
**Microbiology of food and animal feeding
stuffs — Horizontal method for the
enumeration of presumptive *Bacillus
cereus* — Colony-count technique at
30 °C**

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*Microbiologie des aliments — Méthode horizontale pour le
dénombrement de *Bacillus cereus* présomptifs — Technique par
comptage des colonies à 30 °C*

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Contents

Page

Foreword	iv
0 Introduction	v
1 Scope.....	1
2 Normative references	1
3 Terms and definitions.....	1
4 Principle	1
5 Dilution fluid, culture media and reagents	2
6 Apparatus and glassware.....	4
7 Sampling	5
8 Preparation of test sample	5
9 Procedure.....	5
9.1 Test portion, initial suspension and dilutions	5
9.2 Inoculation and incubation	5
9.3 Counting of the colonies.....	5
9.4 Confirmation.....	6
10 Expression of results.....	6
10.1 Count of presumptive <i>B. cereus</i> colonies	6
10.2 No colonies	7
10.3 Precision	7
11 Test report.....	8
Annex A (normative) Confidence limits for the estimation of small numbers of colonies.....	9
Annex B (informative) Results of interlaboratory test.....	10
Bibliography	13

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 7932 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition cancels and replaces the second edition (ISO 7932:1993) and Technical Corrigendum 1 (ISO 7932:1993/Cor.1:1997).

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In this edition the previous confirmation tests [mannitol/egg yolk/polymyxin (MYP) agar medium, glucose fermentation, Voges-Proskauer reaction and nitrate reduction] are replaced by the following:

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— haemolysis reaction; <https://standards.iteh.ai/catalog/standards/sist/142c2e41-f666-4e53-9b99-0ddb4f1e512f/iso-7932-2004>

— MYP agar medium.

This edition introduces precision data obtained during an interlaboratory trial based on ISO 7932:1993 and using the following confirmation tests: MYP agar medium, glucose agar medium, VP medium and nitrate medium.

0 Introduction

0.1 This International Standard is intended to provide general guidance for the microbiological examination of food products not dealt with by existing International Standards and to be taken into account by organizations preparing microbiological test methods 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 in every detail for certain products 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 the 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.

0.2 It appears that the spores of many, if not most, strains of *B. cereus* germinate readily on the surface of culture media used for enumeration. In most cases there does not seem to be a need for heat shock treatment to provoke germination. Sometimes a heat shock procedure is desirable, for example for spore counts or to inhibit growth of vegetative bacterial cells. In such cases, treatment for 10 min at 80 °C is recommended.

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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive *Bacillus cereus* — Colony-count technique at 30 °C

1 Scope

This International Standard specifies a horizontal method for the enumeration of viable presumptive *Bacillus cereus* by means of the colony-count technique at 30 °C. It is applicable to

- products intended for human consumption and the feeding of animals, and
- environmental samples in the area of food production and food handling.

NOTE In order to have a practicable test method, the confirmatory stage has been restricted to the typical aspect on MYP agar and the haemolysis test. Thus the term “presumptive” has been introduced in order to acknowledge the fact that the confirmatory stage does not enable the distinction of *B. cereus* from other closely related but less commonly encountered *Bacillus* species, such as *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*. An additional motility test may help to differentiate *B. cereus* from *B. anthracis* in cases where the presence of the latter is suspected.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1:1999, *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*

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*, and Amd.1:2001

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

presumptive *Bacillus cereus*

microorganism that forms typical colonies on the surface of a selective culture medium and which gives a positive confirmation reaction under the conditions specified in this International Standard

NOTE See Note in Clause 1.

4 Principle

4.1 A specified quantity of the test sample if the initial product is liquid, or a specified quantity of an initial suspension in the case of other products, is surface plated on a solid selective culture medium contained in Petri dishes.

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

4.2 The plates are incubated under aerobic conditions at 30 °C for 18 h to 48 h.

4.3 The number of *B. cereus* per gram or per millilitre of sample is calculated from the number of confirmed colonies obtained on plates at dilution levels chosen so as to give a significant result, and confirmed according to the test specified.

5 Dilution fluid, culture media and reagents

For current laboratory practice, see ISO 7218.

NOTE Commercially prepared ready-to-use reagents may be used.

5.1 Dilution fluid

See ISO 6887-1 and any specific standard dealing with the product to be examined.

