
**Water quality — Detection and enumeration
of intestinal enterococci —**

**Part 2:
Membrane filtration method**

*Qualité de l'eau — Recherche et dénombrement d'entérocoques
intestinaux —*
Partie 2: Méthode par filtration sur membrane

ISO 7899-2:2000

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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 3.

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 part of ISO 7899 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 7899-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This second edition cancels and replaces the first edition (ISO 7899-2:1984), which has been technically revised.

ISO 7899 consists of the following parts, under the general title *Water quality — Detection and enumeration of intestinal enterococci*:

- *Part 1: Miniaturized method (Most Probable Number) for surface and waste water*
- *Part 2: Membrane filtration method*

Annex A of this part of ISO 7899 is for information only.

Introduction

In this part of ISO 7899 a method is described for the isolation of intestinal enterococci. *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae* can be detected and enumerated with the methods described in this part of ISO 7899. In addition, other *Enterococcus* species and some species of the genus *Streptococcus* (namely *S. bovis* and *S. equinus*) may occasionally be detected. These *Streptococcus* species do not survive long in water and are probably not enumerated quantitatively. For purposes of water examination, enterococci can be regarded as indicators of faecal pollution. However it should be noted that some enterococci found in water can occasionally also originate from other habitats.

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Water quality — Detection and enumeration of intestinal enterococci —

Part 2: Membrane filtration method

1 Scope

This part of ISO 7899 specifies a method for the detection and enumeration of intestinal enterococci in water by membrane filtration. This part of ISO 7899 is especially intended for examination of drinking water, water from swimming pools and other disinfected or clean waters. Nevertheless, the method can be applied to all types of water, except when a large amount of suspended matter or many interfering microorganisms are present. It is particularly suitable for the examination of large volumes of water containing only a few intestinal enterococci.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 7899. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 7899 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*.

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 6887-1:1999, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*.

ISO 8199:1988, *Water quality — General guide to the enumeration of micro-organisms by culture*.

ISO/IEC Guide 2:1996, *Standardization and related activities — General vocabulary*.

3 Terms and definitions

For the purposes of this part of ISO 7899, the terms and definitions given in ISO/IEC Guide 2 and the following apply.

3.1

intestinal enterococci

bacteria which are able to reduce 2,3,5-triphenyltetrazolium chloride to formazan and to hydrolyse aesculin at 44 °C on the media (6.3.1 and 6.3.2) specified in this part of ISO 7899

NOTE See also annex A.

4 Principle

4.1 Filtration, incubation and enumeration

The enumeration of intestinal enterococci is based on filtration of a specified volume of water sample through a membrane filter with a pore size (0,45 µm) sufficient to retain the bacteria. The filter is placed on a solid selective medium containing sodium azide (to suppress the growth of Gram-negative bacteria) and 2,3,5-triphenyltetrazolium chloride, a colourless dye, that is reduced to red formazan by intestinal enterococci.

Typical colonies are raised, with a red, maroon or pink colour, either in the centre of the colony or throughout.

4.2 Confirmation

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If typical colonies are observed, a confirmation step is necessary, by transfer of the membrane, with all the colonies, onto bile-aesculin-azide agar, preheated at 44 °C. Intestinal enterococci hydrolyse aesculin on this medium in 2 h. The end-product, 6,7-dihydroxycoumarin, combines with iron(III) ions to give a tan-coloured to black compound which diffuses into the medium.

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5 Apparatus

Except for disposable glassware which is delivered sterile, glassware shall be sterilized in accordance with ISO 8199.

Usual microbiological laboratory equipment and particularly:

5.1 **Membrane filtration apparatus**, according to ISO 8199.

5.2 **Sterile membrane filters**, with a nominal pore size of 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, in accordance with ISO 7704.

5.3 **Incubator**, capable of being maintained at 36 °C ± 2 °C.

5.4 **Incubator**, capable of being maintained at 44 °C ± 0,5 °C.

5.5 **Autoclave**, capable of being maintained at 121 °C ± 3 °C.

5.6 **Sterile forceps**.

5.7 **Hotplate or water bath**, maintained at 100 °C.

6 Culture media and reagents

6.1 Basic materials

WARNING — The selective media described in this part of ISO 7899 contain sodium azide. As this substance is highly toxic and mutagenic, precautions shall be taken to avoid contact with it, especially by the inhalation of fine dust during the preparation of commercially available dehydrated complete media. Azide-containing media should not be mixed with strong inorganic acids, as toxic hydrogen azide (HN_3) may be produced. Solutions containing azide can also form explosive compounds when in contact with metal pipework, for example from sinks.

Azides can be decomposed safely by the addition of an excess of a saturated nitrite solution.

For uniformity of results, in the preparation of media, either use a dehydrated complete medium or use constituents of uniform quality and chemicals of recognized analytical grade. Sodium azide deteriorates with time so that dehydrated media have a limited shelf-life.

NOTE Use of chemicals of another quality is possible provided they are shown to be of equal performance in the test.

6.2 Distilled water or water of equivalent purity, in accordance with ISO 3696, Grade 1.

6.3 Culture media

6.3.1 Slanetz and Bartley medium

6.3.1.1 Basal medium

Tryptose	20,0 g
Yeast extract	5,0 g
Glucose	2,0 g
Dipotassium hydrogenphosphate (K_2HPO_4)	4,0 g
Sodium azide (NaN_3)	0,4 g
Agar	8 g to 18 g ¹⁾
Water	1 000 ml

Dissolve the ingredients in boiling water.

Once dissolution is complete, heat for an additional 5 min.

Cool to 50 °C to 60 °C.

6.3.1.2 TTC solution

2,3,5-triphenyltetrazolium chloride	1 g
Water	100 ml

Dissolve the indicator in the water by stirring.

Sterilize by filtration (0,2 µm).

Protect the solution against the action of light, and discard it if a pink tinge develops.

¹⁾ Depending on the gel strength of the agar.