Australian Standard®

Water microbiology
Method 15: Examination for *Vibrio cholerae*

PREFACE

This Standard was prepared by the Australian members of Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, to supersede AS/NZS 4276.15:1999, *Water microbiology*, Method 15: *Examination for Vibrio cholerae.*

After consultation with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The objective of this revision is to update the method to reflect current practice according to recognized reference methods, as well as any developments in the relevant area of science. This has included the International Technical Specification for the detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* in food, ISO/TS 21872-1. The bibliography lists the methods and references taken into account in the preparation of this Standard.

During the review, the Committee noted there is no published ISO method for the detection of *V. cholerae* in water to be considered for adoption, or in the pipeline.

This method is designed to detect *V. cholerae* in water using conventional culture-based techniques. The enumeration of this microorganism is not normally carried out because its presence, irrespective of numbers, is significant from a public health point of view.

The *V. cholerae* species is composed of numerous O-serotypes. Toxin producing strains of the O1 serotype are responsible for epidemic cholera. However, another serotype, O139, has relatively recently been identified as an epidemic producing strain. Other strains belonging to different serotypes may cause sporadic cases of diarrhoeal illness. This method will detect all strains of *V. cholerae* and allow identification of the cholera epidemic producing O1 and O139 serotypes.

It should be emphasized that the finding of *V. cholerae* O1 or O139 in a water sample would necessitate the prompt notification of the relevant health authority.

In addition, isolates of *V. cholerae* O1 and O139 are now subject to an Australian Commonwealth security sensitive biological agent (SSBA) regulatory scheme (the *National Health Security Act 2007* and the National Health Security Regulations 2008). The aim of the SSBA regulatory scheme is to limit the opportunities for acts of bioterrorism or biocrime to occur using harmful biological agents and to provide a legislative framework for managing the security of SSBAs. The regulatory scheme imposes statutory obligations on laboratories that isolate or receive strains of *V. cholerae* O1 or O139. Laboratories that have an involvement with *V. cholerae,* in whatever form, can familiarize themselves with this regulatory scheme by accessing the Department of Health website: www.health.gov.au/SSBA.

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.

METHOD

1 SCOPE

This Standard sets out a method for detecting *V. cholerae* in water using culture in enrichment broth and on selective agar media.

NOTES:

1 A flow diagram of the procedure is shown in Appendix A.

2 This method is suitable for the isolation of other *Vibrio* species besides *V. cholerae*. However, the NaCl concentration in the enrichment broth should be increased to 1% for isolation of such species.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS
4276 Water microbiology
4276.1 Part 1: General information and procedures (ISO 8199:2005, MOD)

3 DEFINITION

For the purposes of this Standard the following definition applies:

3.1 *V. cholerae*

V. cholerae is a member of the family Vibrionaceae. It is a facultatively anaerobic, gram-negative, non-spore-forming curved rod, about 1.4–2.6 µm long, capable of respiratory and fermentative metabolism. It is oxidase-positive, reduces nitrate, and is motile by means of a single, sheathed, polar flagellum.

V. cholerae is able to grow at pH 8.6 in the presence of bile salts, 1% sodium thiosulphate, and 1% sodium citrate and ferment sucrose without the production of sulphide.

4 PRINCIPLE

V. cholerae is tolerant of alkaline conditions and will grow rapidly in peptone water at an alkaline pH and with 0.5% sodium chloride. The water sample is enriched in alkaline peptone water and plated onto thiosulphate citrate bile salts sucrose agar. Typical colonies are confirmed as *V. cholerae* by biochemical tests. The serotypes responsible for epidemic cholera, O1 and O139, are identified by slide agglutination with specific antisera.

NOTE: This method does not include enumeration. If enumeration of *V. cholerae* in water is required, the most probable number (MPN) procedure may be employed.

See AS 4276.1. The volumes tested depend on the degree of contamination of the water, but the set of 50 mL, 10 mL and 1 mL is a useful starting point. The method commences with enrichment using APW, and then proceeds according to this standard. Calculation of the most probable number per given volume requires the use of MPN tables.

