

---

---

**Milk and milk products — Method for the  
enumeration of *Pseudomonas* spp.**

*Lait et produits laitiers — Méthode de dénombrement des  
Pseudomonas spp.*

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[ISO/TS 11059:2009](https://standards.iteh.ai/catalog/standards/sist/d61cd482-6761-46b1-9f4a-2e7d9d32092a/iso-ts-11059-2009)

<https://standards.iteh.ai/catalog/standards/sist/d61cd482-6761-46b1-9f4a-2e7d9d32092a/iso-ts-11059-2009>



Reference numbers  
ISO/TS 11059:2009(E)  
IDF/RM 225:2009(E)

**PDF disclaimer**

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. Neither the ISO Central Secretariat nor the IDF accepts any liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies and IDF national committees. In the unlikely event that a problem relating to it is found, please inform the ISO Central Secretariat at the address given below.

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

ISO/TS 11059:2009

<https://standards.iteh.ai/catalog/standards/sist/d61cd482-6761-46b1-9f4a-2e7d9d32092a/iso-ts-11059-2009>



**COPYRIGHT PROTECTED DOCUMENT**

© ISO and IDF 2009

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO or IDF at the respective address below.

ISO copyright office  
Case postale 56 • CH-1211 Geneva 20  
Tel. + 41 22 749 01 11  
Fax + 41 22 749 09 47  
E-mail [copyright@iso.org](mailto:copyright@iso.org)  
Web [www.iso.org](http://www.iso.org)

International Dairy Federation  
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels  
Tel. + 32 2 733 98 88  
Fax + 32 2 733 04 13  
E-mail [info@fil-idf.org](mailto:info@fil-idf.org)  
Web [www.fil-idf.org](http://www.fil-idf.org)

Published in Switzerland

## 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote.
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

<https://standards.iteh.ai/catalog/standards/sist/d61ed482-6761-46b1-9f9c-2e7d9d12092a/iso-ts-11059-2009>

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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.

ISO/TS 11059|IDF/RM 225 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

## Foreword

**IDF (the International Dairy Federation)** is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a Standing Committee may decide to publish an other type of normative document which is called by IDF: *Reviewed method*. Such a method represents an agreement between the members of a Standing Committee and is accepted for publication if it is approved by at least 50 % of the committee members casting a vote. A *Reviewed method* is equal to an ISO/PAS or ISO/TS and will, therefore, also be published jointly under ISO conditions.

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

ISO/TS 11059|IDF/RM 225 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

<https://standards.iteh.ai/catalog/standards/sist/d61cd482-6761-46b1-9f4a-5e7484330724/iso-ts-11059-2009>

All work was carried out by the Joint ISO-IDF Action Team on *Microbiological harmonization* of the Standing Committee on *Microbiological methods of analysis* under the aegis of its project leader, Mrs P. Rollier (FR).

# Milk and milk products — Method for the enumeration of *Pseudomonas* spp.

## 1 Scope

This Technical Specification describes a method for the enumeration of *Pseudomonas* spp. in milk and milk products. The method allows the isolation of all pigmented and non-pigmented psychrophilic *Pseudomonas* spp.

This Technical Specification is also applicable to dairy environmental samples.

## 2 Normative references

The following referenced documents are indispensable for the application 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 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

<https://standards.iteh.ai/catalog/standards/sist/d61cd482-6761-46b1-94a-2e7d9d52092a/iso-ts-11059-2009>

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

### 3.1

#### ***Pseudomonas* spp.**

species of bacteria of the genus *Pseudomonas* which form colonies in penicillin and pimaricin agar (PPA) at 25 °C displaying the biochemical characteristics described, when tested as described in this Technical Specification

## 4 Principle

The surface of a solid selective culture medium is inoculated with a specified quantity of the test sample if the product is liquid, or with a specified quantity of the initial suspension in the case of other products. The inoculation is done, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

The plates are incubated aerobically at 25 °C for 48 h.

The number of *Pseudomonas* is calculated per millilitre, or per gram, of sample from the number of colonies obtained on plates at dilution levels chosen so as to give a significant result, and after confirmation of the selected colonies by the oxidase test and glucose fermentation test.

## 5 Diluent, culture media and reagent

### 5.1 General

For current laboratory practice, see ISO/TS 11133-1 and ISO/TS 11133-2.

### 5.2 Diluent

See ISO 6887-5.

### 5.3 Penicillin and pimaricin agar (PPA)

#### 5.3.1 Basic medium

##### 5.3.1.1 Composition

Component	Amount
Enzymatic digest of gelatine	16,0 g
Enzymatic digest of casein	10,0 g
Potassium sulfate (K <sub>2</sub> SO <sub>4</sub> )	10,0 g
Magnesium chloride (MgCl <sub>2</sub> )	1,4 g
Agar	12,0 g to 18,0 g <sup>a</sup>
Water	1 000 ml

<sup>a</sup> Depending on the gel strength of the agar.

##### 5.3.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling. Adjust the pH, if necessary, so that after sterilization it is  $7,2 \pm 0,2$  at 25 °C. Dispense the basic medium into flasks or bottles of appropriate capacity. Sterilize the medium in the autoclave (6.1) at 121 °C for 15 min.

