# INTERNATIONAL STANDARD

# ISO 29981 IDF 220

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# Milk products — Enumeration of presumptive bifidobacteria — Colony count technique at 37 °C

Produits laitiers — Dénombrement des bifidobacteria présumés — Technique par comptage des colonies à 37 °C



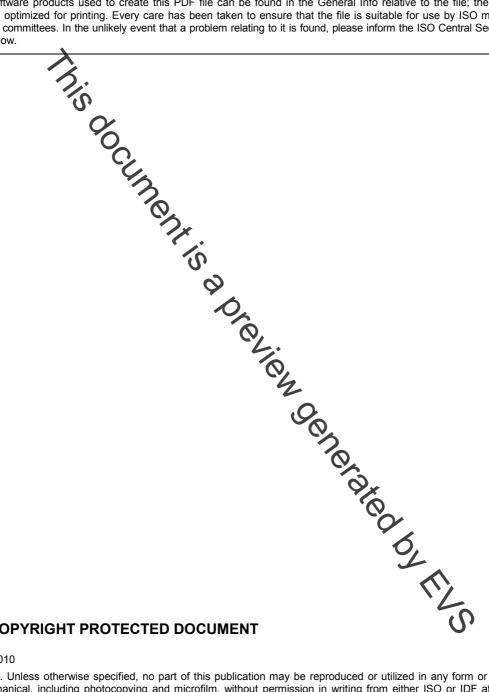
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# Foreword

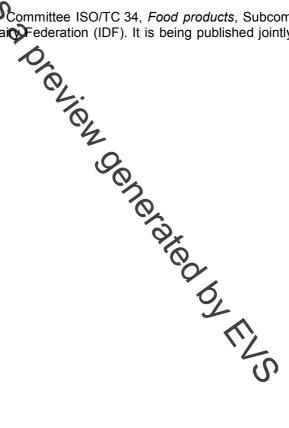
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ISO 29981 IDF 220 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Date Federation (IDF). It is being published jointly by ISO and IDF.



## 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.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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All work was carried out by the Joint ISO- Action Team on *Lactic acid bacteria and starters* of the Standing Committee on *Microbiology methods of analysis* under the aegis of its project leaders, Prof. W. Kneifel (AT) and Dr. U. Zitz (AT).

this document is a preview denerated by EUS

## Milk products — Enumeration of presumptive bifidobacteria — Colony count technique at 37 °C

#### 1 Scope

This International Standard specifies a method for the selective enumeration of presumptive bifidobacteria in milk products by using a colory count technique at 37 °C under anaerobic conditions.

The method is applicable to products such as fermented and non-fermented milks, milk powders, infant formulae, and starter cultures appere these microorganisms are present and viable, and in combination with other lactic acid bacteria. (For coposed quality criteria of dairy products, see, for example, Codex Stan 243:2003<sup>[6]</sup>.)

v be a Preview Generated Bifidobacteria used in milk products userally belong to the species (e.g. see References [7][8][16]):

- a) Bifidobacterium adolescentis;
- b) B. animalis subsp. animalis;
- c) B. animalis subsp. lactis;
- d) B. bifidum;
- e) B. breve;
- f) B. infantis;
- g) B. longum.

#### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cites applies. For undated references, the latest edition of the referenced documents (including any amendments) applies.

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 6887-5, Microbiology of food and animal feeding stuffs — 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 food and animal feeding stuffs — General requirements and guidance for microbiological examinations

ISO 7889 IDF 117, Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C

ISO/TS 11133-1, Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory

ISO 14461-1|IDF 169-1, *Milk and milk products* — *Quality control in microbiological laboratories* — *Part 1: Analyst performance assessment for colony counts* 

ISO 14461-2|IDF 169-2, *Milk and milk products* — *Quality control in microbiological laboratories* — *Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps* 

# 3 Terms and definitions

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

#### 3.1

#### bifidobacteria

anaerobic microorganisms that form leaticular or round whitish colonies, partially star shaped or trilobate of diameter 1 mm to 4 mm on transgalactocylated oligosaccharides-mupirocin lithium salt (TOS-MUP) medium under the conditions specified in this International Standard

#### 4 Principle

**4.1** The antibiotic, mupirocin lithium salt (MUP), inhibits the growth of most lactic acid bacteria commonly used in fermented and non-fermented dairy products.

Owing to the proven selectivity of the MUP antibiotic when added to the medium, usually there is no growth of typical yogurt bacteria (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*), mesophilic cultures (e.g. *Lactococcus lactis*), *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus rhamnosus* on the medium specified.

This property has been tested with a representative number of reference strains and isolates.

Additionally, TOS-agar enhances the growth of bifidobacteria used in dairy products (see Reference [17]).

NOTE 1 Examination under a microscope at a magnification of 100 times and oil immersion in contrast phase illumination shows rods of very varied shapes, usually curved and clubbed, often branched, arranged singly, in pairs, in V-shaped arrangements, in chains, in palisades of parallel cells, or in rosettes occasional exhibiting swollen coccoid forms.

NOTE 2 Bifidobacteria are non-acid-fast, non-spore-forming, gram-positive, non-motile and catalase-negative chemoorganotrophs, which produce acetic acid and lactic 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.

NOTE 3 The optimum growth temperature is between 37 °C and 41 °C. For further details, see Reference [9].

**4.2** Inoculation of appropriate decimal dilutions of the homogenized sample into TOS-agar containing MUP using the pour plate technique, is followed by anaerobic incubation at 37 °C for 72 h.

**4.3** The colonies are counted.

NOTE Optionally, selected isolates from the plates can be confirmed by means of appropriate tests (e.g. F6PPK assay, see References [14][15]).

**4.4** The number of bifidobacteria per gram of sample is calculated from the number of colonies obtained on plates at dilution levels so as to give a significant result.