

---

---

**Milk and milk products — General guidance  
for the preparation of test samples, initial  
suspensions and decimal dilutions for  
microbiological examination**

*Lait et produits laitiers — Lignes directrices générales pour la préparation  
des échantillons pour essai, de la suspension mère et des dilutions  
décimales en vue de l'examen microbiologique*

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

[ISO 8261:2001](https://standards.iteh.ai/catalog/standards/sist/14a8f1d2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001)

<https://standards.iteh.ai/catalog/standards/sist/14a8f1d2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>



Reference numbers  
ISO 8261:2001(E)  
IDF 122:2001(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 8261:2001](https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001)

<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

© ISO and IDF 2001

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.ch](mailto:copyright@iso.ch)  
Web [www.iso.ch](http://www.iso.ch)

International Dairy Federation  
41 Square Vergote  
B-1030 Brussels

Printed in Switzerland

## Contents

Page

Foreword.....	iv
Introduction.....	vi
1 Scope .....	1
2 Normative references .....	1
3 Terms and definitions .....	1
4 Principle.....	2
5 Diluents.....	2
5.1 Basic materials.....	2
5.2 Diluents for general use.....	2
5.3 Diluents for special purposes .....	3
5.4 Distribution, sterilization and storage of diluent.....	5
6 Apparatus .....	6
7 Sampling.....	7
8 Procedure .....	7
8.1 General.....	7
8.2 Preparation of test portion and initial suspension.....	7
8.3 Further decimal dilutions.....	10
8.4 Duration of the procedure.....	11
Bibliography.....	12

iTeh STANDARD PREVIEW

(standards.iteh.ai)

ISO 8261:2001

<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

## 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 3.

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.

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

International Standard ISO 8261 | IDF 122 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition cancels and replaces the first edition (ISO 8261:1989), which has been technically revised.

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

[ISO 8261:2001](https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001)

<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

## Foreword

**IDF (the International Dairy Federation)** is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

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 National Committees casting a vote.

International Standard ISO 8261 | IDF 122 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Preparations of samples and dilutions for microbiological examinations*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Mr L.J.M. Maturin (US).

This edition cancels and replaces the third edition (IDF 122C:1996).

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

[ISO 8261:2001](#)

<https://standards.iteh.ai/catalog/standards/sist/14a8f1d2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

## Introduction

This International Standard is mainly based on ISO 6887-1. The necessary adaptations to microbiological laboratory practice in the dairy industry and instructions specific to dairy products, especially in relation to sample preparation, have been introduced.

The question of which diluent or diluents to specify has been the subject of discussion for some time. In this International Standard the peptone/saline solution, as well as the buffered peptone water solution as used in ISO 6887-1, is specified. Three other diluents which are commonly used in dairy microbiological laboratories are also specified for general use. Furthermore, six diluents are specified for special purposes in dairy microbiological laboratories.

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

[ISO 8261:2001](https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001)

<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

# Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination

## 1 Scope

This International Standard describes general guidelines for the preparation of test samples, initial suspensions and decimal dilutions for the microbiological examination of milk and milk products, including milk-based infant foods.

## 2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

## 3 Terms and definitions

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

### 3.1

#### initial suspension

#### primary dilution

suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed, if necessary, using a blender and observing appropriate precautions, with a nine-fold quantity of dilution fluid (diluent), allowing large particles, if present, to settle

NOTE 1 In certain cases and in particular for products giving an initial 1 + 9 suspension which is too viscous or too thick, it may be necessary to add more diluent. On the other hand, a more concentrated primary dilution than 1 + 9 may be required for results of tests to relate to certain specification criteria. These factors should be taken into account for subsequent operations and/or in the expression of results.

NOTE 2 The use of the first dilution is the most appropriate for fitting the requirement of less than 10 microorganisms per gram. If it is desirable for some enumerations in some products to fall below this threshold, it is possible to use less diluent for the suspension. However, inoculation of this suspension may result in an unbalanced inoculum/medium ratio.

