AS 3896—1991

Australian Standard®

Waters—Examination for legionellae

STANDARDS AUSTRALIA

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The following interests are represented on Committee CH/22/1:

Community and Health Service, A.C.T.

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Department of Health, N.S.W.

Department of Health, Qld

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Fairfield Hospital

Health Department of W.A.

Institute of Medical and Veterinary Scien

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PREFACE

This Standard was prepared by a Subcommittee on Legionella Methodology of the Standards Australia Committee on Methods for Examination of Waters. It stems from a need, expressed by Health Authorities, for a standard method for enumeration of legionellae in waters for investigating outbreaks of legionellosis including Legionnaires' disease and for monitoring the efficacy of measures adopted for preventing the proliferation of legionellae in waters.

The method is based on procedures used in some subcommittee members' laboratories and a bibliography lists the main literature sources of information on the method and culture media used.

The method is designed to estimate the total number of most *Legionella* species in a sample of water and also to determine the number of the species *Legionella pneumophila*. *L. pneumophila* serogroup 1 causes Legionnaires' disease. The method is suitable for use in a laboratory equipped to carry out usual microbiological work.

Identification of a number of *Legionella* spp. other than *L. pneumophila* requires specialized testing using advanced techniques and equipment, e.g. cellular fatty acid profiles by capillary gas/liquid chromatography, plasma membrane ubiquinones and monoclonal subtyping. These tests would not be within the capability of an otherwise well-equipped microbiological laboratory and are not included in this Standard. Laboratories which cannot perform these specialized tests should consult a laboratory with the necessary facilities if further testing, beyond the scope of this Standard, is required to establish the identity of an organism confirmed by this method as a species of *Legionella* or as a legionella-like organism.

The method set out in this Standard should also be suitable for testing environmental samples other than water. Because of the diverse nature of environmental samples and the different methods for their initial treatment before cultural examination, it has not been possible to include such preparative treatments in this Standard.

CONTENTS

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1 SCOPE This Standard sets out a method for detecting and estimating the number of *Legionella pneumophila* and some other *Legionella* species in water.

NOTES:

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

2 This method will identify the species *L. pneumophila* but not all other *Legionella* spp. (see Preface).

3 Conditions which favour the isolation of *L. pneumophila* do not necessarily hold for some other *Legionella* spp. Modifications are suggested (see Notes in Clause 8.2) to enhance the sensitivity of the method for such species.

4 This Standard describes the testing of a sample of water as delivered to the laboratory and does not deal with sample collection or sample size.

2 REFERENCED DOCUMENTS

AS
2031 Selection of containers and preservation of water samples for chemical and microbiological analysis
2031.2 Part 2: Microbiological
2243 Safety in laboratories
2243.3 Part 3: Microbiology

3 PRINCIPLE The sample of water is treated to reduce the concentration of any added biocides and is then cultured on specified media. A presumptive test is carried out on colonies of legionella-like organisms (LLOs) and organisms which are presumed to be *Legionella* spp. are tested to distinguish *L. pneumophila* from other *Legionella* spp. Isolates other than *L. pneumophila* are identified, if necessary, by specialized identification tests.

4 SAFETY PRECAUTIONS The safety precautions to be used in microbiological laboratories described in AS 2243.3 shall be observed.

5 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES

5.1 Culture media (see Appendix B).

5.1.1 *Buffered charcoal yeast extract agar with MWY selective supplement (MWY)*

5.1.2 *Buffered charcoal yeast extract agar with BMPA selective supplement (BMPAα)*

5.1.3 *Buffered charcoal yeast extract agar (BCYEα)*

5.1.4 *Buffered charcoal yeast extract agar without L-cysteine (BCYEα-cys)*

5.1.5 *Trypticase soya + 5% blood agar (TSB)*

5.2 Reagents

5.2.1 *Gram stain reagents*—with carbol fuchsin as the preferred counterstain.

5.2.2 *Latex agglutination reagents*—for identification of the range of *L. pneumophila* serogroups.

NOTE: Kits are commercially available for the latex agglutination test and should be used in accordance with instructions.

5.2.3 *Optional reagents for other identification tests*—other test kits using direct or indirect immunofluorescence or gene probe techniques are available for identifying all known serogroups of *L. pneumophila* and some other *Legionella* spp.

5.3 Reference organisms

5.3.1 *Positive culture—Legionella pneumophila* serogroup 1, ATCC 43111 (NCTC 11404).

5.3.2 *Negative culture—Pseudomonas aeruginosa*, ATCC 10145 (NCTC 10332).

5.3.3 *Use of reference cultures* When testing a sample of water by this standard method, cultures of the positive (5.3.1) and negative (5.3.2) reference organisms shall be submitted to the test procedures at the same time to demonstrate and ensure that typical positive and negative growth characteristics and test reactions are exhibited by the respective reference organisms.

6 APPARATUS

NOTES:

1 The apparatus listed below includes that required for performing biocide reduction (see Clause 8.1) by membrane filtration (Clauses 6.1 to 6.5) and by centrifugation (Clause 6.6).

2 Usual microbiological apparatus and glassware are not listed.