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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

~~DTS stage~~

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 ~~documents~~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).

~~Attention is drawn~~ISO draws attention to the possibility that ~~some of~~ the ~~elements~~implementation of this document may ~~be~~involve the ~~subject~~use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents~~.~~. ISO shall not be held responsible for identifying any or all such patent rights. ~~Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see).~~

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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, *Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR),~~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 of specific DNA sequences (PCR) and real-time detection of specific fluorophores is reproducible between methodologies completed by different laboratories.

~~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.~~

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 ~~may~~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.

~~NOTE 1 The manufacturer and the end users shall fulfil the responsibilities listed in Annex A.~~

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

NOTE ~~2~~ 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. ~~In such cases, validation of the method shall include satisfying this performance requirement.~~

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 ~~terminological~~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
PCR inhibition control
materials and processes used to assess if the sample DNA extract contains (an) 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)~~
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
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 1 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
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* (3.1.13), 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