INTERNATIONAL STANDARD

ISO 13559

IDF 153

First edition
2002-11-01

Butter, fermented milks and fresh cheese — Enumeration of contaminating microorganisms — Colony-count technique at 30 °C

Beurre, laits fermentés et fromage frais — Dénombrement des micro-organismes contaminants — Technique par comptage des colonies à 30 °C

Reference numbers
ISO 13559:2002(E)
IDF 153:2002(E)

© ISO and IDF 2002

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS

Not for Resale

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.

© ISO and IDF 2002

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

Printed in Switzerland

--`,,`,,`-`-`,,`,,`,`,,`---

::

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS

© ISO and IDF 2002 – All rights reserved

Not for Resale

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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 13559 | IDF 153 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

© ISO and IDF 2002 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

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 and AOAC International 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 the National Committees casting a vote.

International Standard ISO 13559 | IDF 153 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Non-Pathogen Contaminants*, under the aegis of its project leader, Mr D. van den Berg (NL).

--`,,`,-`-`,,`,,`,`,,`---

© ISO and IDF 2002 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Butter, fermented milks and fresh cheese — Enumeration of contaminating microorganisms — Colony-count technique at 30 °C

1 Scope

This International Standard specifies a method for the enumeration of contaminating microorganisms by means of the colony-count technique at 30 °C. The method is applicable to butter, fermented milks and fresh cheese.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 8261 | IDF 122:2001, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

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*

3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1
contaminating microorganisms
non-lactic acid bacteria, yeasts and moulds forming countable colonies under the conditions specified in this International Standard

NOTE 1 Product-specific lactic acid bacteria will not be detected by this method.

NOTE 2 In certain types of fermented milks, non-lactic acid bacteria, yeasts or moulds can be part of the microflora contributing to the desired characteristics of the product. In such cases, care should be taken when applying the method described in this International Standard.

4 Principle

4.1 Poured plates are prepared using a specified culture medium, followed by surface plating of a specified quantity of an initial suspension of the product. Under the same conditions, poured plates are prepared, followed by surface plating of a specified quantity of decimal dilutions of an initial suspension.

--`,,`,-`-`,,`,,`,`,,`---

© ISO and IDF 2002 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

4.2 The plates are aerobically incubated at 30 °C ± 1 °C for 72 h.

4.3 The number of microorganisms per gram of test 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 General

For current laboratory practice, see ISO 7218.

5.2 Basic materials

See ISO 8261 | IDF 122:2001, subclause 5.1.

5.3 Diluents for general use

See ISO 8261 | IDF 122:2001, subclause 5.2.

5.4 Diluents for special purposes

See ISO 8261 | IDF 122:2001, subclause 5.3.

5.5 Distribution, sterilization and storage of diluents

See ISO 8261 | IDF 122:2001, subclause 5.4.

5.6 Culture medium

5.6.1 General

All components of the culture medium shall be free from carbohydrates. The absence of carbohydrates in the culture medium used here is essential for the method described. The quality of the carbohydrate-free medium used shall be assured in accordance with ISO/TS 11133-1.

5.6.2 Composition

Peptone from casein	7,5 g
Peptone from gelatin	7,5 g
Sodium chloride (NaCl)	5,0 g
Agar [1)]	10 g to 15 g
Water	1 000 ml

5.6.3 Preparation

5.6.3.1 Preparation from commercial dehydrated complete medium

Follow the manufacturer's instructions. Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,1 at 25 °C. For fermented milks, adjust the pH, if necessary, so that after sterilization it is 8,0 ± 0,1 at 25 °C.

1) Depending on the gel strength of the agar.

© ISO and IDF 2002 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

5.6.3.2 Preparation from dehydrated basic components

Dissolve and disperse by heating in the water, in the following order, the peptone from casein, the peptone from gelatin and the sodium chloride. Add the agar and heat to boiling while stirring frequently until the agar is completely dissolved, or steam for about 30 min. Filter through filter paper, if necessary. Adjust the pH so that, after sterilization, it is 7,5 ± 0,1 at 25 °C. For fermented milks, adjust the pH, if necessary, so that after sterilization it is 8,0 ± 0,1 at 25 °C.

