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МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ

Meat and meat products — Enumeration of micro-organisms — Colony count technique at 30 °C (Reference method)

Viandes et produits à base de viande — Dénombrement des micro-organismes — Méthode par comptage des colonies obtenues à 30 °C (Méthode de référence)

Reference number
ISO 2293 : 1988 (E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 2293 was prepared by Technical Committee ISO/TC 34, *Agricultural food products.*

This second edition cancels and replaces the first edition (ISO 2293 : 1976), of which it constitutes a technical revision.

Printed in Switzerland

Meat and meat products — Enumeration of micro-organisms — Colony count technique at 30 °C (Reference method)

1 Scope

This International Standard specifies the reference method for the enumeration of micro-organisms present in meat and meat products by counting the colonies growing in a solid medium after incubating aerobically at 30 °C. It has been drafted in conformity with ISO 4833, *Microbiology — General guidance for enumeration of micro-organisms — Colony count technique at* 30 °C.

A limitation on the applicability of this International Standard is imposed by the method's susceptibility to a large degree of variability. The method should be applied and the results interpreted in the light of the information given in 10.2.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3100-1: —[1], *Meat and meat products — Sampling and preparation of test samples — Part 1: Sampling.*

ISO 3100-2: 1988, *Meat and meat products — Sampling and preparation of test samples — Part 2: Preparation of test samples for microbiological examination.*

ISO 6887: 1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

3 Definition

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

micro-organisms: Bacteria, yeasts and moulds growing aerobically at 30 °C, under the conditions specified in this International Standard.

4 Principle

4.1 Preparation of two poured plates using a specified culture medium, and using a specified quantity of the test sample if the initial product is liquid (drip), or a specified quantity of an initial supension in the case of other products.

Preparation of other pairs of poured plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

4.2 Aerobic incubation of the plates at 30 °C for 72 h.

4.3 Calculation of the number of micro-organisms per millilitre or per gram of sample from the number of colonies obtained in selected plates (see 10.1).

5 Culture media and dilution fluid

5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the culture media, dehydrated basic components, or a complete dehydrated medium, be used. The manufacturer's instructions shall be rigorously followed.

The chemicals used shall be of recognized analytical quality.

The water used shall be distilled or deionized, and shall be free from substances that might inhibit the growth of micro-organisms under the test conditions.

If the media and dilution fluid are not used immediately, they shall, unless otherwise specified, be stored in the dark at a temperature between 0 °C and +5 °C, and in conditions that prevent any change in their composition. They shall not be kept for longer than 1 month.

5.2 Dilution fluid

Refer to ISO 6887 and to the International Standard dealing with the product under examination.

1) To be published.

5.3 Plate count agar

Composition

Tryptone[1]	5,0 g
Dehydrated yeast extract	2,5 g
Anhydrous D-glucose (anhydrous dextrose)	1,0 g
Agar in powder or flake form	12 g to 18 g[2]
Water	1 000 ml

Preparation

Dissolve the components or the complete dehydrated medium in the water by boiling. Adjust the pH so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into test tubes (6.9), in quantities of 15 ml per tube, or into flasks or bottles (6.9) of capacity not greater than 500 ml, in quantities equal to about half the volume of the respective container.

Sterilize in an autoclave (6.2) at 121 °C ± 1 °C for 15 min. If the medium is to be used immediately, cool it to 45 °C ± 0,5 °C in the water-bath (6.6) before use. If not, before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium in a boiling water-bath, and then cool it to 45 °C ± 0,5 °C in the water-bath (6.6).

5.4 Water-agar medium (if necessary — see 9.2.1.4)

Composition

Agar in powder or flake form	12 g to 18 g[2]
Water	1 000 ml

Preparation

Dissolve the agar in the water by boiling. Adjust the pH, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into test tubes (6.9), in quantities of 4 ml per tube, or into 150 ml flasks or bottles (6.9), in quantities of 100 ml per container.

Sterilize in an autoclave at 121 °C ± 1 °C for 15 min. If the medium is to be used immediately, cool it to 45 °C ± 0,5 °C in the water-bath (6.6) before use. If not, before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium in a boiling water-bath, and then cool it to 45 °C ± 0,5 °C in the water-bath (6.6).

6 Apparatus and glassware

NOTE — Disposable apparatus is an acceptable alternative to glassware if it has similar specifications.

Usual microbiological laboratory apparatus and, in particular, the following.

6.1 Blending equipment.

One of the following shall be used:

a) mechanical meat mincer, laboratory size, capable of being sterilized, fitted with a plate with holes not exceeding 4 mm in diameter;

b) peristaltic-type blender (Stomacher), with sterile plastic bags.

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

Apparatus that will come into contact with the culture media, the dilution fluid or the sample, particularly plastic apparatus, except for apparatus that is supplied sterile, shall be sterilized either

— by being kept at 170 °C to 175 °C for not less than 1 h in the oven, or

— by being kept at 121 °C ± 1 °C for not less than 20 min in the autoclave.

6.3 Incubator, capable of being controlled at 30 °C ± 1 °C.

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

6.5 Pipettes, calibrated for bacteriological use only, with a nominal capacity of 1 ml, graduated in divisions of 0,1 ml and with an outflow opening of diameter 2 mm to 3 mm.

6.6 Water-bath, or similar apparatus, capable of being controlled at 45 °C ± 0,5 °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 1,5X, and a mechanical or electronic digital counter.

6.8 pH meter, having an accuracy of ± 0,1 pH unit at 25 °C.

1) This term is used at present only by certain producers of culture media. Any other casein digest giving comparable results may be used.

2) According to the manufacturer's instructions.