SLOVENSKI STANDARD
SIST ISO 4833:1997

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Mikrobiologija - Splošno navodilo za ugotavljanje števila mikroorganizmov - Tehnika štetja kolonij pri 30 °C

Microbiology -- General guidance for the enumeration of micro-organisms -- Colony count technique at 30 degrees C

Microbiologie -- Directives générales pour le dénombrement des micro-organismes -- Méthode par comptage des colonies obtenues à 30 degrés C

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INTERNATIONAL STANDARD

ISO 4833

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Microbiology — General guidance for the enumeration of micro-organisms — Colony count technique at 30 °C

Microbiologie — Directives générales pour le dénombrement des micro-organismes — Méthode par comptage des colonies obtenues à 30 °C

Reference number
ISO 4833:1991(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 voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 4833 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition cancels and replaces the first edition (ISO 4833:1978), of which it constitutes a technical revision.

Annex A forms an integral part of this International Standard.

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Introduction

This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International Standards and for reference for bodies preparing microbiological methods of test for application to foods or to animal feeding stuffs. Because of the large variety of products within this field of application, these guidelines may not be appropriate for some products in every detail, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply the guidelines provided as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviation from them in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with these guidelines. In cases where International Standards already exist for the product to be tested, they should be followed, but it is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that eventually the only remaining departures from these guidelines will be those necessary for well-established technical reasons.

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Microbiology — General guidance for the enumeration of micro-organisms — Colony count technique at 30 °C

1 Scope

This International Standard gives general guidelines for the enumeration of micro-organisms present in products intended for human consumption or feeding of animals, by counting the colonies growing in a solid medium after aerobic incubation at 30 °C.[1)]

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 indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations.*

3 Definition

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

micro-organisms: Bacteria, yeasts and moulds growing aerobically under the test 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, or using a specified quantity of an initial suspension 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 General

For current laboratory practice, see ISO 7218.

5.2 Dilution fluid

See ISO 6887 and the specific International Standard dealing with the product under examination.

1) A temperature of 30 °C is that most frequently used for the enumeration of total flora and this temperature has been adopted by ISO/TC 34 Subcommittee 9. However, if for particular reasons another temperature is used, it should be indicated in the test report.

5.3 Plate count medium

Composition

tryptone	5,0 g
dehydrated yeast extract	2,5 g
anhydrous glucose	1,0 g
agar	12 g to 18 g[2]
water	1 000 ml

Preparation

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

Dispense the medium into test tubes (6.8), in quantities of 15 ml per tube, or into flasks or bottles (6.8) of capacity not greater than 500 ml, in quantities of approximately half the volume of the container.

Sterilize in an autoclave set at 121 °C for 15 min. If the medium is to be used immediately, cool it in the water-bath (6.5) set at 45 °C 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, then cool it in the water-bath (6.5) set at 45 °C.

5.4 Water agar medium (if necessary — see 9.2.3)

Composition

agar	12 g to 18 g[2]
water	1 000 ml

Preparation

Dissolve the agar in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 7,0 at 25 °C.

Dispense the medium into test tubes (6.8), in quantities of 4 ml per tube, or into flasks or bottles (6.8) of appropriate capacity, in quantities of 100 ml per container.

Sterilize in an autoclave set at 121 °C for 15 min. If the medium is to be used immediately, cool it in the water-bath (6.5) set at 45 °C 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, then cool it in the water-bath (6.5) set at 45 °C.

2) According to the gel strength of the agar.

6 Apparatus and glassware

NOTE 1 Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment 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 30 °C ± 1 °C.

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

6.4 Total delivery pipettes, having a nominal capacity of 1 ml.

6.5 Water-bath, or similar apparatus, capable of operating at 45 °C ± 0,5 °C.

6.6 Colony counting equipment, consisting of an illuminated base and a mechanical or electronic digital counter.

6.7 pH meter, accurate to ± 0,1 pH unit at 25 °C.

6.8 Test tubes, 20 mm × 200 mm or **flasks** or **bottles** of 150 ml capacity and not greater than 500 ml capacity.

7 Sampling

Sampling shall have been carried out in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

8 Preparation of the test sample

Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Test portion, initial suspension and dilutions

See ISO 6887 and the specific International Standard appropriate to the product concerned.

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.3). Using a sterile pipette (6.4), transfer to each dish 1 ml of the test sample, if the product is liquid, or 1 ml of the initial suspension in the case of other products.

Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1 ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products.

Repeat the procedure with the further dilutions, using a fresh sterile pipette for each decimal dilution.

9.2.2 Pour about 15 ml of the plate count medium (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 medium and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface.

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

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

9.2.4 Invert the prepared dishes and incubate them in the incubator set at 30 °C for 72 h ± 3 h.

9.3 Counting of the colonies

After the specified period of incubation (see 9.2.4), count, using the colony counting equipment (6.6), the colonies in each dish containing not more than 300 colonies.

10 Expression of results

10.1 Method of calculation

10.1.1 General case — Dishes containing between 15 and 300 colonies

Retain dishes containing not more 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 millilitre 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

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

$$= \frac{168 + 215 + 14 + 25}{[2 + (0{,}1 \times 2)] \times 10^{-2}}$$

$$= \frac{422}{0{,}022} = 19182$$