Technical Specification

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Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR) —

Part 2: On-site methods

Qualité de l'eau — Détection et quantification de Legionella spp. et/ou Legionella pneumophila par concentration et amplification génique par réaction de polymérisation en chaîne quantitative (qPCR) —

Partie 2: Méthodes sur site

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

A list of all parts in the ISO 12869 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

ISO/TS 12869 provides the guidelines, minimum requirements and performance characteristics intended to guarantee that the quantification of *L. pneumophila* or *Legionella* spp. by amplification of specific DNA sequences (PCR) and real time detection of specific DNA sequences (PCR) and real-time detection of specific fluorophores is reproducible between methodologies completed by different laboratories.

Similar to ISO/TS 12869, this document specifies a method to determine recovery of the bacteria and subsequent DNA amplification (lysis efficiency is not estimated).

Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR) —

Part 2:
On-site methods

1 Scope

This document provides the guidelines, minimum requirements and performance characteristics intended to guarantee that manufactured systems intended for on-site/field use (i.e. outside the laboratory) provide reliable and reproducible results.

This document specifies the requirements for technologies that enable on-site detection and quantification of *Legionella* spp. and *L. pneumophila* using a quantitative polymerase chain reaction assay (qPCR). It specifies general methodological requirements, performance evaluation requirements and quality control requirements. This document is intended to be used by manufacturers of these technologies so that they produce detection systems that end users can operate safely and effectively. End users will be guided by this document to adhere to manufacturer's instructions, to ensure user competency and to perform the necessary controls.

Technical details specified in this document are given for information only. Any other technical solutions complying with the performance requirements are suitable.

NOTE For validation and performance requirements, see Clause 9.

This document is intended to be applied in the bacteriological investigation of all types of water (hot or cold water, cooling tower water, etc.), unless the nature and/or content of suspended matter and/or background microorganisms interfere with the determination. This interference can result in an adverse effect on both the detection limit and the quantification limit.

The results are expressed as the number of genome units of *Legionella* spp. and/or *L. pneumophila* per millilitre (or litre) of sample.

Although the method described in this document is applicable to all types of water, some additives, such as chemicals used for water treatment, can interfere with and/or affect the sensitivity of the method.

The qPCR methods do not give any information about the physiological state of the *Legionella.* However, there are on-site qPCR methodologies which are able to distinguish intact bacteria from free DNA.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 19458, *Water quality — Sampling for microbiological analysis*

ISO/TS 12869:2019, *Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR)*

ISO 11731, *Water quality — Enumeration of Legionella*

3 Terms, definitions, symbols and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 12869 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1.1
***Legionella* spp.**
several species of *Legionella*, including *L. pneumophila*

3.1.2
polymerase chain reaction inhibition control
PCR inhibition control
materials and processes used to assess if the sample DNA extract contains (a) PCR inhibitor(s)

Note 1 to entry: The control can be a plasmid, an oligonucleotide or the *L. pneumophila* genomic DNA. A specific probe shall be used to detect the inhibition control.

3.1.3
bacterial recovery
evaluation of the reported quantity of bacteria by the *on-site qPCR* (3.1.7) system when a known quantity of reference material is tested

3.1.4
working calibration solution
L. pneumophila DNA calibrated solutions, derived from a standard solution, for which accuracy is determined by an independent method (e.g. digital droplet PCR) used to establish the calibration curve

3.1.5
negative control of the method
control for monitoring the whole process in this method (from filtration to extraction to qPCR)

3.1.6
no template control
NTC
control for monitoring qPCR reagent amplification

3.1.7
on-site qPCR
qPCR testing that can occur immediately after sample collection, such that sample preservation is not required (e.g. sodium thiosulfate)

Note 1 to entry: On-site qPCR is validated for use by non-laboratory personnel that have been trained in the procedure.

3.1.8
concentration device
device that prepares a water sample for qPCR amplification

Note 1 to entry: This kind of device is designed such that it can be used safely and effectively by non-laboratory trained personnel.

3.1.9
threshold cycle
C_t
Note 1 to entry: number of PCR cycles (denaturation and amplification) required to replicate the DNA copies originally present in the sample, so that the concentration of DNA exceeds the detection limit

Note 2 to entry: The C_t value is the intercept of the line that represents the DNA concentration of a sample with fluorescent base line.

3.1.10
genome unit
GU
Note 1 to entry: unit representing a single copy of bacterial genomic DNA

3.1.11
graphical user interface
GUI
on-screen controls of the testing equipment which can describe sample concentration and analysis procedure

Note 1 to entry: The interface is designed such that it can be used and understood by non-laboratory personnel that have been trained in the procedure.

