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

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



Reference numbers
ISO 29981:2010(E)
IDF 220:2010(E)

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. Neither the ISO Central Secretariat nor the IDF accepts any liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies and IDF national committees. In the unlikely event that a problem relating to it is found, please inform the ISO Central Secretariat at the address given below.

This document is a preview generated by EVS



COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2010

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO or IDF at the respective address below.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword	iv
Foreword	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle	2
5 Culture media, diluents and reagents	3
5.1 Basic materials	3
5.2 Diluent(s)	3
5.3 Culture medium (TOS-MVP medium)	3
6 Apparatus	5
7 Sampling	5
8 Procedure	6
8.1 General	6
8.2 Preparation of the test portion and primary dilution	6
8.3 Microscopic examination	7
8.4 Preparation of decimal dilutions	7
8.5 Inoculation	7
8.6 Duration of the procedure	8
8.7 Incubation	8
8.8 Counting of the colonies	8
8.9 Reading of the Petri dishes — confirmation	8
9 Calculation and expression of results	8
9.1 Calculation	8
9.2 Expression of results	9
10 Precision	10
10.1 Interlaboratory test	10
10.2 Repeatability	10
10.3 Reproducibility	10
10.4 Precision data collectively defined for dairy products	11
11 Knowledge of use of the method	13
12 Test report	13
Annex A (informative) Interlaboratory trial – A 'bifido' ring trial	14
Bibliography	16

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 29981|IDF 220 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.

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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 29981|IDF 220 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.

All work was carried out by the Joint ISO-IDF 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 generated by EVS

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 colony count technique at 37 °C under anaerobic conditions.

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

Bifidobacteria used in milk products usually 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 cited 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 lenticular or round whitish colonies, partially star shaped or trilobate of diameter 1 mm to 4 mm on transgalactosylated 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 occasionally 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.