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**Yogurt — Enumeration of characteristic
microorganisms — Colony-count
technique at 37 °C**

*Yaourt — Dénombrement des micro-organismes caractéristiques —
Technique de comptage des colonies à 37 °C*

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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 7889|IDF 117 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.

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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.

ISO 7889|IDF 117 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, *Lactic acid bacteria and starters*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Prof. B. Bianchi Salvadori (IT).

This edition cancels and replaces the first edition of IDF 117A:1988, which has been technically revised.

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Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C

1 Scope

This International Standard specifies a method for the enumeration of characteristic microorganisms in yogurt by means of the colony-count technique at 37 °C.

The method is applicable to yogurts in which both characteristic microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) are present and viable.

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

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

3 Terms and definitions

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

3.1

characteristic microorganisms in yogurt

Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* as detected under the conditions specified in this International Standard

3.2

Lactobacillus delbrueckii* subsp. *bulgaricus

thermophilic microorganism which forms lenticular, often sharp-shaped, colonies of diameter 1 mm to 3 mm on acidified MRS medium under the conditions specified in this International Standard

NOTE Under a microscope, these microorganisms appear as rods, generally short, but sometimes in longer forms. They are non-spore forming, Gram-positive, non-motile and catalase-negative.

3.3

Streptococcus thermophilus

thermophilic microorganism which forms lenticular colonies of diameter 1 mm to 2 mm on M17 medium under the conditions specified in this International Standard

NOTE Under a microscope, these microorganisms appear as spherical or ovoid cells (of diameter 0,7 µm to 0,9 µm) in pairs or in long chains. They are Gram-positive and catalase-negative.

4 Principle

4.1 Decimal dilutions of the test sample are inoculated into:

- a) acidified MRS medium, followed by anaerobic incubation at 37 °C ± 1 °C for 72 h, for the count of *Lactobacillus delbrueckii* subsp. *bulgaricus*;
- b) complete medium (M17), followed by aerobic incubation at 37 °C ± 1 °C for 48 h, for the count of *Streptococcus thermophilus*.

4.2 The colonies are counted and confirmed by means of appropriate tests.

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

5 Culture media, diluents and reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. See also ISO 6887-1 and ISO 8261 | IDF 122.

5.1 Diluent

See ISO 6887-1 and ISO 8261 | IDF 122.

5.2 Culture media

Use freshly prepared culture media (MRS and M17) which shall not be exposed to direct sunlight.

If the prepared culture media are not used immediately, they shall, unless otherwise specified, be cooled and stored at between 2 °C and 4 °C for no longer than 1 week and under conditions which do not produce any change in their composition.

As for reagents, see storage conditions specified in ISO 7218.

5.2.1 Acidified MRS medium (see reference [7])

5.2.1.1 Composition

Peptone 1 (tryptic digest of casein)	10,0 g
Meat extract	10,0 g
Yeast extract (dried)	5,0 g
Glucose (C ₆ H ₁₂ O ₆)	20,0 g
Tween 80 (sorbitan mono-oleate)	1,0 ml
Dipotassium hydrogen orthophosphate (K ₂ HPO ₄)	2,0 g
Sodium acetate trihydrate (CH ₃ CO ₂ Na·3H ₂ O)	5,0 g
Diammonium citrate [C ₆ H ₆ O ₇ (NH ₄) ₂]	2,0 g
Magnesium sulfate heptahydrate (MgSO ₄ ·7H ₂ O)	0,2 g
Manganese sulfate tetrahydrate (MnSO ₄ ·4H ₂ O)	0,05 g
Agar	9,0 g to 18,0 g ¹⁾
Water up to	1 000 ml

1) Depending on the gel strength of the agar or according to manufacturer's instructions.

5.2.1.2 Preparation

Separately dissolve each component in a water bath (6.7) set at boiling. Cool in another water bath (6.7) to 50 °C. Adjust the pH so that after sterilization it is $5,4 \pm 0,1$ at $25 \text{ °C} \pm 1 \text{ °C}$ by adding acetic acid (5.3.3) and checking with the pH-meter (6.8). Transfer the medium in 100 ml portions into 150 ml bottles (6.10) or in 200 ml portions into 250 ml bottles (6.10). Sterilize for 15 min in an autoclave at $121 \text{ °C} \pm 1 \text{ °C}$.

NOTE 1 MRS medium is highly sensitive to heat treatment which may cause differences according to the autoclave used.

NOTE 2 Comparative tests have shown that commercially available MRS media may give counts that are lower than those given by the MRS medium prepared in accordance with this International Standard. Therefore, if used, the former should be checked against the medium prepared according to this International Standard. This may cause problems for yogurt producers and the authorities investigating the requested minimum cell count in yogurt products.

Before beginning the bacteriological examination, completely melt the required amount of medium in a water bath (6.7) set at boiling, or by steaming in a partially closed container. Then cool it in another water bath (6.7).

5.2.2 M17 medium (see reference [8])

5.2.2.1 Basic medium

5.2.2.1.1 Composition

Peptone 1 (tryptic digest of casein)	2,5 g
Peptone 2 (peptic digest of meat)	2,5 g
Peptone 3 (papain digest of soya)	5,0 g
Yeast extract (dried)	2,5 g
Meat extract	5,0 g
β -Glycerophosphate (disodium salt) ($\text{C}_3\text{H}_7\text{O}_6\text{PNa}_2$)	19,0 g
Magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0,25 g
Ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$)	0,50 g
Agar	9,0 g to 18,0 g ¹⁾
Water up to	950 ml

5.2.2.1.2 Preparation

Separately, dissolve each component in a water bath (6.7) set at boiling. Cool in another water bath (6.7) to 50 °C. Adjust the pH so that, after sterilization, it is $6,8 \pm 0,1$ at $25 \text{ °C} \pm 1 \text{ °C}$ by using a reagent (5.3) and checking with the pH-meter (6.8). Transfer the medium in 95 ml portions into 150 ml bottles (6.10). Sterilize for 15 min in an autoclave at $121 \text{ °C} \pm 1 \text{ °C}$.

5.2.2.2 Lactose solution

5.2.2.2.1 Composition

Lactose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	10,0 g
Water up to	100 ml

5.2.2.2.2 Preparation

Dissolve the lactose in the water. Dilute with water to 100 ml. Sterilize for 15 min in an autoclave at $121 \text{ °C} \pm 1 \text{ °C}$.