SLOVENSKI PREDSTANDARD

oSIST ISO 11731-2:2006

julij 2006

Kakovost vode - Ugotavljanje prisotnosti in števila legionel – 2. del: Metoda neposredne membranske filtracije za vode z majhnim številom bakterij

Water quality - Detection and enumeration of Legionella - Part 2: Direct membrane filtration method for waters with low bacterial counts

ICS 07.100.20

Referenčna številka
oSIST ISO 11731-2:2006(en)

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INTERNATIONAL STANDARD

ISO 11731-2

First edition
2004-05-01

Water quality — Detection and enumeration of *Legionella* —

Part 2: Direct membrane filtration method for waters with low bacterial counts

Qualité de l'eau — Recherche et dénombrement des Legionella —

Partie 2: Méthode par filtration directe sur membrane pour les eaux à faible teneur en bactéries

Reference number
ISO 11731-2:2004(E)

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Published in Switzerland

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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.

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 11731-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

ISO 11731 consists of the following parts, under the general title *Water quality — Detection and enumeration of* Legionella:

— *Part 2: Direct membrane filtration method for waters with low bacterial counts*

The general method will be the subject of a future Part 1 of ISO 11731.

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Water quality — Detection and enumeration of *Legionella* —

Part 2: Direct membrane filtration method for waters with low bacterial counts

WARNING — Persons using this part of ISO 11731 should be familiar with normal laboratory practice. This part of ISO 11731 does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 11731 describes a monitoring method for the isolation and enumeration of *Legionella* organisms in water intended for human use (e.g. hot and cold water, water used for washing), for human consumption and for treated bathing waters (e.g. swimming pools). It is especially suitable for waters expected to contain low numbers of *Legionella*. As the growth of *Legionella* may be inhibited by overgrowth of other bacterial colonies on the membrane, the method is only suitable for waters containing low bacterial counts.

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 8199:—[1)], *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 11731:1998, *Water quality — Detection and enumeration of Legionella*

3 Terms and definitions

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

3.1
Legionella
genus of Gram-negative bacteria normally capable of growth in no 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

NOTE Some species fluoresce under long wavelength UV light. The colonies have a ground-glass appearance when viewed with a low power stereomicroscope. Growth does not occur in the absence of L-cysteine with the exception of *L. oakridgensis* and *L. spiritensis*. *L. oakridgensis* and *L. spiritensis* require L-cysteine and iron for primary isolation but can grow weakly in the absence of added L-cysteine thereafter.

1) To be published. (Revision of ISO 8199:1988)

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4 Safety

The reagents used in this part of ISO 11731 should be subject to assessment in accordance with control substances hazardous to health.

Legionella species 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 and it is advisable therefore to assess all techniques for their ability to produce aerosols. If in any doubt, carry out the work in a safety cabinet.

5 Principle

5.1 General

Bacteria, including *Legionella* organisms, in the water sample are concentrated by membrane filtration. After filtration, the filter is treated with acid buffer added directly into the funnel to reduce the growth of non-*Legionella* organisms. The filter is subsequently transferred onto a plate of agar medium selective for *Legionella* and incubated. Samples expected to contain sufficient numbers of *Legionella* need not be subjected to concentration prior to culture (9.1).

5.2 Enumeration

After incubation, morphologically characteristic colonies which form on the selective medium are regarded as presumptive *Legionella*.

5.3 Confirmation

Presumptive colonies are confirmed as *Legionella* organisms by subculture to demonstrate their growth requirement for L-cysteine and iron. Further biochemical and serological tests are needed for species identification. Species identification may not be considered necessary for routine monitoring but is indispensable in outbreak situations.

NOTE *L. pneumophila* serogroup 1 is the causative agent of most legionellosis cases and is therefore considered the most "critical" type of *Legionella* to be found in the water system. Since increasing numbers of cases of legionellosis caused by other serogroups of *L. pneumophila* and other *Legionella* species are being described, even the presence of other *Legionella* species in water is considered a potential risk.

6 Culture media and reagents

6.1 General

Use chemicals of analytical grade in the preparation of media and reagents unless otherwise stated. Alternatively, use commercially available dehydrated media and reagents. Prepare the media according to the manufacturer's instruction and add freshly prepared (or thaw the stored material at room temperature prior to use) selective agents or growth supplements at the concentrations recommended. Prepare media using glass distilled water or water of equivalent quality complying with ISO 3696:1987, Grade 3. Other grades of chemicals may be used providing they can be shown to produce the same results.

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6.2 Culture media

6.2.1 Buffered charcoal yeast extract agar medium (BCYE)

6.2.1.1 Composition

Yeast extract (bacteriological grade)	10,0 g
Agar	12,0 g
Activated charcoal	2,0 g
α-Ketoglutarate, monopotassium salt	1,0 g
ACES buffer (*N*-2-acetamido-2-aminoethane sulfonic acid)	10,0 g
Potassium hydroxide (KOH) (pellets)	2,8 g
L-cysteine hydrochloride monohydrate	0,4 g
Iron(III) pyrophosphate [$Fe_4(P_2O_7)_3$]	0,25 g
Distilled water	1 000 ml

NOTE Check manufacturer's recommendations for concentration of agar to be added to provide adequate gelling strength.

6.2.1.2 Preparation

6.2.1.2.1 Cysteine and iron solutions

Prepare fresh solutions of L-cysteine hydrochloride and iron(III) pyrophosphate by adding the 0,4 g and 0,25 g respectively to 10 ml volumes of distilled water. Decontaminate each solution by filtration through a cellulose ester membrane filter with an average pore size of 0,2 µm. Store in clean, sterile containers at (–20 ± 5) °C for no more than 3 months.

6.2.1.2.2 ACES buffer

Add the ACES granules to 500 ml of distilled water and dissolve by standing in a water bath at 45 °C to 50 °C. To a separate 480 ml of distilled water, add all the potassium hydroxide pellets and dissolve with gentle shaking. To prepare the ACES buffer mix the two solutions.

NOTE ACES buffer can cause denaturation of the yeast extract if the following sequence is not followed.

6.2.1.2.3 Final medium

Add sequentially to the 980 ml of ACES buffer, the charcoal yeast extract and α-ketoglutarate. Prepare a 0,1 mol/l solution of potassium hydroxide (KOH) by dissolving 5,6 g in 1 l of distilled water. Prepare a 0,1 mol/l solution of sulfuric acid (H_2SO_4) by carefully adding 5,3 ml of H_2SO_4 (ρ = 1,84, of 95 % to 98 % purity) to 1 l of distilled water. Use the solutions of 0,1 mol/l potassium hydroxide or 0,1 mol sulfuric acid as appropriate to adjust the pH to 6,8 ± 0,2. Add the agar, mix and autoclave at (121 ± 3) °C for (15 ± 1) min (6.2.4). After autoclaving allow to cool to (50 ± 2) °C in a water bath.

Add the L-cysteine and the iron(III) pyrophosphate solutions aseptically, mix well between additions.

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