INTERNATIONAL STANDARD ISO 2293

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Meat and meat products — Aerobic count at 30 °C (Reference method)

Viandes et produits à base de viande — Dénombrement des germes aérobies à 30 °C (Méthode de référence)

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FOREWORD

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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.

International Standard ISO 2293 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products,* and circulated to the Member Bodies in April 1971.

It has been approved by the Member Bodies of the following countries :

Australia	Germany	Portugal
Austria	Hungary	South Africa, Rep. of
Belgium	India	Spain
Brazil	Iran	Sweden
Bulgaria	Ireland	Turkey
Chile	Israel	United Kingdom
Czechoslovakia	Netherlands	
Egypt, Arab Rep. of	Poland	

The Member Bodies of the following countries expressed disapproval of the document on technical grounds :

France
New Zealand

Printed in Switzerland

Meat and meat products — Aerobic count at 30 °C (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the number of viable aerobic micro-organisms in meat and meat products.

The method can be applied to all kinds of meat and meat products.

2 REFERENCES

ISO 3100, *Meat and meat products — Sampling.*

3 DEFINITIONS

3.1 aerobic micro-organisms : Micro-organisms which grow under aerobic conditions when the test is carried out in accordance with the method described.

3.2 aerobic count : The determination of the number of viable aerobic micro-organisms per gram of meat or meat product that is found when the test is carried out in accordance with the method described.

4 PRINCIPLE

Mincing of the meat or meat product, followed by maceration in a mechanical blender with a sterile diluent. Preparation from the macerate of decimal dilutions.

Spreading of 0,1 ml portions of the dilutions on agar plates of a non-selective medium and, concurrently, transfer of 1 ml portions of the undiluted macerate to empty Petri dishes, into which a non-selective medium is poured.

Incubation at 30 °C under aerobic conditions for 3 days.

Calculation, from the number of colonies per Petri dish, of the number of viable aerobic micro-organisms per gram of sample.

5 CULTURE MEDIUM AND DILUTION FLUID

5.1 Basic materials

For uniformity of results, it is recommended that either dehydrated culture medium components of uniform quality and analytical grade chemicals, or a dehydrated complete medium be used.

The basic materials, water, peptone, tryptone and yeast extract powder, shall be of a quality recognized as suitable for microbiological testing.

5.2 Culture medium

The culture medium prescribed in this method is the standard methods agar (American Public Health Association (1960), "Standard methods for the examination of dairy products", 11th edition, APHA Inc., New York, U.S.A.).

5.2.1 *Composition*

Agar	15,0 g
Yeast extract, powder	2,5 g
Tryptone	5,0 g
Glucose	1,0 g
Water	1 000 ml

5.2.2 *Preparation of the culture medium*

Dissolve the dehydrated culture medium components or the dehydrated complete medium in the water, by boiling.

Adjust the pH so that after sterilization it is 7,0 ± 0,1 at 40 °C.

Transfer the culture medium to tubes or flasks (6.2.1) of not more than 500 ml capacity and sterilize them with their contents by heating in an autoclave (6.1.3) for 20 min at 121 ± 1 °C.

The sterility of the medium shall be checked by incubating one plate with medium only, for 3 days at 30 °C at the same time as the inoculated plates.

5.2.3 *Preparation of the agar plates*

To sterile Petri dishes (6.2.3) add about 15 ml of the melted culture medium (5.2) at a temperature of approximately 45 °C, and allow to solidify. Plates prepared in advance shall not be kept longer than 4 h at room temperature or 1 day in a refrigerator.

Immediately before use, dry the plates, preferably with the lids off and the agar surface downwards, in an oven or incubator (6.1.4) at a temperature of 50 °C for 30 min.

5.3 Dilution fluid

5.3.1 *Composition*

Peptone	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

5.3.2 *Preparation of the dilution fluid*

Dissolve the components in the water, by boiling.

Adjust the pH so that after sterilization it is 7,0 ± 0,1 at 20 °C.

5.3.3 *Preparation of dilution tubes and flasks*

Transfer a part of the dilution fluid into culture flasks (6.2.1) in quantities of 100 to 300 ml, for maceration. Transfer the remainder into culture tubes, or flasks, in such quantities that after sterilization each culture tube, or flask, contains 9,0 ml of dilution fluid.

Sterilize the dilution fluid in an autoclave (6.1.3) for 20 min at 121 ± 1 °C.

6 APPARATUS

6.1 General apparatus

6.1.1 Mechanical meat mincer, laboratory size, sterile, fitted with a plate with holes of diameter not exceeding 4 mm.

6.1.2 Mechanical blender, operating at not less than 8 000 rev/min and not more than 45 000 rev/min, with glass or metal blending jars of an appropriate capacity, fitted with lids and resistant to the conditions of sterilization.

6.1.3 Autoclave and, if necessary, **oven,** for sterilization of glassware, blender jars, culture medium, dilution fluid, etc.

6.1.4 Drying cabinet or **incubator** for drying the surface of agar plates, maintained preferably at a temperature of 50 °C.

6.1.5 Incubator for maintaining the inoculated plates at a temperature of 30 ± 1 °C.

6.1.6 Water baths, for heating and cooling solutions and culture media to the appropriate temperatures.

6.2 Glassware

The glassware shall be resistant to repeated sterilization.

6.2.1 Culture tubes and **flasks** for sterilization and storage of the culture medium and dilution fluid, and for preparing dilutions.

6.2.2 Graduated pipettes, with a nominal capacity of 1 ml, subdivided in 0,1 ml and with an outflow opening of diameter 2 to 3 mm.

