

Australian Standard[®]

Water microbiology

Method 14: Detection of *Salmonella* spp. (ISO 19250:2010, MOD)

AS 4276.14:2014

PREFACE

This Standard was prepared by the Australian members of Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, to supersede AS 4276.14—1995, *Water microbiology*, Method 14: *Salmonellae*.

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.

This Standard is an adoption with national modifications and has been reproduced from ISO 19250:2010, *Water quality—Detection of Salmonella spp.*

The objective of this revision is to adopt the ISO Standard with Australian technical variations to the following Clauses of ISO 19250:

- (a) Clause 4.3, Enrichment in selective liquid media.
- (b) Clause 7, Culture media and reagents.
- (c) Clause 8.1, Preparation of the sample.
- (d) Clause 8.5.4, Serological confirmation and serotyping.
- (e) Clause 8.6 (new), Complete serological identification.
- (f) Clause 11, Quality assurance.
- (g) Annex A, Diagram of procedure.
- (h) Annex B, Competition and preparation of culture media and reagents.
- (i) Bibliography.

These variations, which are necessary for Australian conditions, are listed in Appendix ZZ, which is added at the end of the source text.

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

- (i) In the source text 'this International Standard' should read 'this Australian Standard'.
- (ii) A full point substitutes for a comma when referring to a decimal marker.
- (iii) Substitute 'mL' for 'ml' wherever it appears.

References to International Standards should be replaced by references to Australian Standards, as follows:

<i>International Standard</i>		<i>Australian or Australian/New Zealand Standard</i>	
ISO		AS	
6579	Microbiology of food and animal feeding stuffs—Horizontal method for the detection of <i>Salmonella</i> spp.	5013	Food microbiology
		5013.10	Method 10: Microbiology of food and animal feeding stuffs—Horizontal method for the detection of <i>Salmonella</i> spp. (ISO 6579:2002, MOD)
6887	Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination	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
6887-1	Part 1: General rules for the preparation of the initial suspension and decimal dilutions		
7218	Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations	5013.14.1	Method 14.1: General requirements and guidance for microbiological examinations (ISO 7218:2007, MOD)
		AS/NZS	
8199	Water quality—General guidance on the enumeration of micro-organisms by culture	4276	Water microbiology
		4276.1	Part 1: General information and procedures (ISO 8199:2005, MOD)
		AS	
19458	Water quality—Sampling for microbiological analysis	2031	Water quality—Sampling for microbiological analysis (ISO 19458:2006, MOD)

Isolates of *Salmonella* Typhi 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 *Salmonella* Typhi. Laboratories that have an involvement with *Salmonella* Typhi, in whatever form, can familiarize themselves with this regulatory scheme by accessing the Department of Health website: www.health.gov.au/SSBA.

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

CONTENTS

1	Scope	1
2	Normative references	1
3	Terms and definitions	2
4	Principle.....	2
4.1	General	2
4.2	Pre-enrichment in non-selective liquid medium	2
4.3	Enrichment in selective liquid media	2
4.4	Plating out and recognition	3
4.5	Confirmation	3
5	Apparatus	3
6	Sampling.....	4
7	Culture media and reagents	4
8	Procedure.....	5
8.1	Preparation of the sample	5
8.2	Non-selective pre-enrichment.....	5
8.3	Selective enrichment.....	5
8.4	Plating out	6
8.5	Confirmation	6
9	Expression of results	9
10	Test report.....	9
	Annex A (normative) Diagram of procedure	10
	Annex B (normative) Composition and preparation of culture media and reagents.....	11
	Annex C (informative) Results of the interlaboratory trial.....	18
	Bibliography.....	23

INTRODUCTION

Salmonella species are bacteria which are widely distributed all over the world. They are usually classified as pathogens, although their virulence and pathogenesis vary widely. The natural hosts of *Salmonella* include humans, agricultural and domestic livestock, and wild animals including birds. Humans and animals can excrete these bacteria while carrying them asymptotically as well as during disease. It is therefore impossible to eliminate them from the environment. Following the infection of humans, the transmission of *Salmonella* can cause severe disease.

Since water is a recognized vehicle of infection, the presence or absence of *Salmonella* is monitored in water where there is perceived to be a risk of infection. *Salmonella* can be present in all types of domestic and agricultural waste water, freshwaters, including ground and drinking waters, as well as sea water.

The detection of *Salmonella* in water usually requires a concentration step. Since *Salmonella* cells can be present in low numbers and injured in the aqueous environment, their detection in water usually requires a pre-enrichment step.

METHOD

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella*, and especially *S. enterica* subsp. *enterica* ser. Typhi (*Salmonella* ser. Typhi) and *S. enterica* subsp. *enterica* ser. Paratyphi (*Salmonella* ser. Paratyphi), be undertaken only in properly equipped laboratories, under the control of a skilled microbiologist, and that great care be taken in the disposal of all incubated materials.

Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the detection of *Salmonella* spp. (presumptive or confirmed) in water samples. It is possible that, for epidemiological purposes or during outbreak investigations, other media are also required.

WARNING — It is possible that the method does not recover *Salmonella* ser. Typhi and ser. Paratyphi.

NOTE For a semi-quantitative approach, most probable number (MPN) tests can be performed using appropriate sample volumes. For these cases, the volume of the buffered peptone water is adjusted accordingly.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 7701, *Water quality — Evaluation of membrane filters used for microbiological analyses*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 19458, *Water quality — Sampling for microbiological analysis*