

SLOVENSKI STANDARD SIST-TS ISO/TS 12869:2013

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Kakovost vode - Ugotavljanje prisotnosti in števila Legionella spp. in/ali Legionella pneumophila s koncentriranjem in pomnoževanjem genov s kvantitativno verižno reakcijo s polimerazo (qPCR)

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

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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) b4f21d37281e/sist-ts-iso-ts-12869-2013

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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)

iTeh STANDARD PREVIEW Qualité de l'eau — Détection et quantification de Legionella spp. et/ou (**SLegionella pleumophila par** concentration et amplification génique par réaction de polymérisation en chaîne quantitative (qPCR)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.
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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an Sinternational Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years/at-which time it must either be transformed into an International Standard of be withdrawn. 12869-2013

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 12869 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

This Technical Specification specifies a method for the detection and quantification of *Legionella* species (spp.) and *Legionella pneumophila* (*L. pneumophila*) in water using a quantitative polymerase chain reaction (qPCR).

The presence of *L. pneumophila* or *Legionella* spp. in water samples is demonstrated and quantified by amplifying DNA sequences (PCR) with specific oligonucleotides. Specificity of the detection is ensured by using a target sequence specific fluorescent-labelled probe. The increase in the amount of the DNA amplicon can be measured and visualized in real time by a quantitative PCR device with fluorophore specific filters.

A calibration curve is used for quantification purposes. The guidelines, minimum requirements and performance characteristics are intended to guarantee that the results are reliable and reproducible between different laboratories.

This Technical Specification specifies a determination of the recovery of the DNA extraction. The performance of the extraction procedure is not fully covered (lysis efficiency is not estimated).

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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)

WARNING — *Legionella* spp. can be handled safely by experienced microbiologists on the open bench in a conventional microbiology laboratory conforming to containment level 2. Infection is caused by inhalation of the organism; hence it is advisable to assess all techniques for their ability to produce aerosols. If in doubt, carry out the work in a safety cabinet.

1 Scope

This Technical Specification specifies a method for the detection and quantification of *Legionella* spp. and *L. pneumophila* using a quantitative polymerase chain reaction (qPCR). It specifies general methodological requirements, performance evaluation requirements, and quality control requirements.

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

NOTE For performance requirements, see Clause 10. PREVIEW

This Technical Specification is intended to be applied in the bacteriological investigation of all types of water (both hot and cold), unless the nature and/or content of suspended matter and/or accompanying flora interfere with the determination. This interference can result in an adverse effect on both the detection limit and the guantification limit.

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

The method described in this Technical Specification is applicable to all types of water. However, some additives, e.g. 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 live or dead cells.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 19458, Water quality — Sampling for microbiological analysis

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

Legionella

<genotype definition> bacterial genus which can be defined by DNA sequences of genes encoding its specific 16S rRNA

NOTE rRNA is the abbreviation of ribosomal ribonucleic acid.

3.2

Legionella

<phenotype definition> genus of Gram-negative bacteria normally capable of growth in not less than 2 days on buffered charcoal yeast extract agar containing L-cysteine and iron(III), and forming colonies, often white, purple to blue or lime green in colour

Some species fluoresce under long-wavelength UV light. The colonies have a ground-glass appearance NOTE when viewed with a low power stereomicroscope. With a very few exceptions, growth does not occur in the absence of L-cysteine.

3.3

Legionella pneumophila

<genotype definition> species belonging to the *Legionella* genus which can be defined by its specific **DNA** sequences

The distinction between Legionella spp. and L. pneumophila can be made on the basis of the difference NOTE between the nucleotide sequence in the macrophage infectivity potentiator (*mip*) gene.

3.4

Legionella pneumophila

<phenotype definition> species belonging to the Legionella genus giving a positive reaction in the presence of an anti-L. pneumophila serum

NOTE Within *L. pneumophila* at least 15 different groups can be distinguished on the basis of serology. The replication in the environment takes place through intracellular parasitic growth in protozoa.

3.5

DNA

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deoxyribonucleic acid

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genetic material of living organisms consisting of generic and very specific parts

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PCR

enzymatic procedure whereby a specific DNA fragment is replicated by a cyclical iterated process of denaturation, annealing of specific primers and DNA synthesis

3.7

amplification

DNA replication

increase of DNA fragments or amplicons as a result of the PCR reaction

3.8

PCR product

PCR amplicon DNA that is synthesized by the PCR

3.9

quantitative PCR

qPCR

formation of specific DNA fragments which is highlighted by a labelled fluorescent probe and monitored in real time

The intensity of the fluorescence is a measure of the amount of amplicons. By comparison with a NOTE calibration curve, the initial concentration of the DNA target can be determined.

3.10 Ct value threshold cycle

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

The C_t value is the intercept of the line that represents the DNA concentration of a sample with the NOTE fluorescent base line.

3.11 reverse primer forward primer

single-strand DNA fragment (oligonucleotide) that serves as a template for specific DNA replication

The choice of the DNA sequences of both the forward and reverse primers determines which DNA NOTE fragment is replicated. The length of the primer varies from 15 to 30 nucleotides.