5.6.3.3 Distribution, sterilization and storage

Dispense the medium into test tubes (6.8), in quantities of 12 ml to 15 ml per tube, or into flasks or bottles (6.9), in quantities of 100 ml to 150 ml. Sterilize in an autoclave set at 121 °C ± 1 °C for 15 min.

If the medium is to be used immediately, cool it in the water bath (6.5) to between 44 °C and 47 °C. If not used immediately, store it in the dark at between 1 °C and 5 °C for no longer than 3 months. In order to avoid any delay when pouring the medium and before commencing the microbiological examination, completely melt the medium in a boiling water bath, then cool it in another water bath set at between 44 °C and 47 °C before use.

6 Apparatus

Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications. Reusable glassware should be capable of undergoing repeated sterilization and should be chemically inert.

Sterilize all apparatus that will come into contact with the test sample and the diluents or culture medium in accordance with ISO 8261.

Usual microbiological equipment (see ISO 7218 and ISO 8261) and, in particular, the following.

6.1 Incubator, capable of operating at 30 °C ± 1 °C.

6.2 Oven or **incubator**, ventilated by convection, capable of operating at 50 °C ± 1 °C, or a **laminar air-flow cabinet**.

6.3 Petri dishes, made of glass or plastic, of diameter 90 mm to 100 mm, or, when an inoculum of more than 0,1 ml is used, dishes of diameter 140 mm.

6.4 Graduated pipettes, of nominal capacity of 1 ml ± 0,02 ml or 10 ml ± 0,2 ml.

6.5 Water baths, capable of operating at between 44 °C and 47 °C, and capable of boiling.

6.6 Colony-counting equipment, consisting of an illuminated base with a dark background, fitted with a magnifying lens to be used at a magnification of 1,5× and a mechanical or electronic digital counter.

6.7 pH-meter, accurate to ± 0,1 pH unit at 25 °C, with readability to 0,01 units.

6.8 Test tubes, with plugs or caps, of capacity of approximately 20 ml.

6.9 Bottles or **flasks**, with plugs or caps, of nominal capacity 150 ml to 250 ml.

Bottles or flasks with non-toxic metal screw-caps may be used.

6.10 Sterile spreaders, with diameter of approximately 3,5 mm and length of 20 cm, bent at right angles about 3 cm from one end. The cut ends should be made smooth by heating.

--`,,`,,`-`-`,,`,,`,`,,`---
© ISO and IDF 2002 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

8 Procedure

8.1 Preparation of test portion and initial suspension

8.1.1 General

For general requirements see ISO 6887-1, and for specific requirements see ISO 8261.

Take normal aseptic precautions. The operations described in 8.1 and 8.2 shall not be carried out in direct sunlight.

8.1.2 Butter

See ISO 8261:2001, subclause 8.2.6.

8.1.3 Fresh cheese

See ISO 8261:2001, subclause 8.2.4.

8.1.4 Fermented milks

See ISO 8261:2001, subclause 8.2.9.

8.2 Further decimal dilutions

See ISO 8261.

8.3 Preparation of plates

Pour 12 ml to 15 ml of the prepared medium (5.6) into Petri dishes (6.3) and allow to solidify. Dry the plates, preferably with the lids off and the agar surface facing downwards, in an oven or incubator (6.2) set at 50 °C for 30 min. See also ISO 7218.

As an option, Petri dishes with a diameter of 140 mm may be used (6.3) when an inoculum of $>$ 0,1 ml is used.

Instead of drying in an oven or incubator, the plates may also be dried in a laminar air-flow cabinet (6.2) for 30 min.

8.4 Inoculation and incubation

8.4.1 Transfer to each of two prepared plates (8.3), by means of a sterile pipette (6.4), 0,1 ml of the initial suspension of the product.