3.1.12
end user
operator
individual who performs the assay on the test system

3.1.13
critical task
step in the on-site test workflow that can lead to a hazardous situation, such as an incorrect test result and/or injury to the test *operator* (3.1.12), if performed incorrectly

3.1.14
batch
manufacturing production run used to generate one or more lots of finished goods

3.2 Symbols and abbreviated terms

C_t	threshold cycle
D_{opt600}	optical density at 600 nm
L_{DqPCR}	(detection limit of the qPCR) lowest number of genome units that give a positive result in the qPCR with 90 % confidence
L_{QqPCR}	(quantification limit of the qPCR) lowest number of genome units that can be quantified with an accuracy less than or equal to 0,20 $\log_{10}$ unit
V_b	volume of the bacterial sample in the reaction
V_f	final volume of the reaction
V_w	volume of water in the reaction
BCYE	buffered charcoal yeast extract
BSA	bovine serum albumin
DMSO	dimethyl sulfoxide
GMP	good manufacturing practice

GU	genome unit
GUI	graphical user interface
HFT	human factors testing
NTC	no template control
OD	optical density
PPE	personal protective equipment
QC	quality control
UNG	uracil-DNA N-glycosylase

4 Principle

The detection and quantification of *Legionella* spp. or *L. pneumophila* by on-site qPCR are carried out in three phases:

— concentration of bacteria from water samples by the concentration device;

— recovery of the bacteria from the concentration device and transfer of the bacteria to a vessel or apparatus in which bacterial lysis and DNA extraction occur; PCR can then proceed in the same apparatus or an additional step can be required to transfer the extracted DNA to a PCR reaction;

— amplification, detection and quantification of one or more specific DNA sequences belonging to the *Legionella* genus and/or *L. pneumophila* species by real-time PCR.

5 Sampling

Sampling shall be in accordance with ISO 19458, however one of the main advantages of on-site testing is the ability to test samples immediately after collection, thus mitigating the known effects of time on sample quality (see Reference [1]). The manufacturer shall indicate to the end user the acceptable holding times between sample collection and analysis. These time intervals can vary between water sources (e.g. potable water without biocides present versus cooling tower samples that contain biocides). The manufacturer shall validate these holding times, which will be provided in the instructions (see 6.2.2).

Sampling conditions (e.g. water treatment, temperature, turbidity, time that water was run prior to sampling) shall be indicated on the test report if they are known. Manufacturers will validate conditions, including temperature and commonly used chemicals (e.g. biocides, neutralizing agents, anti-corrosives) in intended sample types, that are compatible with the testing system. Manufacturers will indicate the compatible sample conditions to end users. Samples shall not be exposed to conditions that the manufacturer has not validated.

Biocides (bactericides or bacteriostatics) are sometimes used, in particular in cooling tower circuits. The presence of biocides, however, can lead to under quantification of the analyte, therefore the presence of biocides shall thus be declared and indicated on the test report if it is known. When inhibition of PCR sufficient to result in under quantification is detected, the test result shall be suppressed and a warning message specifying that interference was encountered shall be provided to the end user. Where appropriate, sample containers shall contain a suitable neutralizing agent (refer to ISO 19458). As it is not always possible to neutralize these products, minimizing the elapsed time between sample collection and analysis is recommended.

Manufacturers shall indicate to end users the need for a sampling plan and refer users to ISO 5667-1 for guidance.

6 General testing conditions

6.1 General

PCR is a sensitive detection method, the results of which can be affected by aerosols, dust and other particles which can contain contaminating DNA. It is therefore essential to physically separate the different stages of the analysis. The on-site qPCR concentration device shall be designed in such a way to prevent this type of contamination.

The principles to be applied are as follows:

— single use concentration device and qPCR reagent;

— procedures for eliminating DNA traces and amplicons shall be described to the user in the event of accidental contamination of the premises or apparatus;

— regular manufacturing quality controls shall be used to demonstrate the effectiveness of the concentration device and qPCR reagent production procedures with the objective of ensuring that there is no contaminating *Legionella* DNA or PCR products/amplicons (see 10.4).

The manufacturer and the end users shall fulfil the responsibilities listed in Annex A.

6.2 End users

6.2.1 General

All personnel who perform on-site qPCR shall be provided with instructions to operate the system, as well as training materials as needed. Instructions shall be provided as a physical copy of the instructions, a training video or interactive instructions provided by a graphical user interface (GUI).

The test operators shall wear personal protective equipment required for sample collection as per jurisdictional guidelines. Gloves are required. They shall be disposable and talc-free.

As the qPCR results shall be analysed and interpreted by software and expressed to the user via the GUI in appropriate units (e.g. GU/ml or GU/l), the operator shall not require additional advanced training or experience in PCR data analysis. Likewise, the presence of inhibition shall be determined, via PCR inhibition control, by automated analysis of the data by the software.

6.2.2 Manufacturer's instructions

Instructions shall be provided to end users by manufacturers. The instructions shall include clear and specific information necessary to safely and effectively perform tests on-site. The following topics shall be included in the manufacturer's instructions to end users.

— Intended use: Statement of the test system input material(s) and result output(s) and how the results may be used. The latter shall include a statement notifying the end user to be aware of jurisdictional requirements and how they can affect how the results are used. The intended end user shall also be provided.

— Warnings and precautions: Description of safety measures required to avoid any risk of harm to the operator or other individuals when the test is performed. Risks considered shall include the risk of exposure to aerosolized *Legionella* as well as any critical concerns that can lead to incorrect results or expose the operator to risk (such as do not use if damaged, store consumables as indicated, follow critical steps in the instructions/workflow, etc.). Detailed handling and waste disposal instructions shall also be provided.

— Personal protective equipment (PPE): Description of the appropriate PPE required by users to handle and use the system safely, including protection from the risk of exposure to aerosolized *Legionella*.