3.12

probe

single-stranded DNA fragment, targeting a specific sequence, labelled with a fluorophore that can be detected in the real-time PCR device

3.13

Tag DNA polymerase

polymerase obtained from the bacterium *Thermus aquaticus* that lives in hot springs and geysers

This thermo-stable polymerase is used for the DNA synthesis in the PCR. NOTE 1

The use of hot-start polymerase is possible to avoid false-positive results. NOTE 2

3.14

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Legionella spp. genome unit https://standards.iteh.ai/catalog/standards/sist/ef201eaa-8dba-4e17-a398-GU

GU <u>b4f21d37281e/sist-ts-iso-ts-12869-2013</u> unit representing a single copy of the *Legionella* spp. bacterial genomic DNA

3.15

detection limit of the qPCR

LD_{aPCR}

lowest number of genome units that give a positive result in the qPCR with 90 % confidence

3.16

quantification limit of the qPCR

LQ_{aPCR}

lowest number of genome units that can be quantified with an accuracy less than or equal to $0.15\log_{10}$ unit

3.17

macrophage infectivity potentiator gene

mip gene

gene present in Legionella spp. which is essential for the infection of the host (protozoa) and macrophages (humans)

The unique base sequence of the *mip* gene of *L. pneumophila* can be used for the design of the primer NOTE and probe sequences for the specific qPCR detection of *L. pneumophila*.

3.18

PCR inhibition control

calibrated DNA that is required to be co-amplified with the sample DNA extract using the primers needed for *Legionella* spp. or *L. pneumophila* detection

NOTE 1 The PCR inhibition control should reveal any inhibitor presence in the sample DNA extract.

NOTE 2 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.

3.19

recovery

efficiency of the DNA extraction

3.20

Legionella pneumophila DNA primary standard

calibrated DNA solution of *L. pneumophila* (WDCM 00107) with a known quantity of genome units and an associated uncertainty

NOTE 1 The standard is used to adjust the working calibration DNA solutions.

NOTE 2 For the WDCM catalogue, see Reference [3].

3.21

reference material

ready-to-use calibrated DNA solution connected to the *L. pneumophila* DNA primary standard (3.20)

NOTE The reference material shall be processed in each PCR run to check the accuracy of the qPCR.

3.22

amplification series

set of PCR amplifications run while using the same PCR reagent batches, same materials, and same instruments

3.23

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working calibration solutions (standards.iteh.ai)

L. pneumophila (WDCM 00107) DNA calibrated solutions, compared to the *L. pneumophila* DNA primary standard, used to establish the calibration <u>curve_IS ISO/IS 12869.2013</u>

NOTE The procedure is specified in 7.4. bitps://standards/sist/ef201eaa-8dba-4e17-a398b4f21d37281e/sist-ts-iso-ts-12869-2013

4 Principle

The detection and quantification of *Legionella* spp. by PCR is carried out in three phases:

- concentration of water samples by filtration;
- DNA extraction from the filter;
- amplification, detection and quantification of one or more specific DNA sequences belonging to the *Legionella* genus and/or *L. pneumophila* species by real-time qPCR.

5 Sampling

The samples shall be taken in sterile containers with all the necessary precautions. The sampling conditions shall be indicated on the test report if they are known. Perform the sampling in accordance with ISO 19458.

Preferably begin the investigation immediately after the sampling. If samples are delivered to the laboratory within 24 h after sampling, they can be shipped at room temperature. However, if the delay between sampling and arrival to the laboratory is longer than 24 h, the shipment shall be performed at +5 °C ± 3 °C.

Validate the storage of the filter membrane or the sample for a longer time or at another temperature.

For samples from oxidizing biocide-treated water, the sterile container used for collection shall in addition contain a sufficient quantity of sterile sodium thiosulfate to neutralize the oxidizer (e.g. at a concentration of 20 mg/l).

Other biocides (bactericides or bacteriostatics) are sometimes used, in particular in cooling tower circuits. Their presence, which can lead to underestimation, shall thus be declared and indicated on the test report if it is known. However, it is not always possible to neutralize these products.

6 General testing conditions

6.1 General

PCR is a sensitive detection method. Aerosols, dust, and other particles are carriers of contaminating DNA. It is therefore essential to separate in space and/or time the different stages of the analysis. In particular, provide separate dedicated spaces, materials, and equipment for pre- and post-amplification stages.

The principles to be applied are as follows:

- use of disposables compatible with PCR methods is preferred;
- a procedure for eliminating DNA traces shall be implemented in event of accidental contamination of the premises or apparatus;
- regular quality controls checks shall be used to demonstrate the effectiveness of maintenance procedures with the objective of ensuring that there is no contaminating legionella DNA (see 11.4).

6.2 Staff

All personnel who perform aspects of the testing procedures shall be trained to work with PCR and microbiology as appropriate. (standards.iteh.ai)

The staff shall wear separate laboratory coats for microbiology activities involving cultures and molecular biology activities. Any gloves used shall be disposable and talc free.

Laboratory coats shall be changed between the areas of low DNA concentration (pre-amplification) and the areas of high legionella DNA concentration (post-amplification). When laboratory coats are not disposable, then they shall be periodically cleaned and replaced. Only duly equipped staff shall access the specific rooms where these tests are run.

6.3 Premises

The laboratory shall contain at least two physically separated areas (e.g. PCR cabinet), the area including pre-PCR [a) and b) below] and PCR [c) below] activities. Ideally, there should be three physically separated areas a), b), and c) available:

- a) an area for the concentration of samples and DNA extraction;
- b) an area for the preparation of PCR reagents (reaction mixtures);
- c) an area for amplification.

If automated machines are used, then certain activities can be grouped together in the same area. In all cases, check that there is no contamination (see 11.4).

Regardless of the amplicon detection and amplification system used, no tube shall be opened after amplification in areas a), b), and c).

6.4 Apparatus and consumables (excluding reagents)

6.4.1 General

Usual laboratory equipment, and in particular the following.