8.4.2 Repeat this operation using further decimal dilutions.

8.4.3 Carefully spread the inoculum as quickly as possible over the surface of the plate, taking care not to touch the sides of the dish, using a sterile spreader (6.10). Use one sterile spreader for each plate. Leave the plates, with the lids on, for approximately 15 min on the bench to allow absorption of the inoculum into the plates.

© ISO and IDF 2002 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

8.4.4 Invert the prepared dishes and place them in the incubator (6.1) set at 30 °C for 72 h ± 2 h. Do not stack the dishes more than six high. Stacks of plates shall be separated from one another and from the walls and top of the incubator (see ISO 7218).

8.5 Counting of colonies

Using the colony-counting equipment (6.6), count the colonies after incubation (8.4.4). Count the colonies characteristic for contaminating microorganisms in each dish containing not more than 150 colonies. Do not count pin-point colonies as these are not typical for contaminants.

Information as to the source of contamination may be obtained by examination of the colonies present. It can be valuable, therefore, to record the types of colonies present (e.g. pure or mixed, yeasts, moulds, *Bacillus* sp. etc.).

9 Calculation and expression of results

9.1 Calculation

Retain dishes containing more than 10 and less than 150 characteristic colonies at two successive dilutions.

Calculate the number, N, of contaminating microorganisms per gram of test sample using the following equation:

$$N = \frac{\sum c}{(n_1 + 0{,}1\, n_2)\, d}$$

where

Σc is the sum of characteristic colonies counted in all dishes retained;

n_1 is the number of dishes retained at the first dilution;

n_2 is the number of dishes retained at the second dilution;

d is the dilution factor corresponding to the first dilution.

9.2 Expression of results

9.2.1 Round the result obtained in 9.1 to two significant figures. For a three-figure number, round the third figure to the nearest zero. If the third figure is 5, round to the figure below if the second figure is even, and to the figure above in the case of an odd second figure.

EXAMPLE Round:

234 to 230;

235 to 240

225 to 220;

245 to 240.

9.2.2 If there are only counts of less than 10, report the number of microorganisms per gram as “less than $10 \times 1/d$”, where d is the dilution factor corresponding to the lowest dilution.

© ISO and IDF 2002 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

9.2.3 If there are only counts exceeding 150, calculate an estimated count from dishes having a count nearest to 150 colonies and multiply by the reciprocal of the dilution factor corresponding to the highest dilution. Report as the "estimated number of microorganisms per gram".

9.2.4 Express the results as a number from 1,0 to 9,9 multiplied by the appropriate power of 10.

EXAMPLE

At the first (10^{-2}) dilution: 83 and 97 colonies.

At the second (10^{-3}) dilution: 13 and 10 colonies.

Calculation result by using the formula:

$$N = \frac{\sum c}{(n_1 + 0{,}1n)d} = \frac{83 + 97 + 13 + 10}{[2 + (0{,}1 \times 2)]10^{-2}} = 9\ 227$$

Rounding the result to two significant figures means 9 200 or $9{,}2 \times 10^3$ contaminating microorganisms per gram of sample.

10 Precision

10.1 General

See ISO 7218 for information about the confidence limits for the estimation of small numbers of microorganisms.

NOTE No detailed precision data obtained from a collaborative study are available. However, due to the large diversity of contaminating microorganisms, depending on, for example, the type of product, topographic origin and location of production, inclusion of data from a collaborative study based on specific strains is not considered to be relevant.

10.2 Repeatability

Experience indicates that if the higher of two independent tests on the same sample frequently exceeds the lower by 30 %, the analyst should examine the procedures to determine sources of error.

11 Test report

The test report shall specify:

a) all information required for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incident which may have influenced the result(s);

e) the test result(s) obtained; and

f) if the repeatability has been checked, the final quoted results obtained.

© ISO and IDF 2002 – All rights reserved
Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

Bibliography

[1] ISO 707, *Milk and milk products — Guidance on sampling*

© ISO and IDF 2002 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS
Not for Resale

ICS 07.100.30

Price based on 7 pages

© ISO and IDF 2002 – All rights reserved

Copyright International Organization for Standardization
Provided by IHS under license with ISO
No reproduction or networking permitted without license from IHS

Not for Resale