AS 4276.9:2019

Australian STANDARD

Water microbiology

Method 9: Enterococci in water by membrane filtration using membrane—Enterococcus indoxyl-β-D-glucoside agar (mEI)

STANDARDS Australia

AS 4276.9:2019

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Originated as AS 1095.4.1.8—1981.
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Jointly revised and redesignated as AS/NZS 4276.9:2007.
Revised and redesignated as AS 4276.9:2019.

Preface

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology, to supersede AS/NZS 4276.9:2007, *Water microbiology, Method 9: Enterococci—Membrane filtration method (ISO 7899-2000, MOD)*.

The objective of this Standard is to specify a method for the detection and enumeration of enterococci, also known as intestinal enterococci, in water by membrane filtration.

The major changes in this edition are as follows:

(a) Use of a chromogenic medium for the enumeration of enterococci in water types, including recreational, potable, industrial, waste, natural, fresh and marine waters.

(b) Incorporate culture media and reagents.

(c) Update reference cultures.

(d) Update the normative and informative references.

The terms "normative" and "informative" are used in Standards to define the application of the appendix to which they apply. A "normative" appendix is an integral part of a Standard, whereas an "informative" appendix is only for information and guidance.

Contents

Introduction

Enterococci are commonly found in the faeces of humans and other warm-blooded animals. Although some strains are ubiquitous and not related to faecal pollution, the presence of enterococci in water is an indication of faecal pollution and the possible presence of enteric pathogens.

Epidemiological studies have led to the development of criteria to promulgate recreational water standards based on established relationships between health effects and water quality. The significance of finding enterococci in recreational fresh or marine water samples is the direct relationship between the density of enterococci and the risk of gastrointestinal illness associated with swimming (see Clause B.2 of Appendix B).

Enterococci include *Enterococcus faecalis* (*E. faecalis*), *E. faecium*, *E. avium*, *E. gallinarium*, and their variants. The genus *Enterococcus* includes the enterococci formerly assigned to the Group D faecal streptococci.

This Standard method is based on a validated and published US EPA Method 1600: *Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI)* (see Bibliography [1]).

NOTE The preparation of the nalidixic acid and triphenyltetrazolium chloride (TTC) supplements is based on the alternative method described in US EPA Method 1106.1 (mE-EIA) (see Bibliography [2]).

ISO 7899-2:2000 was not adopted because the ISO Standard is not suitable for recreational water monitoring in Australia due to the high demand for quicker analytical turnaround times to ensure public health without compromising test results; e.g. for management of major aquatic sport events and recreational water use. Incidents during these activities may have a negative impact on dependent commercial activities and attract poor publicity.

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1 Scope

This Standard sets out a method for enumerating enterococci bacteria in water by membrane filtration.

NOTE 1 This method may not be suitable for isolation of strains of *Streptococcus bovis* and *Streptococcus equinus* that do not grow at 41 °C ± 0.5 °C.

NOTE 2 Membrane filtration is not suitable for turbid water samples.

NOTE 3 A flow diagram of the procedure is shown in Appendix A.

WARNING — THE SELECTIVE MEDIUM (mEI) USED IN THIS METHOD CONTAINS SODIUM AZIDE AND CYCLOHEXIMIDE. CAUTION SHALL BE EXERCISED DURING THE PREPARATION, USE, AND DISPOSAL OF THIS MEDIUM TO PREVENT INHALATION OR CONTACT.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document.

NOTE Documents referenced for informative purposes are listed in the Bibliography.

AS 2031, *Water quality—Sampling for microbiological analysis (ISO 19458:2006, MOD)*

AS 5140, *Microbiology of food, animal feed and water—Preparation, production, storage and performance testing of culture media (ISO 11133:2014, MOD)*

AS/NZS 4276.1, *Water microbiology, Method 1: General information and procedures (ISO 8199:2005, MOD)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
enterococci
bacteria which express β-glucosidase from a chromogenic cellobiose analog (indoxyl β-D-glucoside) in the mEI agar

Note 1 to entry: Enterococci produce an insoluble indigo blue complex that diffuses into the surrounding media, forming a blue halo around colonies that are greater than or equal to 0.5 mm.

3.2
may
indicates the existence of an option

3.3
shall
indicates that a statement is mandatory

3.4
should
indicates a recommendation