Australian Standard®

Water microbiology

Method 17.2: Spores of *Clostridium perfringens*—Estimation of most probable number (MPN) using the multiple tube dilution technique

AS 4276.17.2:2016

PREFACE

This Standard was prepared by the Standards Australia Committee FT-20, Water Microbiology, to supersede AS/NZS 4276.17.2:2000, *Water microbiology*, Method 17.2: *Spores of sulfite-reducing anaerobes (clostridia) including Clostridium perfringens—Estimation of most probable number (MPN) using the multiple tube dilution technique*.

The objectives of this revision are—

(a) to incorporate an additional more selective medium [oleandomycin polymyxin sulphadiazine perfringens (OPSP) agar] for testing of samples with a greater proportion of competing microflora and to incorporate other technical variations;

(b) to incorporate culture media, reagents and to remove reference to AS 4276.2;

(c) to update reference cultures; and

(d) to update the references.

There is no ISO Standard for estimation of most probable number (MPN) using the multiple tube dilution technique for spores of *Clostridium perfringens*.

The term 'informative' has been used in this Standard to define the application of the appendix to which it applies. An 'informative' appendix is only for information and guidance.

FOREWORD

The spores of sulfite-reducing anaerobes such as *Clostridium perfringens* are widespread in the environment. They are present in human and animal faecal material, in waste water and in soil. Unlike *Escherichia coli* and other coliform bacteria, spores of *Clostridium perfringens* survive in water for months as they are more resistant to the action of chemical agents (e.g. chlorination) and physical conditions (e.g. heat) than vegetative forms and may be used as indicators of water quality.

As *Clostridium perfringens* is associated with faecal contamination, it can indicate remote or intermittent pollution in the absence of other faecal indicator microorganisms.

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 treatment efficiency.

METHOD

1 SCOPE

This Standard sets out a method for determining the most probable number (MPN) of spores of *C. perfringens* in water, using a multiple tube dilution technique, with a confirmation procedure for identifying *C. perfringens*.

Refer to AS/NZS 4276.1 for a description of the general procedure for estimating the MPN of microorganisms.

MPN procedures may be used for the examination of all water samples and are particularly applicable for samples containing sufficient suspended material to make filtration procedures impractical.

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

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS		
2031	Water quality—Sampling for microbiological analysis	
5013	Food microbiology	
5013.16	Method 16:	Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of *Clostridium perfringens*—Colony-count technique
AS/NZS		
4276	Water microbiology	
4276.1	Method 1:	General information and procedures(ISO 8199:2005, MOD)

3 DEFINITIONS

For the purposes of this Standard the following definitions apply:

3.1 Presumptive *C. perfringens*

Bacteria that produce all shades of black or grey to yellow-brown colonies, even if the colour is faint, after anaerobic incubation at 36 ±2°C or 44 ±1°C for 21 ±3 h, on tryptose-sulfite-cycloserine (TSC) agar at 44 ±1°C or oleandomycin polymyxin sulphadiazine perfringens (OPSP) agar at 36 ±2°C or 44 ±1°C.

3.2 Confirmed *C. perfringens*

Anaerobic bacteria that produce characteristic colonies on TSC or OPSP agar, capable of growth at 44 ±1°C, and possess the enzyme acid phosphatase.

4 PRINCIPLE

A series of dilutions are inoculated into the differential reinforced clostridial medium (DRCM) in accordance with the MPN method in AS/NZS 4276.1. Tubes are incubated anaerobically at 44 ±1°C for 48 ±4 h. A loopful of broth from each blackened DRCM tubes is subcultured onto TSC or OPSP medium to obtain isolated colonies for confirmation.

This method consists of the following stages:

(a) A heating procedure to eliminate non-spore-forming microorganisms from the test sample.

(b) A multiple tube dilution cultural procedure to selectively detect H_2S producing anaerobic microorganisms in the heat treated sample.