INTERNATIONAL STANDARD

ISO 16266-2

First edition
2018-07

Water quality — Detection and enumeration of Pseudomonas aeruginosa —

Part 2: Most probable number method

Qualité de l'eau — Recherche et dénombrement de Pseudomonas aeruginosa —

Partie 2: Méthode du nombre le plus probable

Reference number
ISO 16266-2:2018(E)

© ISO 2018



COPYRIGHT PROTECTED DOCUMENT

© ISO 2018

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword v
Introduction vi
1 Scope 1
2 Normative references 1
3 Terms and definitions 2
4 Principle 2
5 Apparatus and glassware 2
6 Culture media, diluents and reagents 3
6.1 Basic materials 3
6.2 Diluent 3
6.3 Antifoam B 3
7 Sampling 4
8 Procedure 4
8.1 Transport and storage of the samples 4
8.2 Preparation of the sample and inoculation of media 4
8.2.1 Preparation of 100 ml samples 4
8.2.2 Preparation of 250 ml samples 4
8.3 Incubation and differentiation 4
8.4 Examination of results 5
9 Expression of results 5
10 Quality assurance 5
11 Test report 5
Annex A (informative) Further microbiological information about *Pseudomonas aeruginosa* 7
Annex B (normative) The Quanti-Tray Sealer and calculation of results 8
Annex C (normative) Composition of the Pseudalert medium 120
Annex D (informative) Performance characteristics 121
Bibliography 122

© ISO 2018 – All rights reserved

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbibiological methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

© ISO 2018 – All rights reserved

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen of man that is capable of growth in water at very low nutrient concentrations. At source and during marketing, a natural mineral water or a spring water is to be free from *Pseudomonas aeruginosa* in any 250 ml sample examined (see, for example, Council Directive 2009/54/EC, Reference [1]). Other bottled waters offered for sale are also to be free of *Pseudomonas aeruginosa* in any 250 ml sample (see e.g. Council Directive 98/83/EC, Reference [2]). Other waters, including swimming and spa pool waters, water for human consumption and hospital waters, may sometimes be tested for *Pseudomonas aeruginosa* for reasons of public health. In these cases, it is typical to examine 100 ml volumes.

The method described in this document can be applied to a range of types of water, for example, hospital waters, drinking water and non-carbonated bottled waters intended for human consumption, groundwater, swimming pool and spa pool waters including those containing high background counts of heterotrophic bacteria (see References [3], [4], [5], [6] and [7]).

© ISO 2018 – All rights reserved

Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Part 2: Most probable number method

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document are carried out by suitably qualified staff.

1 Scope

This document specifies a method for the enumeration of *Pseudomonas aeruginosa* in water. The method is based on the growth of target organisms in a liquid medium and calculation of the most probable number (MPN) of organisms by reference to MPN tables.

This document is applicable to a range of types of water. For example, hospital waters, drinking water and non-carbonated bottled waters intended for human consumption, groundwater, swimming pool and spa pool waters including those containing high background counts of heterotrophic bacteria.

This document does not apply to carbonated bottled waters, flavoured bottle waters, cooling tower waters or marine waters, for which the method has not been validated. These waters are, therefore, outside the scope of this document. Laboratories can employ the method presented in this document for these matrices by undertaking appropriate validation of performance of this method prior to use.

The test is based on a bacterial enzyme detection technology that signals the presence of *P. aeruginosa* through the hydrolysis of a 7-amino-4-methylcoumarin aminopeptidase substrate present in a special reagent. *P. aeruginosa* cells rapidly grow and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the reagent. Actively growing strains of *P. aeruginosa* have an enzyme that cleaves the 7-amido-coumarin aminopeptidase substrate releasing a product which fluoresces under ultraviolet (UV) light. The test described in this document provides a confirmed result within 24 h with no requirement for further confirmation of positive wells.

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. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8199, *Water quality — General guide to the enumeration of micro-organisms by culture*

ISO 11133, *Microbiology of food, animal feeding stuffs, food production, environment and water — Preparation, production, storage and performance testing of culture media*

ISO 19458, *Water quality — Sampling for microbiological analysis*

© ISO 2018 – All rights reserved

ISO/IEC Guide 2, *Standardization and related activities — General vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 2 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
Pseudomonas aeruginosa
species of microorganism that is capable of growing in a selective broth and capable of hydrolyzing a diagnostic 7-amino-4-methylcoumarin aminopeptidase substrate present in the reagent

Note 1 to entry: See Annex A for further information on *P. aeruginosa*.

4 Principle

A snap pack of dehydrated medium is added to a sample of water (100 ml or 250 ml), or to a dilution of a sample made up to 100 ml. Sample plus medium is gently shaken to ensure adequate mixing and to afford dissolution of the medium. When enumeration is required, the sample plus medium (100 ml) is then aseptically poured into either a Quanti-Tray[1)] or Quanti-Tray/2000[1)] to enumerate up to 201 organisms or 2 419 organisms respectively per 100 ml sample. The procedure for the enumeration of 250 ml samples is described in 8.2. Trays are sealed with a Quanti-Tray[1)] Sealer. Quanti-Trays[1)] or vessels (for presence/absence tests) are then incubated at (38 ± 0,5) °C for 24 h to 28 h. Results are confirmed at 24 h but may be read up to 28 h.

After incubation, vessels or Quanti-Tray[1)] sample wells that exhibit any degree of blue fluorescence under long wavelength ultraviolet light (365 nm) are considered positive for *P. aeruginosa*.

By means of statistical tables, or a simple computer program, the MPN of *P. aeruginosa* in 100 ml or 250 ml of the sample can be determined.

This method is also suitable as a qualitative procedure.

5 Apparatus and glassware

Usual microbiological laboratory equipment, and, in particular, the following equipment.

[1)] Quanti-Tray is a trademark or registered trademark of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

© ISO 2018 – All rights reserved