NOTE 3 For appropriate precautions, see 8.1.

NOTE 4 For details of diluents, see clause 5.

**3.2**  
**further decimal dilutions**

suspensions, solutions or emulsions obtained by mixing a specific volume of the primary dilution (3.1) with a nine-fold volume of diluent, and by repeating this operation with every dilution thus prepared, until a decimal dilution series, suitable for the inoculation of culture media, is obtained

NOTE See 8.1.

**4 Principle**

An initial suspension (3.1) is prepared and, if necessary, further decimal dilutions (3.2) are prepared to reduce the number of microorganisms per unit volume to facilitate microbiological examination.

**5 Diluents**

**5.1 Basic materials**

In order to improve the precision of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation be used. The manufacturer's instructions shall be rigorously followed.

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent quality (see ISO 7218).

Any adjustment necessary to the pH of the media shall be made with solutions of sodium hydroxide (NaOH) or hydrochloric acid (HCl) of appropriate molarities to minimize the change in media volume and thus composition; i.e. in general, the lower the volume of medium, the higher the molarity.

**5.2 Diluents for general use**

**5.2.1 Peptone-salt solution**

**5.2.1.1 Composition**

Peptone of enzymatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

**5.2.1.2 Preparation**

Dissolve the components in the water, by heating slightly on a hot plate (6.13) if necessary. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $7,0 \pm 0,2$  at 25 °C.



## 5.2.2 Quarter-strength Ringer's solution

### 5.2.2.1 Composition

Sodium chloride (NaCl)	2,25 g
Potassium chloride (KCl)	0,105 g
Calcium chloride, anhydrous (CaCl <sub>2</sub> )	0,06 g
Sodium hydrogen carbonate (NaHCO <sub>3</sub> )	0,05 g
Water	1 000 ml

### 5.2.2.2 Preparation

Dissolve the salts in the water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $6,9 \pm 0,2$  at 25 °C.

## 5.2.3 Peptone solution

### 5.2.3.1 Composition

Peptone	1,0 g
Water	1 000 ml

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

### 5.2.3.2 Preparation

Dissolve the peptone in the water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $7,0 \pm 0,2$  at 25 °C.

ISO 8261:2001

<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-c011-4bd0-a17a-64338ec380ee/iso-8261-2001>

## 5.2.4 Phosphate buffer solution

### 5.2.4.1 Composition

Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	42,5 g
Water	1 000 ml

### 5.2.4.2 Preparation

Dissolve the salt in 500 ml of water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $7,2 \pm 0,2$  at 25 °C. Dilute to 1 000 ml. Store the stock solution under refrigeration.

Add 1 ml of this stock solution (at 20 °C) to 1 000 ml of water for use as diluent.

## 5.3 Diluents for special purposes

These diluents shall only be used for the preparation of initial suspensions.

### 5.3.1 Pre-enrichment medium: Buffered peptone water

#### 5.3.1.1 Composition

Peptone of enzymatic digest of animal tissues	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate (Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O)	9,0 g
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1,5 g
Water	1 000 ml

#### 5.3.1.2 Preparation

Dissolve the components in the water by heating slightly on a hot plate (6.13) if necessary. Adjust the pH with the appropriate solution (5.1) so that after sterilization it is  $7,0 \pm 0,2$  at 25 °C.

This diluent is recommended for cases where tests for detecting *Salmonella* or *Listeria monocytogenes* are also to be undertaken (see ISO 6579).

### 5.3.2 Sodium citrate solution [for cheese and (roller) dried milk]

#### 5.3.2.1 Composition

Trisodium citrate dihydrate (Na <sub>3</sub> C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> ·2H <sub>2</sub> O)	20,0 g
Water	1 000 ml

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

<https://standards.iteh.ai/catalog/standards/sist/14a8f1d2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

#### 5.3.2.2 Preparation

Dissolve the salt in water by heating on a hot plate (6.13) at a temperature of between 45 °C to 50 °C. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $7,5 \pm 0,2$  at 25 °C.

