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Kakovost vode - Ugotavljanje števila Clostridium perfringens - Metoda membranske filtracije

Water quality - Enumeration of Clostridium perfringens - Method using membrane filtration

Qualité de l'eau - Dénombrement de Clostridium perfringens - Méthode de filtration sur membrane

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INTERNATIONAL STANDARD

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Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration

Qualité de l'eau — Dénombrement de Clostridium perfringens — *Méthode de filtration sur membrane*

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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

Clostridium perfringens is widely recognized as a valuable indicator for faecal pollution. Within the intestinal tract of animals and man, these Gram-positive bacteria form spores which are resistant to heating compared with vegetative cells. *C. perfringens* in the intestine exists both as spores and vegetative cells, spores are also found in environmental samples. The spores of *C. perfringens* survive in water for months, much longer than vegetative faecal indicator bacteria and consequently their presence may indicate remote or intermittent faecal pollution. Monitoring of *C. perfringens* has proven useful for the assessment of the quality of water resources and to check the stages of water treatment to evaluate the treatment-works performance. The spores are not always inactivated by routine disinfection procedures (e.g. chlorination).

Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration

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 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 staff.

1 Scope

This International Standard specifies a method for the enumeration of vegetative cells and spores of *Clostridium perfringens* by the membrane filtration method in samples of water intended for human consumption. However, the method can be applied to all types of water samples provided they do not contain particulate or colloidal matter that interferes with filtration.

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

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

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

ISO/IEC Guide 2:2004, *Standardization and related activities — General vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 2 and the following apply:

3.1
presumptive *Clostridium perfringens*
bacteria which produce all shades of black or grey to yellow brown colonies on tryptose-sulfite-cycloserine agar, even if the colour is faint, after anaerobic incubation at (44 ± 1) °C for (21 ± 3) h

Note 1 to entry: Unlike colonies growing directly on the agar medium, colonies on the membrane do not always display a distinct blackening, so faint colonies are included in the count.

3.2
confirmed *Clostridium perfringens*
bacteria that produce characteristic colonies on tryptose-sulfite-cycloserine agar and possess the enzyme acid phosphatase

4 Principle

A measured volume of the sample, or a dilution of it, is filtered through a membrane with a pore size of 0,45 µm sufficient to retain spores of clostridia. The membrane is incubated on a selective/differential agar (tryptose-sulfite-cycloserine agar) anaerobically at (44 ± 1) °C for (21 ± 3) h. *C. perfringens* usually produce black or grey to yellow brown colonies as a result of the reduction of sulfite to sulfide which reacts with a ferric salt in the medium. Characteristic colonies are counted and confirmatory tests are carried out. The result is calculated as the colony count per sample volume. If a count of spores alone is required the sample is first pre-treated at (60 ± 2) °C to inactivate vegetative bacteria.

NOTE 1 The medium contains cycloserine as a selective agent to inhibit *Bacillus* species.

NOTE 2 Incubation at 44 °C increases the selectivity of the test for *C. perfringens.*

5 Apparatus and glassware

Except for disposable glassware or plastics ware which is delivered sterile, sterilize glassware as specified in ISO 8199.

Usual microbiological equipment and particularly:

5.1 Membrane filtration apparatus, as specified in ISO 8199.

5.2 Sterile filter funnels

Use funnels either delivered sterile or sterilized as specified in ISO 8199. Alternatively flaming of funnels made of metal prior to their use is acceptable.

NOTE For this method it is insufficient to disinfect funnels by boiling.

5.3 Sterile membrane filters, nominal pore size 0,45 µm.

The quality of membrane filters may vary from brand to brand or even from batch to batch. It is therefore advisable to check the quality on a regular basis.

5.4 Incubators, capable of being maintained at (36 ± 2) °C and at (44 ± 1) °C.

5.5 Water bath (optional), capable of being maintained at (60 ± 2) °C equipped with a means of circulating the water.

5.6 Autoclave, capable of being maintained at (121 ± 3) °C.

5.7 Sterile forceps

5.8 Anaerobic jars, or similar equipment.

5.9 Anaerobic gas generating system, to generate an atmosphere of approximately 90 % hydrogen and 10 % carbon dioxide.

6 Culture media and reagents

6.1 Basic materials

For uniformity of results, in the preparation of media, use constituents of uniform quality and chemicals of recognized analytical grade. For preparation of the media use glass-distilled water or deionized water of equivalent purity, as specified for water grade 3 in ISO 3696[1].

Alternatively, use commercially available dehydrated complete medium and reagents prepared and used according to the manufacturer's instructions.

Other grades of chemicals may be used provided they can be shown to lead to the same results.

6.2 Culture media

See Annex A.

6.2.1 Tryptose sulfite cycloserine agar (TSC agar), References [6][11][12]

See A.1.

6.2.2 Blood agar or Columbia agar base or another suitable nutrient-rich agar

See A.2.

6.2.3 Acid phosphatase reagent

See A.3.

7 Sampling

Carry out sampling as specified in ISO 19458.

8 Procedure

8.1 Transport and storage of the sample

Start examination as soon as possible after the collection of the sample preferably within the same working day. Samples should be cooled during transport ideally at (5 ± 3) °C. The recommended maximum sample storage time including transport is for vegetative bacteria 12 h and for spores 24 h. The sample storage time including transport shall not exceed 18 h for vegetative bacteria and 72 h for spores.

8.2 Heat pre-treatment to select spores

If it is the intention to count only spores, mix the sample well and then heat it to (60 ± 2) °C in a water bath for (15 ± 1) min. The volume heated should be greater than the volume to be analysed. The temperature should be monitored by placing an appropriate thermometer in a reference bottle of the same size as the sample bottle and containing the same volume of water at the same initial temperature as the sample being treated. The time taken to reach (60 ± 2) °C shall not exceed 15 min and can be minimized by ensuring the water in the water bath is circulated to maximize heat exchange.

8.3 Sample dilution

A test volume of sample or dilution of it - after heat treatment if required - should be chosen to yield, if possible, between 10 and 80 colonies on a membrane 47 mm to 50 mm in diameter. Test volumes or dilutions should be prepared as described in ISO 8199.