

AS 4276.13:2021



# Water Microbiology

Method 13: *Pseudomonas aeruginosa* — Membrane filtration method



AS 4276.13:2021

This Australian Standard™ was prepared by Technical Committee FT-020, Water Microbiology. It was approved on behalf of the Council of Standards Australia on 18 November 2021.

This Standard was published on 17 December 2021.

The following are represented on Committee FT-020:

- ACT Health Directorate
- Australian Society for Microbiology
- Australian Water Association
- Chemistry Australia
- National Association of Testing Authorities, Australia
- National Measurement Institute
- NSW Health
- PathWest Laboratory Medicine, WA
- Queensland Health
- Queensland University of Technology
- University of Melbourne
- Victorian Water Industry Association
- Water NSW
- Water Services Association of Australia

Additional Interests

- Swimming Pool and Spa Association

This Standard was issued in draft form for comment as DR AS 4276.13:2020.

### **Keeping Standards up-to-date**

Ensure you have the latest versions of our publications and keep up-to-date about Amendments, Rulings, Withdrawals, and new projects by visiting:

[www.standards.org.au](http://www.standards.org.au)

ISBN 978 1 76113 635 1

# Water Microbiology

## Method 13: *Pseudomonas aeruginosa* — Membrane filtration method

Originated as AS 1095.5.1.13—1981.  
Revised as AS 4276.13—1995.  
Jointly revised and designated as AS/NZS 4276.13:2008.  
Revised and redesignated AS 4276.13:2021.

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 (Cth).

## Preface

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology to supersede AS/NZS 4276.13:2008, *Water Microbiology, Method 13: Pseudomonas aeruginosa—Membrane Filtration method*.

The objective of this document is to specify a method, using membrane filtration, for enumerating *Pseudomonas aeruginosa* in water.

The major changes in this edition are as follows:

- (a) Available culture media and reagents have been reviewed.
- (b) Reference cultures have been updated.
- (c) Normative and informative references have been updated.

Due to occupational health and safety concerns, ISO 16266, *Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration* has not been adopted. Refer to ISO 16266-2, *Water quality — Detection and enumeration of Pseudomonas aeruginosa — Part 2: Most probable number method* for MPN methods.

The terms “normative” and “informative” are used in Standards to define the application of the appendices to which they apply. A “normative” appendix is an integral part of a Standard, whereas an “informative” appendix is only for information and guidance.

# Contents

<b>Preface</b> .....	<b>ii</b>
<b>Introduction</b> .....	<b>iv</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principle</b> .....	<b>2</b>
<b>5 Reagents and culture media</b> .....	<b>2</b>
<b>6 Apparatus</b> .....	<b>2</b>
<b>7 Reference culture</b> .....	<b>2</b>
7.1 General.....	2
7.2 Positive culture.....	3
<b>8 Samples</b> .....	<b>3</b>
8.1 Laboratory samples.....	3
8.2 Dilutions.....	3
<b>9 Procedure</b> .....	<b>3</b>
9.1 Filtration procedure.....	3
9.2 Selective isolation and incubation.....	3
9.3 Confirmatory tests.....	3
9.3.1 General.....	3
9.3.2 Procedure.....	4
<b>10 Calculation</b> .....	<b>4</b>
<b>11 Quality assurance</b> .....	<b>4</b>
11.1 General.....	4
11.2 Performance testing of culture media.....	4
<b>12 Test report</b> .....	<b>6</b>
<b>Appendix A (informative) Flow diagram</b> .....	<b>7</b>
<b>Appendix B (informative) Culture media and reagents</b> .....	<b>8</b>
<b>Bibliography</b> .....	<b>12</b>

## Introduction

*Pseudomonas* is a large and complex genus of Gram-negative bacteria that includes species with both clinical and environmental importance. They are aerobic, non-spore forming, Gram-negative rods which are straight or slightly curved.

*Pseudomonas aeruginosa* is an environmental organism that can be readily isolated from water, soil, and vegetation, and behave as an opportunistic pathogen. *P. aeruginosa* is an oxidase-positive microorganism that hydrolyses casein and usually produces the pigments pyocyanin and fluorescein.

*P. aeruginosa* is known to cause ear, eye, minor and major wound and skin infections; and may result in bacteraemia in susceptible individuals. Two aquatic locations from which *P. aeruginosa* infections can be acquired are from poorly maintained swimming pools and spas, where either infected individuals or those carrying *P. aeruginosa* asymptotically can contaminate the pool water and pass *P. aeruginosa* onto others.

# Australian Standard®

## Water Microbiology

### Method 13: *Pseudomonas aeruginosa* — Membrane filtration method

#### 1 Scope

This document sets out a method, using membrane filtration for enumerating *Pseudomonas aeruginosa* in water.

NOTE 1 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.

NOTE 2 A flow diagram of the procedure is shown in [Appendix A](#).

NOTE 3 Culture media preparation notes are shown in [Appendix B](#).

#### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document.

NOTE Documents referenced for informative purposes are listed in the Bibliography.

AS 2031, *Water quality—Sampling for microbiological analysis (ISO 1948:2006, MOD)*

AS 4276.1, *Water microbiology, Method 1: Water quality — General requirements and guidance for microbiological examinations by culture (ISO 8199:2018, MOD)*

AS 5140, *Microbiology of food, animal feed and water—Preparation, production, storage and performance testing of culture media (ISO 11133:2014, MOD)*

#### 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

##### 3.1

##### ***Pseudomonas aeruginosa***

environmental microorganism that can be readily isolated from water, soil and vegetation, and is considered an opportunistic pathogen

Note 1 to entry: It is an oxidase-positive microorganism that usually produces the pigments pyocyanin and fluorescein, and hydrolyzes casein.

Note 2 to entry: *P. aeruginosa* can grow at  $41.5\text{ °C} \pm 0.5\text{ °C}$  on selective culture media containing certain antibiotics. It hydrolyses casein at  $36\text{ °C} \pm 2\text{ °C}$  in the presence of cetrимide (cetyl trimethylammonium bromide, a quaternary ammonium compound), produces the blue-green non-fluorescent pigment pyocyanin and/or fluoresces under ultraviolet radiation at approximately 360 nm.

##### 3.2

##### **may**

indicates the existence of an option

##### 3.3

##### **shall**

indicates a statement that is mandatory