filtration	2

The table below shows the DNA extraction kits used.

Table A22. List of DNA extraction kits used

DNA extraction kit	Number of laboratories
NucliSens easyMAG	2

The table below shows the volume of extracted DNA for use in assays.

Table A23. Volume of extracted DNA used in assays

Volume (mL)	Number of laboratories
10	1
Other	1

The table shows the commercial assay that was used.

Table A24. Commercial assays used

Commercial assay	Number of laboratories
Other	2

The table below shows the amplification platforms used.

Table A25. Amplification platforms used

Amplification platform	Number of laboratories
Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System	1
Other	1

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