6.2.3 Petri dishes, glass, with the following dimensions :

Dish, internal diameter 90 ± 2 mm;
external height not less than 18 mm;
the rim shall be ground in a plane parallel to the base;
the bottom of the dish shall be flat and be parallel to the base.

Lid, external diameter not more than 102 mm.

Plastics Petri dishes, though of slightly different dimensions, may also be used.

6.2.4 Glass spreaders (hockey sticks), made from glass rod approximately 3,5 mm in diameter and 200 mm long, bent at right angles about 30 mm from one end; the cut ends shall be made smooth by heating.

6.3 Sterilization of glassware, etc.

Sterilize the glassware, etc., by one of the following methods :

– wet sterilization at not less than 121 °C for not less than 20 min;

– dry sterilization at not less than 170 °C for not less than 1 h.

7 SAMPLING

Proceed from a representative sample of at least 200 g. See ISO 3100.

The sample may be stored at a temperature of 0 to 5 °C, but not for longer than 1 h.

8 PROCEDURE

8.1 Pre-treatment of the sample

Grind and mix the sample twice in the meat mincer (6.1.1). Start the examination of the pre-treated sample as soon as possible; it may be stored, if necessary, at a temperature between 0 and 5 °C, but not for longer than 1 h.

8.2 Maceration and dilution

8.2.1 Weigh, to the nearest 0,1 g, about 10 g of the minced meat or meat product (8.1), into a blender jar and add 9 times the quantity, by mass, of dilution fluid (5.3).

Operate the blender (6.1.2) for sufficient time to give a total number of 15 000 to 20 000 revolutions.

Thus, even with the slowest blender, this time will not exceed 2,5 min.

8.2.2 Directly after maceration, take duplicate portions of 1 ml of the macerate (8.2.1) with a sterile pipette (6.2.2) and add each portion to 9 ml of sterile dilution fluid, in a culture tube or flask (see 5.3.3), avoiding contact between the pipette and the dilution fluid.

Carry out the operations in 8.2.3, 8.2.4 and 8.3.1 on each diluted portion.

8.2.3 Mix the liquids carefully by aspirating ten times with a fresh sterile pipette.

Transfer with the same pipette 1 ml of this dilution (10^{-2}) to another dilution tube containing 9 ml of sterile dilution fluid, avoiding contact between the pipette and the dilution fluid.

8.2.4 Mix the liquids with a fresh sterile pipette as in 8.2.3 and repeat the operations twice more so that the required number of dilutions up to 10^{-5} has been made.

8.3 Inoculation

8.3.1 *Inoculation with diluted macerate*

With a fresh sterile pipette, transfer in duplicate to agar plates (5.2.3) 0,1 ml of the macerate (8.2.1) and of each of the dilutions of both dilution series. Start with the highest dilution selected, and proceed to the lowest, filling and emptying the pipette three times before transferring the 0,1 ml portions to the plates.

Carefully spread this quantity as quickly as possible on the surface of the agar plate using a glass spreader (6.2.4) and leave the plate for about 15 min on the bench for impregnation to take place. Use a fresh sterile spreader for each plate.

8.3.2 *Inoculation with undiluted macerate*

With a fresh sterile pipette, transfer 1 ml of the macerate (8.2.1) in duplicate to empty sterile Petri dishes.

Within 5 min pour into each dish 15 to 20 ml of the melted culture medium (5.2), cooled beforehand to a temperature of about 45 °C.

Mix the contents of the Petri dishes thoroughly, immediately after the addition of the culture medium. Make sure that the dishes are in a horizontal position while the mixture is solidifying.

8.4 Incubation of the plates

Keep the plates prepared according to 8.3.1 and 8.3.2, with the bottom of the Petri dishes uppermost, for 3 days in an incubator (6.1.5) at a temperature of 30 ± 1 °C.

8.5 Counting of colonies

At the end of the incubation period count the number of colonies on each plate.

The results shall be considered void when half or more than half of the surface area of the plate is overgrown.

When part, but less than half, of the surface area of the plate is overgrown, count the number of colonies on the clear half, and multiply the result by two to obtain the number in relation to the total surface area of the plate.

9 EXPRESSION OF RESULTS

9.1 Use for the calculation plates on which have developed between 30 and 300 colonies, preferably those inoculated with the maximum amount of the sample examined.

9.2 Calculate the average number of colonies from the counts made on duplicate inoculated agar plates at the chosen dilutions of both dilution series.

9.3 Round the result as follows :

a) if the figure is less than 100, round to the nearest multiple of 5;

b) if the figure is more than 100, and does not end with a 5, round it to the nearest multiple of 10;

c) if the figure is more than 100 and ends with a 5, round it to the nearest multiple of 20.

9.4 Calculate the number of micro-organisms per gram of sample by multiplying the number obtained according to 9.3 by 10 and by the dilution factor (10, 10^2, 10^3, 10^4 or 10^5) in the case of spread plates, and only by the dilution factor 10 for plates which are poured according to 8.3.2.

9.5 Calculate the average of the results obtained from each of the duplicate dilution series (see 8.2.2).

Note the result as the number of micro-organisms per gram of meat or meat product, expressed as a number between 1,0 and 9,9 multiplied by the appropriate power of 10 (for example an average count of 15 000 would be reported as $1,5 \times 10^4$ per gram). In the event of finding less than 300 micro-organisms per gram, note the result as "less than $3,0 \times 10^2$ per gram".

10 TEST REPORT

The report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances which may have influenced the result.

The report shall include all details required for complete identification of the sample.