

**Mjök och mjökprodukter – Allmän vägledning
för provberedning, homogenisering och spädning
för mikrobiologisk undersökning
(ISO 8261:2001)**

**Milk and milk products – General guidance
for the preparation of test samples, initial
suspensions and decimal dilutions for micro-
biological examination
(ISO 8261:2001)**

ICS 07.100.30

Språk: engelska

Tryckt i januari 2002

Europastandarden EN ISO 8261:2001 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 8261:2001.

The European Standard EN ISO 8261:2001 has the status of a Swedish Standard. This document contains the official English version of EN ISO 8261:2001.

Dokumentet består av 19 sidor.

Upplysningar om **sakinnehållet** i standarden lämnas av SIS, Swedish Standards Institute, tel 08 - 555 520 00.

Standarder kan beställas hos SIS Förlag AB som även lämnar **allmänna upplysningar** om svensk och utländsk standard.

Postadress: SIS Förlag AB, 118 80 STOCKHOLM
Telefon: 08 - 555 523 10. *Telefax:* 08 - 555 523 11
E-post: sis.sales@sis.se. *Internet:* www.sisforlag.se

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 8261

June 2001

ICS 07.100.30

English version

**Milk and milk products - General guidance for the preparation of
test samples, initial suspensions and decimal dilutions for
microbiological examination (ISO 8261:2001)**

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 (ISO 8261:2001)

Milch und Milchprodukte - Allgemeiner Leitfaden für die
Vorbereitung von Untersuchungsproben und die
Herstellung von Anschüttelungen und
Dezimalverdünnungen für mikrobiologische
Untersuchungen (ISO 8261:2001)

This European Standard was approved by CEN on 15 June 2001.

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, 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

Contents	Page
Foreword	3
Introduction	4
1 Scope	5
2 Normative references	5
3 Terms and definitions	5
4 Principle	6
5 Diluents	6
5.1 Basic materials	6
5.2 Diluents for general use	6
5.3 Diluents for special purposes	7
5.4 Distribution, sterilization and storage of diluent	9
6 Apparatus	10
7 Sampling	11
8 Procedure	11
8.1 General	11
8.2 Preparation of test portion and initial suspension	11
8.3 Further decimal dilutions	14
8.4 Duration of the procedure	15
Bibliography	16
Annex ZA	17

Foreword

The text of the International Standard ISO 8261:2001 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis", the secretariat of which is held by NEN.

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 December 2001, and conflicting national standards shall be withdrawn at the latest by December 2001.

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, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 8261:2001 was approved by CEN as a European Standard without any modification.

NOTE: Normative references to International Standards are listed in annex ZA (normative).

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.

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.