### 5.3.3 Dipotassium hydrogen phosphate solution [for cheese, (roller) dried milk, fermented milk, caseinates, dried acid whey and sour cream]

#### 5.3.3.1 Composition

Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	20,0 g
Water	1 000 ml

#### 5.3.3.2 Preparation

Dissolve the salt in the water by heating on a hot plate (6.13) at a temperature of between 45 °C to 50 °C. For acid whey powder, adjust the pH with the appropriate solution (5.1) so that for the primary dilution, after sterilization, it is  $8,4 \pm 0,2$  at 25 °C. For cheese, roller-dried milk, fermented milk, caseinates and sour cream, adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is  $7,5 \pm 0,2$  at 25 °C.

**5.3.4 Dipotassium hydrogen phosphate solution with antifoam agent** (for acid casein, lactic casein and rennet caseins)

**5.3.4.1 Composition**

Dipotassium hydrogen phosphate ( $K_2HPO_4$ )	20,0 g
Water	1 000 ml

**5.3.4.2 Composition of antifoam stock solution**

Polyethylene glycol 2000 (BDH)	1 g
Water	1 000 ml

**5.3.4.3 Preparation**

Dissolve the salt in the water by heating at a temperature of between 45 °C to 50 °C. Add 1 ml of the antifoam stock solution to 1 litre of the  $K_2HPO_4$  solution. Adjust the pH with the appropriate solution (5.1) so that for the primary dilution of both acid and lactic casein, after sterilization, it is  $8,4 \pm 0,2$  at 25 °C, and for rennet casein, after sterilization, it is  $7,5 \pm 0,2$  at 25 °C.

**5.3.5 Tripolyphosphate solution** (alternative solution for rennet caseins with solubilization problems)

**5.3.5.1 Composition**

Sodium tripolyphosphate ( $Na_3O_{10}P_3$ )	20,0 g
Water	1 000 ml

ITC STANDARD PREVIEW  
 (standards.iteh.ai)  
<https://standards.iteh.ai/catalog/standards/sist/14a8fd2-cc1b-4bda-a17a-64338ec380ee/iso-8261-2001>

**5.3.5.2 Preparation**

Dissolve the salt in the water by heating slightly on a hot plate (6.13), if necessary. Dispense the tripolyphosphate solution in bottles in portions of 90 ml and sterilize them in the autoclave (6.1) set at 121 °C for 20 min. The medium may be preserved at a temperature between 0 °C and + 5 °C for a maximum of 1 month.

**5.3.6 Diluent for general use with  $\alpha$ -amylase solution** (for infant food with high starch contents)

Add 12,5 mg of  $\alpha$ -amylase (EC 3.2.1.1)<sup>1)</sup> with a specific activity of approximately 400 units<sup>2)</sup> per milligram to 225 ml of the diluent for general use (see 5.2). This diluent is used for 25 g of the test sample. Use analogous amounts of reagents for the preparation of other amount of test samples (e.g. for a 10 g sample, add 5 mg of  $\alpha$ -amylase to 90 ml of the diluent for general use).

**5.4 Distribution, sterilization and storage of diluent**

Dispense the diluent (5.2 or 5.3), preheated to 45 °C if necessary, for the primary dilution into flasks or bottles (6.4). Dispense the diluent for decimal dilutions (5.2) into test tubes (6.5), flasks or bottles (6.4).

1) The EC number refers to the Enzymatic Classification number as given by the Nomenclature Committee of the International Union of Biochemistry in Enzyme Nomenclature Recommendation (1978), Academic Press, New York, 1979.

2) This unit (often called the International Unit or Standard Unit) is defined as the amount of enzyme which catalyses the transformation of 1  $\mu$ mol of substrate per minute under standard conditions.