INTERNATIONAL STANDARD

ISO 17410

First edition
2001-05-15

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of psychrotrophic microorganisms

Microbiologie des aliments — Méthode horizontale pour le dénombrement des micro-organismes psychrotrophes

Reference number
ISO 17410:2001(E)

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Printed in Switzerland

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 17410 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of psychrotrophic microorganisms

1 Scope

This International Standard specifies a method for the enumeration of psychrotrophic microorganisms by means of the colony-count technique at 6,5 °C. The method is applicable to products intended for human consumption or for animal feeding stuffs.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 6887-1, *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.*[1)]

ISO 7218*, Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*.

ISO 8261, *Milk and milk products — Preparation of samples and dilutions for microbiological examination*.

3 Term and definition

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

3.1
psychrotrophic microorganism
bacteria, yeasts and moulds forming countable colonies under the conditions specified in this International Standard

4 Principle

4.1 Two agar plates are prepared using a solid non-selective 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. In order to prevent heat stress, surface inoculation is used.

Other pairs of agar plates are prepared, under the same conditions, using decimal dilutions of the test sample or the initial suspension.

1) Parts 2 to 5 are in preparation, see biliography.

4.2 The plates are subjected to aerobic incubation at 6,5 °C for 10 days.

4.3 The number of microorganisms per millilitre or per gram of sample is calculated from the number of colonies obtained on the plates chosen.

5 Culture media and dilution fluid

For current laboratory practice, see ISO 7218.

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

If the prepared culture media and reagents are not used immediately, store them, unless otherwise stated, in the dark at a temperature of 3 °C ± 2 °C for no longer than 1 month, under conditions which do not produce any change in their composition.

5.1 Dilution fluid

See ISO 6887-1.

5.2 Plate count agar (PCA)

5.2.1 Composition

Enzymatic digest of casein	5,0 g
Yeast extract	2,5 g
Glucose	1,0 g
Agar	9 g to 18 g[a]
Water	1 000 ml

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

When dairy products are examined, it is recommended to add 1,0 g of skimmed milk powder per litre of the culture medium. The skimmed milk powder shall be free from inhibitory substances.

5.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary. Mix thoroughly and leave to stand for several minutes. Adjust the pH (6.9), if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into flasks or bottles (6.10) in appropriate quantities. Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

If the medium is to be used immediately, cool it before use in the water bath (6.7) set at 44 °C to 47 °C. If not, allow the medium to solidify in the flask or bottle. Before use, melt the medium completely in a boiling water bath, then cool it in the water bath (6.7) set at 44 °C to 47 °C.

Place in each of an appropriate number of Petri dishes (6.5) about 15 ml of the freshly prepared, or remelted, complete medium. Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in an incubator (6.3) set between 37 °C and 55 °C until the surface of the agar is dry. The agar plates may also be dried in a laminar-flow safety cabinet for 30 min with half-open lids, or overnight with the lids in place (see ISO 7218).

6 Apparatus and glassware

For general requirements, see ISO 7218.

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

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

6.1 **Autoclave**, capable of operating at a temperature of 121 °C.

6.2 **Oven for dry sterilization**, capable of operating at a temperature between 170 °C and 175 °C for 1 h.

6.3 **Incubator**, capable of operating at a temperature of 37 °C to 55 °C.

6.4 **Incubator**, capable of operating at a temperature of 6,5 °C ± 1 °C.

6.5 **Petri dishes**, made of glass or plastic, of approximately 90 mm to 100 mm diameter.

6.6 **Pipettes**, calibrated for bacteriological use, having nominal capacities of 0,1 ml, 1 ml and 10 ml.

6.7 **Water baths**, or similar apparatus, one capable of operating at a temperature of 44 °C to 47 °C and another capable of boiling water.

6.8 **Colony-counting equipment**, consisting of an illuminated base and, optionally, a mechanical or electronic digital counter.

6.9 **pH-meter**, capable of being read to the nearest 0,01 pH unit at 25 °C, enabling measurements to be made which are accurate to ± 0,1 pH unit.

