



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



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- Australian Society for Microbiology
- Australian Water Association
- National Association of Testing Authorities
- National Measurement Institute
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- PathWest Laboratory Medicine, WA
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- Queensland Health
- Queensland University of Technology
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- VicWater
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- Water Services Association of Australia

Additional Interests:

- Commercial Testing Laboratories
-

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Australian Standard®

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PREFACE

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology, as a new Standard to complement AS 3896:201X, *Waters—Examination for Legionellae spp including Legionella pneumophila*. This Standard stems from a need, expressed by health authorities and other stakeholders, for a standard method for the enumeration of legionellae in waters with low background microflora in treated water systems, particularly in reticulated water systems supplying warm water systems.

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* because these two ISO Standards are currently under review.

This Standard is suitable for testing water samples where low microbial loads are expected. The target water matrix for this Standard is samples from reticulated water systems where colonization by *Legionella* spp. is of public health concern. While this Standard is not restricted to this matrix, it may not be suitable for some environmental samples, such as industrial, waste and natural waters. This is because samples are concentrated and the high microbial loads in these samples could interfere with the detection of *Legionella* spp., leading to the possibility of false negative results.

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.

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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 with low background microflora, particularly in reticulated warm water systems. Such systems have been implicated in cases and outbreaks of legionellosis. This Standard provides a tool for investigating water systems linked to legionellosis, as well as for monitoring the effectiveness of measures adopted for preventing the proliferation of legionellae in such systems.

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

Legionella pneumophila serogroup 1 causes ‘Legionnaire’s’ disease. However, some other *Legionella* are potentially pathogenic and may cause legionellosis. This 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.

Some *Legionella* can be identified with commercially available systems. Identification of other species may require specialized testing that is 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*—Using concentration

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 with low microbial loads. The target matrix for this Standard is water samples from reticulated water systems where colonisation by *Legionella* spp. is of public health concern. While this Standard is not restricted to this matrix, it may not be suitable for some environmental and recreational samples, such as industrial, waste and natural waters. This is because samples are concentrated and the high microbial loads in these samples could interfere with the detection of *Legionella* spp., leading to the possibility of false negative results.

This Standard has a lower limit of detection than AS 3896. Therefore, it is suitable when—

- (a) the detection of lower levels is sought; or
- (b) higher precision is desired for levels close to the lower limit of detection of AS 3896.

NOTES:

- 1 A flow chart of the procedure is shown in Appendix A.
- 2 This method will isolate *L. pneumophila* but not all other *Legionella* spp. *L. pneumophila* serogroup 1 accounts for the majority of legionellae infections.
- 3 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)

3896 Waters—Examination for *Legionella* spp. including *Legionella pneumophila*

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 concentrated by membrane filtration. Bacteria trapped on the membrane filter are resuspended in 10 mL of diluent. The concentrated sample is cultured, directly and following heat and acid treatments, onto specified media. Presumptive *Legionella* spp. colonies then undergo confirmatory testing. Confirmed *Legionella* isolates can be speciated and typed as required.