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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.

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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.

6.9 Test tubes, 18 mm × 180 mm, or **flasks** or **bottles** of suitable capacity (see 5.3 and 5.4).

7 Sampling

See ISO 3100-1.

8 Preparation of the test sample

Take a test sample of at least 200 g in accordance with ISO 3100-2 using the blending equipment (6.1).

Start the examination of the pretreated sample as soon as possible; it may be stored, if necessary, at a temperature between 0 °C and +5 °C, but for not longer than 1 h.

9 Procedure

9.1 Test portion, initial suspension and dilutions

See ISO 6887.

9.2 Counting technique

9.2.1 Inoculation

9.2.1.1 Take two Petri dishes (6.4). Using a pipette (6.5), transfer to each dish 1 ml of the test sample, if liquid, or 1 ml of the initial suspension in the case of other products.

9.2.1.2 Take two other Petri dishes. Using a fresh pipette, transfer to each dish 1 ml of the 10^{-1} dilution (liquid products) or 1 ml of the 10^{-2} dilution (other products).

Repeat the procedure described in the preceding paragraph with further dilutions as necessary.

9.2.1.3 Pour about 15 ml of the plate count agar (5.3), at 45 °C ± 0,5 °C, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium (5.3) is poured into the dishes shall not exceed 15 min.

Carefully mix the inoculum with the agar and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface.

9.2.1.4 Only in those cases where it is suspected that the product under examination contains micro-organisms whose colonies will overgrow the surface of the medium, after complete solidification, pour about 4 ml of the water-agar medium (5.4), at 45 °C ± 0,5 °C, onto the surface of the inoculated agar. Allow to solidify as described above.

This operation, if carried out, shall be mentioned in the test report.

9.2.2 Incubation

Invert the prepared dishes and place them in the incubator (6.3) at 30 °C ± 1 °C. Leave them for 72 h ± 3 h.

9.2.3 Interpretation

After the specified period of incubation (see 9.2.2), count, using the colony-counting equipment (6.7), the colonies in each dish containing less than 300 colonies.

10 Expression of results

10.1 Method of calculation

10.1.1 Retain dishes containing less than 300 colonies at two consecutive dilutions. It is necessary that one of these dishes contains at least 15 colonies.

Calculate the number N of micro-organisms per millilitre or per gram of product, depending on the case, using the following equation:

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

where

$\sum C$ is the sum of colonies counted on all the dishes retained;

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

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

d is the dilution factor corresponding to the first dilution.

Round the result calculated to two significant figures.

Take as the result the number of micro-organisms per millillitre or per gram of product, expressed as a number between 1,0 and 9,9 multiplied by 10^x, where x is the appropriate power of 10.

EXAMPLE

A micro-organisms count at 30 °C gave the following results:

— at the first dilution retained (10^{-2}): 168 and 215 colonies

— at the second dilution retained (10^{-3}): 14 and 25 colonies

$$N = \frac{\sum C}{(n_1 + 0{,}1 n_2)\, d} = \frac{168 + 215 + 14 + 25}{[2 + (0{,}1 \times 2)] \times 10^{-2}}$$

$$= \frac{422}{0{,}022} = 19\,182$$

Rounding the result as specified above gives 19 000 or $1{,}9 \times 10^4$ micro-organisms per millilitre or per gram of product.

10.1.2 If the two dishes, corresponding to the test sample (liquid products) or the initial suspension (other products), contain less than 15 colonies, calculate the arithmetic mean m of the colonies counted on both dishes.

Report the result as follows:

— estimated number of micro-organisms per millilitre:

$N_E = m$ (liquid products)

— estimated number of micro-organisms per gram:

$N_E = m \times \frac{1}{d}$ (other products), where d is the dilution factor of the initial supension

10.1.3 If the two dishes corresponding to the test sample (liquid products) or the initial suspension (other products) contain no colonies, report the result as follows:

— less than 1 micro-organism per millilitre (liquid products)

— less than $1 \times 1/d$ micro-organism per gram (other products), where d is the dilution factor of the initial suspension.

10.1.4 If there are plates containing between 15 and 30 colonies at two consecutive dilutions, calculate the number of micro-organisms for each dilution and take as the result the arithmetic mean of the two values obtained. If the ratio of the higher value to the lower value is greater than two, take the lower value as the result.

10.2 Precision

For statistical reasons alone, in 95 % of cases the confidence limits of this method vary from ± 12 % to ± 37 %[1]. In practice, even greater variation may be found, especially between results obtained by different microbiologists.

11 Test report

The test report shall specify the method used and the results obtained, and shall indicate the addition of water-agar medium if applicable. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results.

The test report shall include all information necessary for the complete identification of the sample.

1) Taken from COWELL and MORISETTI, *J. Sci. Food Agric.*, 1969 (Vol. 20), p. 573.

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