# SLOVENSKI STANDARD
# SIST EN 12780:2003

**01-januar-2003**

---

**Kakovost vode - Ugotavljanje prisotnosti in števila Pseudomonas aeruginosa z membransko filtracijo**

Water quality - Detection and enumeration of Pseudomonas aeruginosa by membrane filtration

Wasserbeschaffenheit - Nachweis und Zählung von Pseudomonas aeruginosa durch Membranfiltration



Qualité de l'eau - Détection et dénombrement de Pseudomonas aeruginosa par filtration sur membrane



**Ta slovenski standard je istoveten z:** **EN 12780:2002**

---

**ICS:**

| | | |
|---|---|---|
| 13.060.70 | Preiskava bioloških lastnosti vode | Examination of biological properties of water |

**SIST EN 12780:2003** **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

**EN 12780**

May 2002

ICS 07.100.20

English version

# Water quality - Detection and enumeration of Pseudomonas aeruginosa by membrane filtration

Qualité de l'eau - Détection et dénombrement de Pseudomonas aeruginosa par filtration sur membrane

Wasserbeschaffenheit - Nachweis und Zählung von Pseudomonas aeruginosa durch Membranfiltration

This European Standard was approved by CEN on 17 February 2002.

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 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 Management Centre has the same status as the official versions.



CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.



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

**Management Centre: rue de Stassart, 36 B-1050 Brussels**



Ref. No. EN 12780:2002 E

# Contents

## Foreword

This document EN 12780:2002 has been prepared 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 November 2002, and conflicting national standards shall be withdrawn at the latest by November 2002.

Annexes A and B are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

# Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen of man that is capable of growth in water at very low concentrations of nutrients. At source and during marketing, a natural mineral water or a spring water should be free from *Pseudomonas aeruginosa* in any 250 ml sample examined (Council Directives 80/777/EEC and 96/70/EC). Other bottled waters offered for sale must also be free from *Pseudomonas aeruginosa* in any 250 ml sample (Council Directive 98/83/EC). Other waters including pool waters and water for human consumption can sometimes be tested for *Pseudomonas aeruginosa* for reasons of public health when it is usual to examine 100 ml volumes.

**WARNING — Persons using this standard should be familiar with normal laboratory practice. This standard 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 and to ensure compliance with any national regulatory conditions.**

## 1 Scope

This European Standard specifies a method for the isolation and enumeration of *Pseudomonas aeruginosa* in bottled water samples by a membrane filtration technique. This method can also be applied to other types of water with a low background flora, for example pool waters and waters intended for human consumption.



## 2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN 25667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes (ISO 5667-1:1980).*

EN 25667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques (ISO 5667-2:1991).*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987).*

prEN ISO 5667-3 *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples (ISO 5667-3:1994).*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions.*

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

## 3 Terms and definitions

For the purposes of this European Standard, the following term and definition apply.

**3.1**
***Pseudomonas aeruginosa***
micro-organisms that grow on selective media containing cetrimide and produce pyocyanin, or micro-organisms that grow on selective media containing cetrimide, are oxidase positive, fluoresce under UV light (360 ± 20) nm, and are able to produce ammonia from acetamide

## 4 Principle

### 4.1 Filtration

A measured volume of the water sample, or a dilution of the sample, is filtered through a membrane filter that has filtration characteristics equivalent to a rated pore diameter of 0,45 µm. The membrane filter is placed on the selective medium and incubated under the conditions specified for the medium.

### 4.2 Enumeration

The numbers of presumptive *Pseudomonas aeruginosa* are obtained by counting the number of characteristic colonies on the membrane filter after incubation. Pyocyanin-producing colonies are considered as confirmed *Pseudomonas aeruginosa* but other fluorescing or reddish brown colonies require confirmation.



### 4.3 Confirmation



Subcultures of colonies requiring confirmation are made from the membrane filter onto plates of nutrient agar (see annex B). After incubation, cultures that were not fluorescent initially are tested for the oxidase reaction and oxidase-positive cultures are tested for the production of fluorescein and the ability to produce ammonia from acetamide. Cultures that were fluorescent initially are tested for the ability to produce ammonia from acetamide.

