
**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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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.

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).

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. 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 www.iso.org/patents).

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).

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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 $(44 \pm 1)^\circ\text{C}$ 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