### 5.3.2 Inhibitor solutions

#### 5.3.2.1 Penicillin solution

##### 5.3.2.1.1 Composition

Component	Amount
Penicillin G, potassium salt	10 <sup>6</sup> IU
Water	10 ml

### 5.3.2.1.2 Preparation

Dissolve the penicillin in the water and sterilize the solution by filtration.

The penicillin solution can be stored at  $5\text{ °C} \pm 3\text{ °C}$  for one week or frozen in aliquots at  $-20\text{ °C}$  for 6 months.

### 5.3.2.2 Pimaricin solution

#### 5.3.2.2.1 Composition

Component	Amount
Pimaricin (natamycin)	0,1 g
Water	10 ml

#### 5.3.2.2.2 Preparation

Dissolve the pimaricin in the water. Sterilize the solution in the autoclave (6.1) at  $110\text{ °C}$  for 20 min.

Pimaricin in solution is not stable, and has to be protected from light. Therefore, use the pimaricin solution on the day of its preparation. Alternatively, the solution can be stored frozen in aliquots at  $-20\text{ °C}$  for 6 months.

### 5.3.3 Complete medium

#### 5.3.3.1 Composition

Volume	Final concentration
Basic medium 100 ml	
Penicillin solution 0,1 ml	100 000 IU/l
Pimaricin solution 0,1 ml	0,01 g/l

#### 5.3.3.2 Preparation

Under aseptic conditions, add the inhibitor solutions to the basic medium, melted and maintained at between  $44\text{ °C}$  and  $47\text{ °C}$ . Carefully mix the thus prepared complete medium.

### 5.3.4 Preparation of PPA agar plates

For preparation and drying of the plates, see ISO/TS 11133-1. If prepared in advance, the agar plates can be kept in the dark at  $5\text{ °C} \pm 3\text{ °C}$  for no longer than 1 day.

### 5.3.5 Performance testing for quality control

#### 5.3.5.1 Productivity

Incubation: At 25 °C ± 1 °C for 48 h ± 2 h  
Strain: *Pseudomonas fluorescens* ATCC 13525 or  
*Pseudomonas aeruginosa* ATCC 27853  
Reference medium: Soybean-casein digest agar medium (TSA)  
Method of control: Quantitative  
Criteria: Productivity ratio,  $P_R > 0,5$  and  
non-characteristic colonies

#### 5.3.5.2 Selectivity

Incubation: At 25 °C ± 1 °C for 48 h ± 2 h  
Strain: *Escherichia coli* ATCC 25922 or  
*Staphylococcus aureus* ATCC 25923  
Method of control: Qualitative  
Criteria: Total inhibition

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

### 5.4 Nutrient agar

#### 5.4.1 Composition

ISO/TS 11059:2009

<https://standards.iteh.ai/catalog/standards/sist/d61ed482-6761-46b1-9f4a-332092a/iso-ts-11059-2009>

Component	Amount
Meat extract	3,0 g
Enzymatic digest of animal tissues	5,0 g
Agar	12,0 g to 18,0 g <sup>a</sup>
Water	1 000 ml

<sup>a</sup> Depending on the gel strength of the agar.

#### 5.4.2 Preparation

Dissolve the dehydrated components or the dehydrated complete medium in the water by boiling. Adjust the pH, if necessary, so that after sterilization it is  $7,0 \pm 0,2$  at 25 °C. Dispense the culture medium into tubes or bottles of appropriate capacity. Sterilize the nutrient agar in the autoclave (6.1) at 121 °C for 15 min.

#### 5.4.3 Preparation of nutrient agar plates

For preparation, drying and storage of the plates, see ISO/TS 11133-1.



#### 5.4.4 Performance testing for quality control

Productivity details are given below.

Incubation:	At 25 °C ± 1 °C for 24 h ± 2 h
Strain:	<i>Pseudomonas fluorescens</i> ATCC 13525 or <i>Pseudomonas aeruginosa</i> ATCC 27853, <i>Escherichia coli</i> ATCC 25922 or <i>Staphylococcus aureus</i> ATCC 25923
Reference medium:	Soybean-casein digest agar medium (TSA)
Method of control:	Quantitative
Criteria:	Productivity ratio, $P_R > 0,7$ and non-characteristic colonies

#### 5.5 Glucose agar

##### 5.5.1 Composition

Component	Amount
Enzymatic digest of casein	10,0 g
Yeast extract	1,5 g
Sodium chloride	5,0 g
Glucose	10,0 g
Bromocresol purple	0,015 g
Agar	12,0 g to 18,0 g <sup>a</sup>
Water	1 000 ml

<sup>a</sup> Depending on the gel strength of the agar.

##### 5.5.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, heating if necessary. Adjust the pH, if necessary, so that after sterilization it is  $7,0 \pm 0,2$  at 25 °C.

Dispense the medium in 10 ml amounts into test tubes (6.5). Sterilize the glucose agar in the autoclave (6.1) at 121 °C for 15 min. Leave the tubes in a vertical position.

Just before use, heat in boiling water or flowing steam for 15 min. Then cool rapidly to the incubation temperature.

##### 5.5.3 Performance testing for quality control

###### 5.5.3.1 Positive test

Incubation:	At 25 ± 1 °C for 24 h ± 2 h
Strain:	<i>Escherichia coli</i> ATCC 25922
Method of control:	Qualitative
Criteria:	Positive