Australian Standard®

Water microbiology
Method 17.1: Spores of *Clostridium perfringens*—Membrane filtration method

PREFACE

This Standard was prepared by Standards Australia Committee FT-0/ Water Microbiology, to supersede AS/NZS 4276.17.1:2000, *Water microbiology*, Method 17.1: *Spores of sulphite-reducing anaerobes (clostridia) including Clostridium perfringens—Membrane filtration method*.

The objectives of this revision are—

(a) to incorporate an additional more selective medium (oleandomycin polymyxin sulphadiazine perfringens 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.

The Committee considered ISO 14189:2013, *Water quality—Enumeration of Clostridium perfringens—Method using membrane filtration*, for adoption. ISO 14189 has not been adopted, as the method needs to cover an additional, more selective medium for testing of samples with a greater proportion of competing microflora.

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 enumerating spores of *C. perfringens* in water, using membrane filtration.

Refer to AS 4276.1 for a description of the general procedure for determining counts of microorganisms by the membrane filtration technique.

NOTES:

1 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.

2 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 *Clostridium perfringens*

Bacteria which produce all shades of black or grey to yellow-brown colonies, even if the colour is faint, after anaerobic incubation 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*

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

4 PRINCIPLE

A measured volume of the sample, or a dilution, is filtered through a membrane with a pore size of 0.45 µm sufficient to retain spores of clostridia. The membrane is incubated anaerobically on a selective/differential agar (TSC agar at 44 ±1°C, or OPSP agar at 36 ±2°C or 44 ±1°C, as appropriate) for 21 ±3 h. *C. perfringens* produce black or grey to yellow-brown colonies as a result of the reduction of sulfite to sulfide which reacts with a ferric salt in both media. Characteristic colonies are counted and confirmatory tests are carried out. The count is calculated and the result reported as colony forming units per 100 mL.