



# International Standard

**ISO 29981**

**IDF 220**

## **Milk products — Enumeration of bifidobacteria — Colony-count technique**

*Produits laitiers — Dénombrement des bifidobacteria présumés  
— Technique par comptage des colonies*

**Second edition  
2024-11**

This document is a preview generated by FMS



**COPYRIGHT PROTECTED DOCUMENT**

© ISO and IDF 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester:

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11

Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

International Dairy Federation  
Silver Building • Bd Auguste Reyers 70/B  
B-1030 Brussels  
Phone: +32 2 325 67 40  
Fax: +32 2 325 67 41  
Email: [info@fil-idf.org](mailto:info@fil-idf.org)  
Website: [www.fil-idf.org](http://www.fil-idf.org)

# Contents

	Page
<b>Foreword</b> .....	<b>iv</b>
<b>Introduction</b> .....	<b>vi</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>2</b>
<b>4 Principle</b> .....	<b>2</b>
4.1 General.....	2
4.2 Preparation of initial suspension and decimal dilutions.....	2
4.3 Isolation and selection for confirmation.....	2
4.4 Confirmation.....	2
4.5 Calculation.....	3
<b>5 Culture media and reagents</b> .....	<b>3</b>
<b>6 Equipment and consumables</b> .....	<b>3</b>
<b>7 Sampling</b> .....	<b>4</b>
<b>8 Preparation of test sample</b> .....	<b>4</b>
<b>9 Procedure</b> .....	<b>4</b>
9.1 General.....	4
9.2 Preparation of the test portion and primary dilution.....	4
9.2.1 Dried milk products (e.g. infant milk formulae) including dehydrated starter and probiotic cultures.....	4
9.2.2 Non-dried fermented (e.g. yoghurt) and non-fermented milk-based products (e.g. pasteurized milks).....	4
9.3 Microscopic examination of initial suspension or primary dilution (optional).....	5
9.4 Preparation of decimal dilution series.....	5
9.5 Inoculation and incubation.....	5
9.6 Counting of colonies.....	5
9.7 Confirmation.....	6
<b>10 Expression of results</b> .....	<b>6</b>
<b>11 Validation of the method</b> .....	<b>6</b>
11.1 Validation in accordance with ISO 17468.....	6
11.2 Performance characteristics.....	6
<b>12 Test report</b> .....	<b>7</b>
<b>13 Quality assurance</b> .....	<b>7</b>
<b>Annex A (normative) Flow diagram of the procedure</b> .....	<b>8</b>
<b>Annex B (normative) Culture media and reagents</b> .....	<b>9</b>
<b>Annex C (informative) Performance characteristics of the method</b> .....	<b>13</b>
<b>Bibliography</b> .....	<b>15</b>

## 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at [www.iso.org/patents](http://www.iso.org/patents). ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document 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.

This second edition cancels and replaces the first edition (ISO 29981 | IDF 220:2010), which has been technically revised.

The main changes are as follows:

- diluents which can be used have been added;
- preparation of the test portion and primary dilution in cases of dried milk products has been added;
- a new culture medium, TOS agar, has been introduced;
- storage of incubated plates has been included;
- expression of results has been changed to be in accordance with ISO 7218;
- performance testing of the culture media has been introduced;
- performance characteristics, with the results of an interlaboratory study, which are based on the method of this second edition, have been included as [Annex C](#).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

**ISO 29981:2024(en)**  
**IDF 220:2024:2024(en)**

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

IDF draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). IDF takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, IDF had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at [www.iso.org/patents](http://www.iso.org/patents). IDF shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

This document was prepared by IDF *Standing Committee on Methods for Dairy Microbiology* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF/ISO Action Team (D09) of the *Standing Committee on Methods for Dairy Microbiology* under the aegis of its project leader Masamichi Muto (JP).

## Introduction

Bifidobacteria are non-acid-fast, non-spore-forming, Gram-positive, non-motile and catalase-negative chemoorganotrophs bacilli, which produce acetic acid, lactic acid and formic acid. Glucose is degraded exclusively and characteristically by the fructose-6-phosphate shunt in which fructose-6-phosphate phosphoketolase (F6PPK, EC 4.1.2.22) cleaves fructose-6-phosphate into acetyl phosphate and erythrose-4-phosphate.

Many reports show that bifidobacteria have various physiological functions and bifidobacteria are widely applied to foods in milk products such as yoghurt, infant formula and milk powders, and also in non-milk products such as starter and probiotic cultures. Many bifidobacteria-containing products describe the bacterial cell counts on the product label which is an important indicator of the functionality. An accurate bifidobacteria enumeration method, such as the one given in this document, is important to guarantee the bacterial cell counts.

The main technical changes listed in the Foreword, introduced in this document compared to ISO 29981 | IDF 220:2010, are considered as major (see ISO 17468). These technical changes have a major impact on the performance characteristics of the method.

# Milk products — Enumeration of bifidobacteria — Colony-count technique

## 1 Scope

This document specifies a method for the selective enumeration of bifidobacteria in milk products by using a colony-count technique at 37 °C under anaerobic conditions.

The method is applicable to milk products, such as fermented (e.g. yoghurts) and non-fermented milks (e.g. pasteurized milks, skim milks, whey protein concentrates), milk powders and formulae (e.g. infant formulae, follow-up formulae for older infants, products for young children) where these microorganisms are present and viable, in combination with other lactic acid bacteria or alone. The method is also applicable to starter and probiotic cultures. For proposed quality criteria of dairy products, see, for example, CXS 243-2003<sup>[6]</sup>.

Bifidobacteria used in milk products usually belong to the following species (e.g. References [7] and [10]):

- *Bifidobacterium adolescentis*;
- *B. animalis* subsp. *animalis*;
- *B. animalis* subsp. *lactis*;
- *B. bifidum*;
- *B. breve*;
- *B. longum* subsp. *infantis*;
- *B. longum* subsp. *longum*.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6887-1, *Microbiology of the food chain — 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 6887-5, *Microbiology of the food chain — 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 the food chain — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 19036:2019, *Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations*