

Australian/New Zealand Standard™

Water microbiology

Method 9: Enterococci—Membrane filtration method (ISO 7899-2:2000, MOD)

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology to supersede AS 4276.9:1995, *Water microbiology, Method 9: Faecal streptococci—Membrane filtration method*.

This Standard is an adoption with national modifications and has been reproduced from ISO 7899-2:2000, *Water quality—Detection and enumeration of intestinal enterococci—Part 2: Membrane filtration method*. Additional requirements and guidance for Australian/New Zealand conditions are listed in Appendix ZZ which is added at the end of the source text.

The additional requirements are included to bring this Standard into line with others in the AS/NZS 4276 series to introduce quality control procedures to the method.

These technical variations apply to the following clauses of ISO 7899-2:2000:

- (a) Clause 6, Culture media and reagents
- (b) Clause 8.3, Confirmation and enumeration.

The objective of this Standard is to specify a method for the detection and enumeration of intestinal enterococci in water by membrane filtration. Intestinal enterococci are defined by Clause 3.1 of the Standard method. Slanetz and Bartley medium is also known as m-Enterococcus medium.

Appendix ZA provides a flow diagram of the procedure for information.

As this Standard is reproduced from an International Standard, the following applies:

- (i) Its number does not appear on each page of text and its identity is shown only on the cover and title page.
- (ii) In the source text 'this part of ISO 7899' should read 'this Australian/New Zealand Standard'.
- (iii) A full point should be substituted for a comma when referring to a decimal marker.

References to International Standards should be replaced by references to Australian or Australian/New Zealand Standards, as follows:

<i>Reference to International Standard</i>		<i>Australian/New Zealand Standard</i>	
ISO		AS	
6887	Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination	5013	Food microbiology
6887-1	Part 1: General rules for the preparation of the initial suspension and decimal dilutions	5013.11.1	Method 11.1: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions

ISO		AS/NZS	
8199	Water quality—General guidance on the enumeration of microorganisms by culture	4276 4276.1	Water microbiology Method 1: General information and procedures (ISO 8199:2005, MOD)
5677 5677-3	Water quality—Sampling Part 3: Guidance on the preservation and handling of samples	2031	Selection of containers and preservation of water samples for microbiological analysis

The other international references have not been adopted as an Australian/New Zealand Standard.

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

The terms ‘normative’ and ‘informative’ have been used in this Standard to define the application of the annex to which they apply. A ‘normative’ annex is an integral part of a Standard, whereas an ‘informative’ annex is only for information and guidance.

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.

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

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.