



**Waters—Examination for *Legionella*
spp. including *Legionella pneumophila***



This Australian Standard® was prepared by Committee FT-020, Water Microbiology. It was approved on behalf of the Council of Standards Australia on 2 March 2017. This Standard was published on 10 May 2017.

The following are represented on Committee FT-020:

- ACT Health
- Australian Society for Microbiology
- Australian Water Association
- National Association of Testing Authorities
- National Measurement Institute
- NSW Forensic and Analytical Science Service
- PathWest Laboratory Medicine, WA
- Plastics and Chemicals Industries Association
- Queensland Health
- Queensland University of Technology
- University of Melbourne
- VicWater
- Water NSW
- Water Research Australia
- Water Services Association of Australia

Additional Interests:

- Commercial Testing Laboratories
-

This Standard was issued in draft form for comment as DR AS 3896:2016.

Standards Australia wishes to acknowledge the participation of the expert individuals that contributed to the development of this Standard through their representation on the Committee and through the public comment period.

Keeping Standards up-to-date

Australian Standards® are living documents that reflect progress in science, technology and systems. To maintain their currency, all Standards are periodically reviewed, and new editions are published. Between editions, amendments may be issued.

Standards may also be withdrawn. It is important that readers assure themselves they are using a current Standard, which should include any amendments that may have been published since the Standard was published.

Detailed information about Australian Standards, drafts, amendments and new projects can be found by visiting www.standards.org.au

Standards Australia welcomes suggestions for improvements, and encourages readers to notify us immediately of any apparent inaccuracies or ambiguities. Contact us via email at mail@standards.org.au, or write to Standards Australia, GPO Box 476, Sydney, NSW 2001.

Australian Standard®

**Waters—Examination for *Legionella*
spp. including *Legionella pneumophila***

Originated as AS 3896—1991.
Previous edition AS/NZS 3896:2008.
Revised and designated as AS 3896:2017.

COPYRIGHT

© Standards Australia Limited

All rights are reserved. No part of this work may be reproduced or copied in any form or by any means, electronic or mechanical, including photocopying, without the written permission of the publisher, unless otherwise permitted under the Copyright Act 1968.

Published by SAI Global Limited under licence from Standards Australia Limited, GPO Box 476, Sydney, NSW 2001, Australia

ISBN 978 1 76035 735 1

PREFACE

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology, to supersede AS/NZS 3896:2008.

The objectives of this revision are—

- (a) to review the selective media and the volume of the inoculum used in this method;
- (b) to incorporate culture media and reagents and to remove reference to AS 4276.2;
- (c) to update reference cultures; and
- (d) to update the references.

Committee FT-020 did not recommend the adoption of ISO 11731:1998, *Water quality—Detection and enumeration of Legionella*, and ISO 11731-2:2004, *Water quality—Detection and enumeration of Legionella, Part 2: Direct membrane filtration method for waters with low bacterial counts*, either alone or combined as Australian Standards because these two ISO Standards do not align with current health policy and legislative requirements.

This Standard is suitable for the testing of treated and untreated water samples. If low levels of *Legionella* spp. are anticipated in a water sample, AS 5132, *Waters—Examination for Legionella spp. including Legionella pneumophila—Using concentration*, may be more appropriate.

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.

CONTENTS

| | <i>Page</i> |
|---|-------------|
| FOREWORD..... | 4 |
| 1 SCOPE..... | 5 |
| 2 REFERENCED DOCUMENTS..... | 5 |
| 3 PRINCIPLE..... | 5 |
| 4 SAFETY PRECAUTIONS..... | 5 |
| 5 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES..... | 6 |
| 6 APPARATUS..... | 7 |
| 7 SAMPLING, TRANSPORT AND STORAGE..... | 7 |
| 8 PROCEDURE..... | 7 |
| 9 CALCULATIONS..... | 10 |
| 10 TEST REPORT..... | 11 |
| APPENDICES | |
| A FLOW CHART FOR <i>LEGIONELLA</i> SPP. TESTING..... | 12 |
| B CULTURE MEDIA AND REAGENTS..... | 13 |
| C EXAMPLES OF TYPICAL <i>LEGIONELLA</i> COLONIES..... | 20 |
| BIBLIOGRAPHY..... | 23 |

FOREWORD

This Standard stems from a need, expressed by health authorities and other stakeholders, for a standard method for the enumeration of legionellae in waters to facilitate the monitoring of the effectiveness of measures adopted for preventing the proliferation of legionellae in waters.

Legionella pneumophila serogroup 1 causes ‘Legionnaire’s’ disease. However, some other *Legionella* are potentially pathogenic and may cause legionellosis. This revised Standard is designed to estimate the number of *L. pneumophila* and a range of other *Legionella spp.*, the presence of which may indicate poorly managed water systems.

The method is suitable for use in a laboratory equipped to carry out routine microbiological work.

Some *Legionella* can be identified with commercially available systems, while others may require specialized testing not normally available in routine testing laboratories.

Strain characterization of *Legionella* isolates required as part of outbreak investigations is not within the scope of this Standard. This is normally carried out by public health microbiology laboratories. Other laboratories that isolate *Legionella* strains related to outbreaks should refer any *Legionella* isolates to a laboratory with *Legionella* strain characterization capability.

STANDARDS AUSTRALIA

Australian Standard

Waters—Examination for *Legionella* spp. including *Legionella pneumophila*

1 SCOPE

This Standard sets out a method for isolating and estimating the number of *Legionella pneumophila* and a range of other *Legionella* spp. in water. This method is applicable to all water samples, including recreational, industrial, waste and natural waters.

NOTES:

- 1 A flow chart of the procedure is shown in Appendix A.
- 2 While this method is suitable for the testing of all waters, there is a separate method (AS 5132) that is designed specifically for concentration of water where a lower limit of detection is required due to public health concerns.
- 3 This method will isolate the species *L. pneumophila* but not all other *Legionella* spp. *L. pneumophila* serogroup 1 accounts for the majority of legionellae infections.
- 4 Conditions that favour the isolation of *L. pneumophila* do not necessarily apply to some *Legionella* spp. However, treatments and media included in this method are designed to enhance the recovery of other species in addition to *L. pneumophila*.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

- 2031 Water quality—Sampling for microbiological analysis (ISO 19458:2006, MOD)
5132 Waters—Examination for *Legionella* spp. including *Legionella pneumophila*—Using concentration

AS/NZS

- 2243 Safety in laboratories
2243.3 Part 3: Microbiological safety and containment
4276 Water microbiology
4276.1 Method 1: General information and procedures (ISO 8199:2005, MOD)

3 PRINCIPLE

The sample of water is cultured on specified media directly as well as after heat and acid treatments. Presumptive *Legionella* colonies are taken through confirmation tests. Confirmed *Legionella* isolates can be speciated and serotyped as required.

4 SAFETY PRECAUTIONS

The safety precautions for microbiological laboratories, as described in AS/NZS 2243.3, shall be observed.

Legionella cultures on solid culture media can be handled safely by trained staff on the open bench in a conventional microbiology laboratory conforming to Containment Level 2. Infection is caused by inhalation of the microorganism and it is advisable therefore to assess all procedures for their ability to produce aerosols. If in doubt, carry out the work in a biological safety cabinet.