SLOVENSKI STANDARD
SIST EN ISO 11731:2017

01-december-2017

Nadomešča:
SIST EN ISO 11731-2:2008

Kakovost vode - Ugotavljanje števila legionel (ISO 11731:2017)

Water quality - Enumeration of Legionella (ISO 11731:2017)

Wasserbeschaffenheit - Zählung von Legionellen (ISO 11731:2017)

Qualité de l'eau - Dénombrement des Legionella (ISO 11731:2017)

Ta slovenski standard je istoveten z: EN ISO 11731:2017

ICS:

07.100.20	Mikrobiologija vode	Microbiology of water

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EUROPEAN STANDARD

NORME EUROPÉENNE

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EN ISO 11731

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English Version

Water quality - Enumeration of Legionella (ISO 11731:2017)

Qualité de l'eau - Dénombrement des Legionella (ISO 11731:2017)

Wasserbeschaffenheit - Zählung von Legionellen (ISO 11731:2017)

This European Standard was approved by CEN on 12 February 2017.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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European foreword

This document (EN ISO 11731:2017) has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with Technical Committee CEN/TC 230 “Water analysis” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2017, and conflicting national standards shall be withdrawn at the latest by December 2017.

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

This document supersedes EN ISO 11731-2:2008.

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Endorsement notice

The text of ISO 11731:2017 has been approved by CEN as EN ISO 11731:2017 without any modification.

INTERNATIONAL STANDARD

ISO 11731

Second edition
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Water quality — Enumeration of *Legionella*

Qualité de l'eau — Dénombrement des Legionella

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

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

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

This second edition of ISO 11731 cancels and replaces ISO 11731:1998 and ISO 11731-2:2004, which have been technically revised.

Introduction

After the first recognized outbreak of Legionnaires' disease in 1976, the isolated bacterium was named *Legionella pneumophila*. Legionellae are widely found in natural and artificial aquatic environments, soils, composts and can cause legionellosis. Legionellae can grow intracellularly in protozoa like *Acanthamoeba castellanii*, *Hartmannella* species or *Naegleria* species. At least 61 different *Legionella* species have been described. In 26 of these species, some strains infecting humans have been reported. *Legionella pneumophila* can be subtyped into at least 15 different serogroups; nine other species also can be subtyped into at least two separate serogroups. Monitoring for legionellae is important for public health reasons to identify environmental sources which can pose a risk of legionellosis, such as evaporative cooling towers, hot- and cold-water distribution systems in buildings and associated equipment such as spa pools, dental units, air conditioning units, etc. Monitoring is also important for validation of control measures and ongoing verification that controls remain effective.

Water quality — Enumeration of *Legionella*

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified and competent staff.

1 Scope

This document specifies culture methods for the isolation of *Legionella* and estimation of their numbers in water samples.

These methods are applicable to all kinds of water samples including potable, industrial, waste and natural waters. These methods can be used for water related matrices, e.g. biofilms, sediments, etc.

Not all *Legionella* species are culturable; therefore, the methods described in this document do not recover all species of *Legionella*.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7704, *Water quality — Evaluation of membrane filters used for microbiological analyses*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

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

3 Terms and definitions

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

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

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

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

3.1
Legionella
genus of microorganisms normally capable of growth on buffered charcoal yeast extract (BCYE) agar containing L-cysteine and iron(III) salts

Note 1 to entry: For a more detailed description of *Legionella* species, see Annex A.

4 Principle

4.1 General

Legionellae in the water sample are concentrated by membrane filtration, diluted or directly plated depending on the origin/characteristics of the sample. The desired level of detection can vary depending on (inter)national legislation and the reason for sampling or investigation. Samples expected to contain high numbers of legionellae, such as those obtained during outbreak investigations, can be processed with and/or without the concentration steps. To reduce the growth of the concentrated non-target bacteria, which can interfere with the recovery of the target legionellae, portions of the water samples are also subjected to heat treatment, acid treatment or a combination of both treatments.

Dilution is necessary when high concentrations of *Legionella* and/or other bacteria are expected. Separate portions of the diluted sample should be pre-treated; one with heat and a second with acid solution or, in case of extremely contaminated samples, with a combination of acid solution and heat before culturing on selective media.

Treated and/or untreated portions of the sample are transferred onto plates of the chosen culture medium selective for *Legionella* and incubated.

NOTE Mechanical treatment of the sample can enhance the recovery of *Legionella*.

4.2 Examination

After incubation, morphologically characteristic colonies on the selective culture media are regarded as presumptive *Legionella*.

4.3 Confirmation

Presumptive colonies are confirmed as *Legionella* by subculture to demonstrate their growth requirement for L-cysteine and iron(III).

NOTE If species and serotype identification are requested, further tests are needed (see Annex G). These tests are not part of the standardized methods described in this document.

5 Apparatus and glassware

Usual laboratory equipment and in particular:

5.1 Sterile Petri dishes.

5.2 Incubator, capable of being maintained at (36 ± 2) °C.

5.3 Ultraviolet lamp, emitting light of wavelength (360 ± 20) nm.

5.4 Membrane filtration equipment, suitable for filtering water volumes of 10 ml up to 1 000 ml.

5.5 Membrane filter.

5.5.1 Membrane filter for concentration and elution, polycarbonate or polyethersulfone membrane filters, diameter 47 mm to 142 mm with rated pore sizes of 0,2 µm; see Reference [6]. These types of membrane filters are used for concentration followed by a washing procedure.

5.5.2 Membrane filter for direct placing on culture media, membrane filters from cellulose nitrate or mixed cellulose esters, diameter 47 mm to 50 mm with rated pore sizes of 0,2 µm or 0,45 µm. These

types of membrane filters are used for direct placing onto the culture media after filtration. Filters shall be evaluated prior to use in accordance with ISO 7704.

NOTE Black membrane filters contrast better with the white *Legionella* colonies than light-coloured membrane filters.

5.6 pH meter, with an accuracy of ± 0,1 at 20 °C to 25 °C.

5.7 Vortex mixer.

5.8 Ultrasonic water bath, suitable for ensuring that the level of diluent covering the membrane filter is below the level of water in the water bath.

5.9 Water bath, capable of being maintained at (50 ± 1) °C.

5.10 Glassware, sterilized according to ISO 8199.

5.11 Dissection microscope, stereoscopic, with magnification of at least 4× and with oblique incident illumination.

NOTE Also, a hand lens (magnification at least 4×) can be used.

5.12 Disinfected forceps, for handling of membrane filters.

NOTE Forceps with round ends are generally used in order not to damage the membrane during handling.

5.13 Screw cap sterile container, with or without sterile glass beads. To ensure maximum removal of the legionellae from the membrane filter, sterile glass beads (diameter 2 mm to 3 mm) can be added to the sterile container. Add sufficient glass beads to the sterile container just enough to cover the bottom of the container.

6 Culture media and reagents

Use chemicals of analytical grade in the preparation of culture media and reagents unless otherwise stated (see the Note). Prepare the culture media and reagents according to the instructions given in Annexes B, C and D. Prepare culture media using distilled or demineralized water, which is free from substances that might affect growth of microorganisms under the test conditions. The water shall comply with the requirements of ISO 3696, grade 3.

Alternatively, use commercially available culture media and reagents prepared and used according to the manufacturer's instructions.

NOTE Chemicals of other grades can be used, providing they are shown to be of equal performance in the test.

6.1 Culture media.

See Annex B.

6.1.1 Buffered charcoal yeast extract (BCYE) agar.

See B.1.