
**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

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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).

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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.

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]).

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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*