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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**

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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](#).

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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.

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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.