6.10 **Bottles** or **flasks**, of appropriate capacity, for preparation, sterilization and, if necessary, storage of culture media.

6.11 **Glass** or **plastic spreaders**, sterile, for spreading inoculation material on the surface of the culture medium.

7 Sampling

It is important that the laboratory receive a sample which is truly representative of the product and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. See 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.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6887-1 or, for dairy products, ISO 8261.

9 Procedure

9.1 Test portion, initial suspension and dilutions

Prepare the test portion, initial suspension (primary dilution) and further dilutions in accordance with ISO 6887-1 or, for dairy products, ISO 8261.

9.2 Inoculation and incubation

9.2.1 Prepare two dishes from the test sample (if liquid) or from the primary initial solution of other products and from each dilution chosen. Transfer using a sterile pipette (6.6) 0,1 ml of liquid product, the initial suspension (primary dilution) or the appropriate dilutions to the centre of each labelled Petri dish containing the PCA medium (5.3).

9.2.2 Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish, with a spreader (6.11) until there is no longer any liquid visible on the agar surface. A control plate without inoculum should be included for checking sterility. Use a fresh spreader for each plate.

It is possible to use the same spreader for all dilutions from one sample beginning with the highest dilution and progressing in order to the dilution having the greatest amount of test material.

9.2.3 Invert the dishes prepared in 9.2.2 and place them in the incubator (6.4) set at 6,5 °C. Incubate them for 10 days.

9.3 Enumeration

After the specified period of incubation (see 9.2.3), count the colonies in each Petri dish containing not more than 150 colonies, using the colony-counting equipment (6.8). It is important that pinpoint colonies be included in the count but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies.

Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading colonies, count the colonies on the unaffected part of the dish and calculate the corresponding number for the entire dish. If more than one-quarter of the dish is overgrown by spreading colonies, discard the count for that dish.

10 Calculation and expression of results

10.1 Calculation

See ISO 7218.

For a result to be valid, in general it is considered that it is necessary to count the colonies on at least one dish containing as a minimum 15 colonies.

Calculate the number, N, of colony-forming units (CFU) of psychrotrophic microorganisms per gram or per millilitre of sample, using equation (1):

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

where

$\sum C$ is the sum of all the colonies counted on all the dishes retained from two successive dilutions, one of which contains at least 15 colonies;

V is the volume, in millilitres, of inoculum applied to each dish;

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

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

d is the dilution factor corresponding to the first dilution retained.

NOTE The lowest dilution is the dilution with the highest content of test sample.

10.2 Expression of results

10.2.1 General case: Dishes containing between 15 and 150 colonies

Round off the results to two significant figures. In order to do this, if the third figure is less than 5, do not modify the preceding figure; if the third figure is greater than or equal to 5, increase the preceding figure by one unit. For example, 28 500 is rounded to 29 000 and 11 500 is rounded to 12 000.

Take as the result the number of CFU of psychrotrophic microorganisms per millilitre (liquid products) or per gram (other products), expressed preferably as a number between 1,0 and 9,9 multiplied by the appropriate power of 10, or a whole number with two significant figures.

10.2.2 Estimation of low numbers

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 of the colonies counted on both dishes.

Express the result as follows:

— estimated number of psychrotrophic microorganisms, N_E, per millilitre (liquid products) or per gram (other products) using equation (2):

$$N_E = \frac{\sum C}{(V \cdot n \cdot d)} \qquad (2)$$

where

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

V is the volume, in millilitres, of inoculum applied to each dish;

n is the number of retained dishes (in this case $n = 2$);

d is the dilution factor of the initial suspension or of the dilution inoculated.

10.2.3 No colonies present

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 $1/(V \cdot d)$ psychrotrophic microorganisms per millilitre (liquid products) or per gram (other products);

where

V is the volume of the inoculum applied to each dish in millilitres;

d is the dilution factor corresponding to the suspension.

10.2.4 Estimation of large numbers

If there are only dishes containing more than 150 colonies, calculate an estimated arithmetic mean count from dishes having the nearest to 150 colonies.