

Australian/New Zealand Standard™

Water microbiology

Method 13: *Pseudomonas aeruginosa*— Membrane filtration method

AS/NZS 4276.13:2008

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee, Water Microbiology, FT-020, to supersede AS 4276.13—1995.

The Australian/Standard New Zealand Technical Committee reviewed International Standards Organization Draft Standard ISO/DIS 16266, *Water quality—Detection and enumeration of Pseudomonas aeruginosa—Membrane filtration method*, to determine its suitability to replace AS 4276.13—1995. It was decided not to adopt Draft Standard ISO/DIS 16266 due to occupation health and safety concerns regarding the potential carcinogenicity of acetamide. Furthermore, a study conducted by two Australian State Health Laboratories trialing the recommended primary isolation medium concluded that the medium was less selective and that the morphological characteristics of the target microorganism were more variable than colonies on mPA-C agar.

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 microorganism that can be readily isolated from moist environments and can behave as an opportunistic pathogen. *P. aeruginosa* is an oxidase-positive microorganism that usually produces the pigments pyocyanin and fluorescein, and hydrolyzes casein.

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test. The use of positive control is part of this system.

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.

METHOD

1 SCOPE

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

NOTES:

- 1 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.
- 2 A flow diagram of the procedure is shown in Appendix A.
- 3 Culture media preparation notes are shown in Appendix B.

2 REFERENCED DOCUMENT

The following document is referred to in this Standard:

AS/NZS

4276 Water microbiology

4276.1 Method 1: General information and procedures

3 DEFINITION

For the purpose of this Standard the following definition applies:

3.1 *Pseudomonas aeruginosa*

Grows at $41.5 \pm 0.5^\circ\text{C}$ on selective culture media containing antibiotics. It hydrolyses casein at $36 \pm 2^\circ\text{C}$ in the presence of cetrimide (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.

4 PRINCIPLE

A measured quantity of water sample is filtered through a $0.45\ \mu\text{m}$ gridded membrane filter composed of cellulose acetate. Microorganisms to be enumerated are retained on the membrane surface. The membrane is placed, face upward on the surface of the selective, differential medium, mPA-C agar. After incubation, presumptive isolates are enumerated and then confirmed by sub-culturing colonies onto milk agar containing cetrimide to demonstrate; hydrolysis of casein, production of the blue-green pigment pyocyanin and/or the production of fluorescein demonstrated by yellow-green fluorescence under ultraviolet radiation at approximately 360 nm.

5 CULTURE MEDIA AND REAGENT

5.1 mPA-C Agar

5.2 Milk agar with cetrimide (cetyl trimethylammonium bromide)

6 APPARATUS

6.1 Apparatus and materials for membrane filtration

As described in AS/NZS 4276.1.

6.2 Incubators or water baths

Able to maintain incubation temperatures of $36 \pm 2^\circ\text{C}$ and $41.5 \pm 0.5^\circ\text{C}$

6.3 Long wave ultraviolet light (of approximately 360 nm)

7 REFERENCE CULTURE

7.1 *Pseudomonas aeruginosa*, NCTC 10332 or ATCC 10145 or ACM 495 or NZRM 3007 or a culture traceable to any of these strains

This organism produces positive reactions for *P. aeruginosa*.

7.2 Use of reference culture

The reference culture is used in media and analytical quality control procedures relevant to this method. The purpose of the reference culture is to demonstrate and ensure that typical growth characteristics and test reactions are exhibited by the culture on the media and in the tests used in this method.

As a minimum, the reference culture shall be used to assure that the method has been used properly. Each day samples are processed by this method, a suspension of the positive reference culture is submitted to the test procedure. Growth of the reference culture obtained is taken through the procedure as far as any isolates obtained from the samples, including confirmation tests.

If the reference culture does not give appropriate results an investigation shall be undertaken. Test results shall be regarded as invalid unless the investigation reveals reasons for this failure that invalidate the quality control procedures, e.g. prepared suspensions shown not to contain target bacteria.

8 SAMPLES

8.1 Laboratory samples

See AS/NZS 4276.1 for guidance.

8.2 Dilutions

Dilutions of the laboratory sample shall be prepared as required, using the procedure described in AS/NZS 4276.1.

9 PROCEDURE

9.1 Filtration procedure

Using the instructions for sample volume and procedure for membrane filtration described in AS/NZS 4276.1, filter an appropriate volume of sample or dilution thereof through the membrane filtration apparatus.

9.2 Selective isolation and incubation

The procedure shall be as follows:

- (a) Immediately after filtration is completed, turn off the suction, remove the membrane from the funnel using sterilized forceps and roll the membrane, grid-side upwards, onto mPA-C agar, taking care to avoid entrapping air bubbles.
- (b) Incubate plates of mPA-C in an inverted position, in a humid atmosphere at $41.5 \pm 0.5^\circ\text{C}$ for 44 ± 4 h.
- (c) Count target colonies on the membrane and record as presumptive *P. aeruginosa*. *P. aeruginosa* colonies on mPA-C agar are approximately 0.8 mm to 2.2 mm in diameter, flat in appearance with light outer rim and brownish to green-black centre.

If the background growth of other organisms is greater than the *P. aeruginosa* growth and is considered to have interfered with its recovery of all *P. aeruginosa* on the filter, the count may be underestimated and this shall be reported.

NOTE: Morphological characteristics may vary on some commercially supplied media. Refer to manufacturers' technical specifications.

9.3 Confirmatory tests

The procedure shall be as follows:

- (a) Select a representative number of discrete colonies as per AS/NZS 4276.1 for confirmation.
- (b) Inoculate representative colonies from the mPA-C cultures onto plates of milk agar with cetrimide.
- (c) Incubate at $36 \pm 2^\circ\text{C}$ for 21 ± 3 h.