5.2 Agar medium (see [1])

5.2.1 Base medium

5.2.1.1 Composition

Beef extract	1,0 g
Enzymatic digest of casein	10,0 g
D-Mannitol	10,0 g
Sodium chloride (NaCl)	10,0 g
Phenol red	0,025 g
Agar	12 g to 18 g ^a
Water	900 ml

^a Depending on the gel strength of the agar.

5.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that the pH of the complete medium (5.2.4), after sterilization, is $7,2 \pm 0,2$ at 25 °C.

Dispense the medium in quantities of 90 ml into flasks of appropriate capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

5.2.2 Polymyxin B solution

5.2.2.1 Composition

Polymyxin B sulfate	10 ⁶ IU
Water	100 ml

5.3 Sheep blood agar

5.3.1 Base medium: Blood agar base No. 2

5.3.1.1 Composition

Proteose peptone or equivalent peptone	15 g
Liver hydrolysate	2,5 g
Yeast extract	5 g
Sodium chloride (NaCl)	5 g
Agar	12 g to 18 g ^a
Water	1 000 ml

^a Depending on the gel strength of the agar.

5.3.1.2 Preparation

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

Adjust the pH, if necessary, so that after sterilization it is $7,0 \pm 0,2$ at 25 °C.

Dispense into flasks and sterilize for 15 min at 121 °C.

5.3.2 Defibrinated sheep blood

5.3.2.1 Complete medium

5.3.2.1.1 Composition

Base medium (5.3.1)	100 ml
Defibrinated sheep blood	5 ml to 7 ml

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5.3.2.1.2 Preparation

After cooling to 44 °C to 47 °C, add to the base medium (5.3.1) the defibrinated sheep blood. Mix.

Pour at least 12 ml portions of the complete medium into sterile Petri dishes (6.6) and allow to solidify.

6 Apparatus and glassware

NOTE Disposable apparatus is an acceptable alternative to re-usable glassware if it has similar 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 Drying cabinet or incubator, ventilated by convection, for drying the agar plates, capable of operating between $37\text{ °C} \pm 1\text{ °C}$ and $55\text{ °C} \pm 1\text{ °C}$.

6.3 Incubator, capable of operating at $30\text{ °C} \pm 1\text{ °C}$.

6.4 Water baths, capable of being maintained at 44 °C to 47 °C.

6.5 pH-meter, accurate to within $\pm 0,1$ pH units at 25 °C.

6.6 Petri dishes, made of glass or plastic of diameter 90 mm to 100 mm or, if necessary, 140 mm.

6.7 Graduated pipettes, calibrated for bacteriological use only, of nominal capacities 10 ml and 1 ml, graduated respectively in divisions of 0,5 ml and 0,1 ml, and with an outflow opening of nominal diameter 2 mm to 3 mm.

6.8 Spreaders (hockey-stick type), made of glass or plastic rod of diameter approximately 3,5 mm and length 20 cm, bent at right angles about 3 cm from one end; the cut ends shall be made smooth by heating.

7 Sampling

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

Sampling is not part of the method specified in this International Standard. If there is no specific International Standard dealing with sampling of the product concerned, 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 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.

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9 Procedure

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9.1 Test portion, initial suspension and dilutions

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See ISO 6887-1 and the specific International Standard appropriate to the product concerned.

9.2 Inoculation and incubation

9.2.1 Transfer, by means of a sterile pipette (6.7), 0,1 ml of the test sample if the product is liquid, or of the initial suspension in the case of other products, to each of two agar plates (5.2.5). Repeat the procedure using further decimal dilutions if necessary.

9.2.2 When, for certain products, it is desirable to estimate low numbers of *B. cereus*, the limits of detection may be raised by a factor of 10 by examining 1,0 ml of the test sample if the initial product is liquid, or 1,0 ml of the initial suspension for the other products. Distribute the 1 ml of inoculum either on the surface of a large Petri dish (140 mm) or over the surface of three small dishes (90 mm) using a sterile spreader (6.8). In both cases, prepare duplicates by using two large plates or six small plates.

9.2.3 Carefully spread the inoculum as quickly as possible over the surface of the agar plate without touching the sides of the dish with the spreader (6.8). Use a fresh sterile spreader for each plate. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.

9.2.4 Invert the prepared plates (9.2.3) and incubate them for 18 h to 24 h in an incubator (6.3) set at 30 °C. If colonies are not clearly visible, incubate the plates for an additional 24 h before counting.

9.3 Counting of the colonies

After the period of incubation (9.2.4), select plates, preferably at two successive dilutions, containing less than 150 colonies.