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**Milk products — Enumeration of  
presumptive *Lactobacillus acidophilus* on  
a selective medium — Colony-count  
technique at 37 °C**

*Produits laitiers — Dénombrement de Lactobacillus acidophilus  
présomptifs sur un milieu sélectif — Technique de comptage des  
colonies à 37 °C*

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Reference numbers  
ISO 20128:2006(E)  
IDF 192:2006(E)

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Published in Switzerland

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## 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.

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 20128|IDF 192 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.

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## 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 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 IDF National Committees casting a vote.

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 20128|IDF 192 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 *Lactic acid bacteria and starters*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Mrs D. Ellekaer (DK).

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## Introduction

Because of the large variety of fermented and non-fermented milks, this method may not be appropriate in every detail for certain products.

This could be the case where the number of presumptive *Lactobacillus acidophilus* is very much lower than the number of other microorganisms such as *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus helveticus* and yeasts.

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# Milk products — Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium — Colony-count technique at 37 °C

## 1 Scope

This International Standard specifies a method for the enumeration of presumptive *Lactobacillus acidophilus* in milk products on a selective medium by using a colony-count technique at 37 °C.

The method is applicable to fermented and non-fermented milks, milk powders and infant formulae where presumptive *L. acidophilus* is present and in combination with other lactic acid bacteria and bifidobacteria.

The method is not applicable when the number of presumptive *L. acidophilus* is less than 10<sup>4</sup> CFU/g and the numbers of *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus paracasei* subsp. *paracasei* are greater than 10<sup>6</sup> CFU/g.

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## 2 Normative references (standards.iteh.ai)

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

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

## 3 Terms and definitions

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

### 3.1

#### presumptive *Lactobacillus acidophilus*

microorganism forming flat, mat, rough, grey to whitish colonies with more or less irregular edges and a diameter of 1 mm to 3 mm depending on the number of colonies when grown on a solid selective medium under the conditions specified in this International Standard

NOTE *L. acidophilus* is closely related to *Lactobacillus johnsonii*, *Lactobacillus gasseri* and *Lactobacillus crispatus*. The method specified in this International Standard cannot distinguish between these four species and, therefore, only presumptive *L. acidophilus* is mentioned.

## 4 Principle

4.1 The antibiotics clindamycin and ciprofloxacin both inhibit the growth of the most common microorganisms used in fermented milks, non-fermented milks and infant formulae, such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Streptococcus thermophilus*, bifidobacteria, lactococci, *Lactobacillus casei*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Leuconostoc* species.

4.2 A known amount of sample is homogenized with diluent and decimal dilutions are prepared.

4.3 Appropriate dilutions are spread plated on MRS-agar with the addition of clindamycin and ciprofloxacin.

4.4 The plates are incubated anaerobically at 37 °C for 72 h ± 3 h.

4.5 Typical colonies are counted.

4.6 The number of characteristic microorganisms per gram of sample is calculated from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

## 5 Diluents, culture media and reagents

### 5.1 Basic materials

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. See ISO 7218.

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### 5.2 Diluent

See ISO 8261|IDF 122. <https://standards.iteh.ai/catalog/standards/sist/934e03a7-999a-4289-9680-f9526be13f6a/iso-20128-2006>

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### 5.3 Culture media

#### 5.3.1 MRS/clindamycin/ciprofloxacin agar (MRS/CL/CIP agar)

MRS/CL/CIP agar consists of MRS agar (5.3.2) with the addition of 0,1 mg of clindamycin and 10,0 mg of ciprofloxacin per litre of medium (see 5.3.4).

### 5.3.2 Basic medium: MRS agar

#### 5.3.2.1 Composition

Peptone 1 (enzymatic digest of casein)	10,0 g
Meat extract	10,0 g
Yeast extract (dried)	5,0 g
Glucose	20,0 g
Tween 80 (sorbitan mono-oleate)	1,0 ml
Dipotassium hydrogen phosphate ( $K_2HPO_4$ )	2,0 g
Sodium acetate trihydrate ( $NaCH_3CO_2 \cdot 3H_2O$ )	5,0 g
Triammonium citrate ( $(NH_4)_3HC_6H_5O_7$ )	2,0 g
Magnesium sulfate heptahydrate ( $MgSO_4 \cdot 7H_2O$ )	0,2 g
Manganese sulfate tetrahydrate ( $MnSO_4 \cdot 4H_2O$ )	0,05 g
Agar	12 g to 18 g <sup>a</sup>
Water	1 000 ml
<sup>a</sup> Depending on the gel strength of the agar.	

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#### 5.3.2.2 Preparation

Suspend the ingredients in the water. Heat the suspension to boiling with frequent agitation until a complete solution is obtained. Distribute the medium in portions of  $100 \text{ ml} \pm 1 \text{ ml}$  into bottles (6.9) of 150 ml capacity or in portions of  $200 \text{ ml} \pm 2 \text{ ml}$  into bottles (6.9) of 250 ml capacity.

If needed, adjust the pH (6.8) so that, after sterilization, it is  $6,2 \pm 0,2$ . Sterilize in the autoclave (6.6) set at  $121 \text{ }^\circ\text{C}$  for 15 min. If the medium is to be used immediately, cool it in a water bath (6.7) to between  $44 \text{ }^\circ\text{C}$  and  $47 \text{ }^\circ\text{C}$ . Do not expose the medium to direct sunlight.

The thus-prepared MRS agar may be stored in the dark at  $1 \text{ }^\circ\text{C}$  to  $5 \text{ }^\circ\text{C}$  for 6 months.

NOTE The complete MRS agar is commercially available but the results obtained may differ significantly from one supplier to another (See also ISO/TS 11133-1 and ISO/TS 11133-2.)

### 5.3.3 Clindamycin stock solution

#### 5.3.3.1 Composition

Clindamycin hydrochloride	2,0 mg
Water up to	10,0 ml

#### 5.3.3.2 Preparation

Dissolve the clindamycin hydrochloride in the water. Filter the solution then sterilize through a  $0,22 \text{ } \mu\text{m}$  filter (6.13) into a sterile test tube (6.14).

If the solution is not to be used immediately, distribute it in small sterile cryotubes (6.17) and keep the tubes at  $-20 \text{ }^\circ\text{C}$ . The frozen solution may be stored for 6 weeks.