## 5 Diluents, culture media and reagents

### 5.1 General

Use reagents of analytical reagent quality in the preparation of culture media and diluents, unless otherwise specified. Prepare the medium as follows and add the selective agents as supplements at the given concentrations or use commercially available media and reagents prepared according to the manufacturer's instructions. Prepare media and reagents using glass distilled water or water of equivalent purity, in accordance with EN ISO 3696 Grade 3 and free from substances which might inhibit growth under the conditions of the test.

### 5.2 Culture medium

Use the following medium for the determination of *Pseudomonas aeruginosa*.

### 5.2.1 Pseudomonas Agar Base/CN-agar

#### 5.2.1.1 Composition

| | |
|---|---|
| Gelatin peptone | 16,0 g |
| Casein hydrolysate | 10,0 g |
| Potassium sulfate (anhydrous) ($K_2SO_4$) | 10,0 g |
| Magnesium chloride (anhydrous) ($MgCl_2$) | 1,4 g |
| Glycerol | 10 ml |
| Agar | 11,0 g to 18,0 g |
| Water (distilled or equivalent) | 1 000 ml |

The amount of agar required is dependent on the gel strength. Follow the manufacturer's instructions for the agar used.



| | |
|---|---|
| CN Supplement | |
| Hexadecyltrimethyl ammonium bromide (cetrimide) | 0,2 g |
| Nalidixic Acid | 0,015 g |



#### 5.2.1.2 Preparation

Suspend the peptone, casein hydrolysate, potassium sulfate, magnesium chloride and agar in 1 000 ml of distilled water (or equivalent). Add 10 ml of glycerol. Bring to the boil to dissolve completely and sterilize by autoclaving at (121 ± 3) °C for 15 min. Allow the medium to cool to (45 to 50) °C. Add the CN supplement rehydrated in 2 ml of sterile distilled water, mix well and add to the sterile molten basal medium. Mix well and pour into sterile Petri dishes to give a depth of at least 5 mm of agar. The final pH of the solidified medium should correspond to 7,1 ± 0,2 at 25 °C. Store prepared plates in the dark protected from dessication at (5 ± 3) °C and use within 1 month. Do not keep the agar molten for more than 4 h. Do not remelt the medium.

## 5.3 Confirmatory media and reagents

### 5.3.1 King's B Medium

#### 5.3.1.1 Composition

| | |
|---|---|
| Peptone | 20,0 g |
| Glycerol | 10 ml |
| Di-potassium hydrogen phosphate ($K_2 \cdot HPO_4$) | 1,5 g |
| Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) | 1,5 g |
| Agar | 15,0 g |
| Water (distilled or equivalent) | 1 000 ml |

#### 5.3.1.2 Preparation

Dissolve the ingredients in the water by heating. Cool down to (45 to 50) °C and adjust the pH corresponding to 7,2 ± 0,2 at 25 °C, using either hydrochloric acid or sodium hydroxide. Dispense the medium in 5 ml aliquots into culture tubes which are capped and autoclaved at (121 ± 3) °C for 15 min. Allow the tubes to cool and solidify in slants.



Store in the dark at (5 ± 3) °C and use within 3 months.



### 5.3.2 Acetamide broth

#### 5.3.2.1 Composition

**Solution A**

| | |
|---|---|
| Potassium di-hydrogenphosphate ($KH_2PO_4$) | 1,0 g |
| Magnesium sulfate anhydrous ($MgSO_4$) | 0,2 g |
| Acetamide | 2,0 g |
| Sodium Chloride (NaCl) | 0,2 g |
| Water (distilled or equivalent, ammonia free) | 900 ml |

Dissolve the ingredients in water and then adjust the pH to correspond to 7,0 ± 0,5 at 25 °C with either hydrochloric acid or sodium hydroxide.

**CAUTION — Acetamide is carcinogenic and irritant - appropriate precautions shall be taken when weighing out, preparing and discarding the medium.**