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

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

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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web 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
Web www.fil-idf.org

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Foreword

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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;
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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order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn.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again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.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.

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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.

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).

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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*

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 ₂ SO ₄)	10,0 g
Magnesium chloride (MgCl ₂)	1,4 g
Agar	12,0 g to 18,0 g ^a
Water	1 000 ml

^a 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 ⁶ IU
Water	10 ml