INTERNATIONAL STANDARD

ISO 7899-2

Second edition
2000-04-15

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

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.

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.

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 samping 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 : 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

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.

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.

6.3.1.3 Complete medium

Basal medium (6.3.1.1)	1 000 ml
TTC solution (6.3.1.2)	10 ml

Add the TTC solution to the basal medium cooled to 50 °C to 60 °C.

Adjust the pH if necessary so that after sterilization it is 7,2 ± 0,1 at 25 °C, with a solution of sodium carbonate (100 g/l) or of sodium hydroxide (40 g/l) or of hydrochloric acid (36,5 g/l).

Pour 20 ml of medium into Petri dishes of 9 cm diameter (or an equivalent amount in a dish of another size) and allow to set on a cool, horizontal surface.

Poured plates can be stored in the dark for up to 2 weeks at 5 °C ± 3 °C.

6.3.2 Bile-aesculin-azide agar

Tryptone	17,0 g
Peptone	3,0 g
Yeast extract	5,0 g
Ox-bile, dehydrated	10,0 g
Sodium chloride (NaCl)	5,0 g
Aesculin	1,0 g
Ammonium iron(III) citrate	0,5 g
Sodium azide (NaN_3)	0,15 g
Agar	8 g to 18 g[1)]
Water	1 000 ml

Dissolve the ingredients in the water by boiling.

Adjust the pH so that after sterilization it is 7,1 ± 0,1 at 25 °C.

Sterilize for 15 min at 121 °C ± 3 °C.

Cool to 50 °C to 60 °C and pour into Petri dishes to a depth of 3 mm to 5 mm and allow to set on a cool, horizontal surface.

Poured plates can be stored at 5 °C ± 3 °C for up to 2 weeks.

7 Sampling

Sampling shall be carried out in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3.

8 Procedure

8.1 Preparation of the sample

Prepare the sample, filter and inoculate on isolation media in accordance with the instructions given in ISO 8199 and ISO 6887-1. Start the examination preferably immediately after taking the samples. If the samples are kept at ambient temperatures, the examination shall begin within 6 h after taking the sample. Under exceptional circumstances, it is permissible for the samples to be kept at 5 °C ± 3 °C for up to 24 h prior to examination.

If sample dilutions are necessary, prepare these dilutions in accordance with ISO 8199.

8.2 Filtration and incubation

For a general description of the membrane filtration technique, see ISO 8199.

Filter a volume of water appropriate for the water being examined.

Place the membrane filter on Slanetz and Bartley medium (6.3.1).

Incubate the plates at 36 °C ± 2 °C for 44 h ± 4 h.

8.3 Confirmation and enumeration

After incubation, consider all raised colonies which show a red, maroon or pink colour, either in the centre or throughout the colony, as typical.

If there are typical colonies, transfer the membrane and the colonies, with sterile forceps, without inverting it, onto a plate of bile-aesculin-azide agar which has been preheated to 44°C.

Incubate at 44°C ± 0,5°C for 2 h.

Read the plate immediately.

Regard all typical colonies showing a tan to black colour in the surrounding medium as giving a positive reaction, and count them as intestinal enterococci.

NOTE Uneven distribution of colonies or the presence of high background counts may interfere with the differenciation of positive colonies, due to the diffusion of the colour to adjacent colonies.

9 Quality assurance

The laboratory shall have a clearly defined quality control system to ensure that the materials, reagents and techniques are suitable for the test.

10 Expression of results

Calculate the results in accordance with ISO 8199.

11 Test report

The test report shall include the following information:

a) a reference to this part of ISO 7899;

b) all details necessary for complete identification of the sample;

c) the number of colonies confirmed as intestinal enterococci;

d) particular phenomena observed during the analysis and any operation not specified in the method, or considered optional, that could have modified the results.