

<b>STN</b>	<b>Kvalita vody</b> <b>Dôkaz a stanovenie <i>Pseudomonas aeruginosa</i></b> <b>Časť 2: Metóda najpravdepodobnejšieho počtu</b> <b>(ISO 16266-2: 2018)</b>	<b>STN</b> <b>EN ISO 16266-2</b>  75 7838
------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------

Water quality - Detection and enumeration of *Pseudomonas aeruginosa* - Part 2: Most probable number method (ISO 16266-2:2018)

Táto norma obsahuje anglickú verziu európskej normy.  
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 01/22

Obsahuje: EN ISO 16266-2:2021, ISO 16266-2:2018

**134359**

EUROPEAN STANDARD

**EN ISO 16266-2**

NORME EUROPÉENNE

EUROPÄISCHE NORM

November 2021

ICS 13.060.70

English Version

## Water quality - Detection and enumeration of *Pseudomonas aeruginosa* - Part 2: Most probable number method (ISO 16266-2:2018)

Qualité de l'eau - Recherche et dénombrement de  
*Pseudomonas aeruginosa* - Partie 2: Méthode du  
nombre le plus probable (ISO 16266-2:2018)

Wasserbeschaffenheit - Nachweis und Zählung von  
*Pseudomonas aeruginosa* - Teil 2: Verfahren zur  
Bestimmung der wahrscheinlichsten Keimzahl (ISO  
16266-2:2018)

This European Standard was approved by CEN on 15 November 2021.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

**EN ISO 16266-2:2021 (E)**

<b>Contents</b>	<b>Page</b>
<b>European foreword.....</b>	<b>3</b>
<b>Endorsement notice .....</b>	<b>3</b>

## European foreword

The text of ISO 16266-2:2018 has been prepared by Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16266-2:2021 by Technical Committee CEN/TC 230 "Water analysis" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## Endorsement notice

The text of ISO 16266-2:2018 has been approved by CEN as EN ISO 16266-2:2021 without any modification.

**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

**ISO 16266-2:2018(E)****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](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

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

## ISO 16266-2:2018(E)

### 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](http://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](http://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](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological 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](http://www.iso.org/members.html).



## **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]).



# 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 16266-2:2018(E)**

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

**koniec náhľadu – text ďalej pokračuje v platenej verzii STN**

---

<sup>1)</sup> 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.