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Milk — Enumeration of colony-forming units of psychrotrophic microorganisms — Colony-count technique at 6,5 °C

Lait — Dénombrement des unités formant colonie de micro-organismes psychrotrophes — Technique par comptage des colonies à 6,5 °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 6730|IDF 101 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 edition of ISO 6730|IDF 101 cancels and replaces ISO 6730:1992, of which it constitutes a minor revision.

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.

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 IDF National Committees casting a vote.

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

ISO 6730 | IDF 101 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts on *Non-pathogenic contaminants with classical techniques* (E 22), under the aegis of its chairman, Mr H. Asperger (AT).

This edition cancels and replaces IDF 101A:1991.

Milk — Enumeration of colony-forming units of psychrotrophic microorganisms — Colony-count technique at 6,5 °C

1 Scope

This International Standard specifies a method for the enumeration of colony-forming units (CFU) of psychrotrophic microorganisms in raw and heat-treated milk by means of the colony-count technique at 6,5 °C.

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 6887-1:1999, *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 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261:2001, *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
psychrotrophic microorganisms
bacteria, yeasts and moulds forming countable colonies under the conditions specified in this International Standard

4 Principle

4.1 Poured plates are prepared using a specified culture medium and a specified quantity of the test sample. Other plates are prepared under the same conditions, using decimal dilutions of the test sample.

4.2 The plates are aerobically incubated at 6,5 °C for 10 days.

4.3 The number of colony-forming units (CFU) of microorganisms per millilitre of sample is calculated from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

5 Diluents and culture medium

5.1 General

For general guidance, see ISO 7218.

5.2 Basic materials

See ISO 8261.

5.3 Diluents for general use

See ISO 8261.

5.4 Distribution, sterilization and storage

See ISO 8261.

5.5 Culture medium

5.5.1 Components

Tryptone	5,0 g
Yeast extract	2,5 g
Glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$)	1,0 g
Skimmed milk powder [a]	1,0 g
Agar	10 g to 15 g [b]
Water	1 000 ml

[a] The skimmed milk powder shall be free from inhibitory substances. This should be proved by comparative tests using skimmed milk powder known to be free from such substances.

[b] Depending on the gel strength of the agar.

5.5.2 Preparation

5.5.2.1 Preparation from commercial dehydrated complete medium

Follow the manufacturer's instructions but, in all cases, add the skimmed milk powder, even if the manufacturer considers such an addition unnecessary.

Adjust the pH, if necessary, so that after sterilization it is 7,0 at 25 °C.

5.5.2.2 Preparation from dehydrated basic components

Dissolve and disperse in the water, in the following order: the yeast extract, tryptone, glucose and, finally, the skimmed milk powder.

NOTE Heating the water will assist in this procedure.

Add the agar and heat to boiling, stirring frequently until the agar is completely dissolved, or steam for about 30 min. If the solution is not clear, filter it through filter paper.

Adjust the pH, if necessary, so that after sterilization it is 7,0 at 25 °C.

5.5.2.3 Distribution, sterilization and storage

Dispense the medium into test tubes (6.9) 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 (6.1) at 121 °C ± 1 °C for 15 min. If the medium is to be used immediately, cool it to 45 °C in the water bath (6.5). If not, before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium in a hot water bath (6.6) then cool it to 45 °C in the water bath (6.5). (See also 8.5.4.)

Store the medium in the dark at a temperature between 0 °C and +5 °C for no longer than 3 months after preparation.

In order to check the temperature of the agar, it is recommended to place a thermometer into a portion of 15 g/l agar solution in a separate container identical to that used for the medium. This temperature control solution should be exposed to the same heating and cooling operations as the medium itself.

6 Apparatus and glassware

CAUTION — Sterilize all apparatus that will come into contact with the test sample, the diluent, the dilutions or the culture medium in accordance with ISO 8261.

Disposable apparatus is an acceptable alternative to re-usable glassware if it has suitable specifications.

Usual microbiological laboratory equipment, the apparatus required for the preparation of test samples and dilutions as specified in ISO 8261 and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave).

See ISO 7218.

6.2 Incubator, capable of operating at 6,5 °C ± 0,5 °C.

6.3 Petri dishes, made of glass or plastic, of 90 mm to 100 mm diameter.

6.4 Graduated pipettes, plugged with cotton wool, calibrated to deliver 1 ml ± 0,02 ml or 10 ml ± 0,2 ml or 11 ml ± 0,2 ml.

6.5 Water bath, capable of operating at 45 °C ± 1 °C.

6.6 Water bath, capable of operating at more than 100 °C.

6.7 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.

6.8 Temperature-compensated pH-meter, accurate to within ±0,1 pH units at 25 °C.

6.9 Test tubes, of approximately 20 ml capacity (or flasks or bottles of suitable capacity), and **flasks** or **bottles** of 150 ml to 250 ml capacity, for sterilization and storage of the culture medium.

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