INTERNATIONAL STANDARD

ISO 7932

Third edition
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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

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

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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).

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:

— haemolysis reaction;

— 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.

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 ± 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

5.2.2.2 Preparation

Dissolve the polymyxin B sulfate in the water. Sterilize by filtration.

5.2.3 Egg yolk emulsion

Use fresh hens' eggs with their shells intact. Wash the eggs in liquid detergent using a brush. Rinse under running water, dip in 95 % (by volume) ethanol for 30 s and dry. Using aseptic procedures, break each egg and separate the yolk from the white by repeatedly transferring the yolk from one half of the egg shell to the other. Put the yolks into a sterile measuring cylinder and add four parts by volume of sterile water. Transfer aseptically into a sterile flask and mix vigorously.

Heat the mixture for 2 h in a water bath (6.4) set at 44 °C to 47 °C. Then leave for 18 h to 24 h at 5 °C ± 3 °C to allow a precipitate to form.

Collect the supernatant emulsion aseptically.

The emulsion may be stored at 5 °C ± 3 °C for not longer than 72 h.

5.2.4 Complete medium (MYP agar)

5.2.4.1 Composition

Base medium (5.2.1)	90 ml
Polymyxin B solution (5.2.2)	1,0 ml
Egg yolk emulsion (5.2.3)	10,0 ml

5.2.4.2 Preparation

Melt the base medium and cool it in a water bath (6.4) set at 44 °C to 47 °C.

Add the other liquids, mixing well after each addition.

Cool the complete medium in a water bath (6.4) set at 44 °C to 47 °C.

5.2.5 Preparation of agar plates

Pour 15 ml to 20 ml portions of the complete medium (5.2.4) into sterile Petri dishes (6.6) and allow to solidify.

The plates may be stored prior to drying at between 5 °C ± 3 °C for up to 4 days.

Immediately before use, dry the plates, preferably with the lids off and the agar surface downwards, in a drying cabinet or incubator (6.2) set between 37 °C and 55 °C until the agar surface is dry.

5.2.6 Performance testing

See ISO/TS 11133-2:2003, Annex B.

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 ± 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

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 °C ± 1 °C and 55 °C ± 1 °C.

6.3 Incubator, capable of operating at 30 °C ± 1 °C.

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

6.5 pH-meter, accurate to within ± 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.

9 Procedure

9.1 Test portion, initial suspension and dilutions

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.

Count the presumptive *B. cereus* colonies on each plate. The presumptive colonies are large, pink (indicating that mannitol fermentation has not occurred, see Note 1) and generally surrounded by a zone of precipitation (indicating the production of lecithinase, see Note 2).

If there are less than 15 characteristic colonies on plates inoculated with the liquid product or the lowest dilution for other products, it is possible to make an estimated count as described in Clause 10.

NOTE 1 If the plates contain numerous mannitol-fermenting microorganisms leading to the production of acid, then the characteristic pink colour of *B. cereus* colonies may be reduced or disappear entirely.

NOTE 2 Some strains of *B. cereus* produce only little or no lecithinase. Colonies of these strains will not be surrounded by a precipitation zone. These colonies should also be subjected to confirmation tests.

If a 1,0 ml inoculum was spread over three plates (see 9.2.2), treat these plates as one in all subsequent counting and confirmation procedures.

9.4 Confirmation

9.4.1 Selection and purification of colonies for confirmation

Select five presumptive colonies from each plate selected as in 9.3. If there are less than five colonies on the plate, take all presumptive colonies present. Confirm these colonies as specified in 9.4.2 and 9.4.3.

If the plates are overcrowded and it is not possible to select well-isolated colonies, streak five presumptive colonies on plates with complete medium (5.2.4). Incubate in an incubator (6.3) set at 30 °C for 18 h to 24 h.

Select from each plate at least one well-isolated colony with a pink colour. Confirm this colony as specified in 9.4.2 and 9.4.3.

9.4.2 Haemolysis test on sheep blood agar

Streak, stab or spot the selected colonies (9.4.1) onto the surface of sheep blood agar (5.3) in a manner which allows good interpretation of the haemolysis reaction.

Incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.

9.4.3 Biochemical interpretation

See Table 1.

Table 1 — Results of tests

Test	Result confirming presumptive *Bacillus cereus*
MYP agar (9.4.1)	Formation of pink colonies surrounded by precipitate (see Note 1 in 9.3)
Haemolysis (9.4.2)	Positive reaction [a]
[a] The width of the haemolysis zone may vary.	

10 Expression of results

10.1 Count of presumptive *B. cereus* colonies

See ISO 7218:1996/Amd.1:2001 for calculation.

10.2 No colonies

If the two dishes corresponding to the test sample (liquid products) or the initial suspension (other products) contain no colonies of presumptive *B. cereus*, report the result as follows:

— less than 1 microorganism per millilitre (liquid products);

— less than 1/d microorganism per gram (other products), where d is the dilution factor of the initial suspension.

10.3 Precision

10.3.1 Interlaboratory test

Details of the interlaboratory test on the precision of the method are published (see [7] and [8]) and are summarized in Annex B. Repeatability and reproducibility limits were determined using three types of food contaminated at various levels and reference materials. The values derived from the interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

10.3.2 Repeatability limit

The absolute difference between two independent single ($\log_{10}$-transformed) test results (number of *B. cereus* per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material in the same laboratory by the same operator using the same apparatus within the shortest feasible time interval, will in not more than 5 % of cases exceed the repeatability limit r.

As a general indication of repeatability limit (r), the following values may be used when testing food samples in general:

$r = 0{,}29$ (expressed as a difference between $\log_{10}$-transformed test results), or

$r = 2{,}0$ (expressed as a ratio between test results).

For reference materials (see [4]), the following values may be used:

$r = 0{,}12$ (expressed as a difference between $\log_{10}$-transformed test results), or

$r = 1{,}3$ (expressed as a ratio between test results).

EXAMPLE A first test result of 10 000 or $1{,}0 \times 10^4$ *B. cereus* per gram of food product was observed. Under repeatability conditions, the ratio between the first and second test result should not be greater than 2,0. So the second result should be between 5 000 (= 10 000/2,0) and 20 000 (10 000 × 2,0) *B. cereus* per gram.

10.3.3 Reproducibility limit

The absolute difference between two single ($\log_{10}$-transformed) test results (number of *B. cereus* per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit R.

As a general indication of reproducibility limit (R), the following values may be used when testing food samples in general:

$R = 0{,}42$ (expressed as a difference between $\log_{10}$-transformed test results), or

$R = 2{,}6$ (expressed as a ratio between test results).

For reference materials (see [4]), the following values may be used:

$R = 0{,}23$ (expressed as a difference between $\log_{10}$-transformed test results), or

$R = 1{,}7$ (expressed as a ratio between test results).

EXAMPLE 1 A test result of 10 000 or $1{,}0 \times 10^4$ *B. cereus* per gram of food product was observed from the first laboratory. Under reproducibility conditions, the ratio between the test results from the first and second laboratories should not be greater than 2,6. So the result from the second laboratory should be between 3 800 (= 10 000/2,6) and 26 000 (10 000 × 2,6) *B. cereus* per gram.

EXAMPLE 2 A laboratory wants to know the maximum level it may find that is still in compliance with a pre-set limit (e.g. a limit of 100 000 or $\log_{10}$ 5). For this, the R value (on the log scale) has to be multiplied by a factor of 0,59. This value is 0,25 (0,42 × 0,59) as a difference between $\log_{10}$-transformed test results or 1,78 ($10^{0,25}$) as a ratio between test results. So results up to $\log_{10}$ 5,25 ($\log_{10}$ 5 + $\log_{10}$ 0,25) or 178 000 (100 000 × 1,78) do not indicate non-compliance with the limit. The factor 0,59 reflects the fact that a test with a one-sided 95 % interval is used to test whether the limit is exceeded. The factor 0,59 is obtained from the following formula:

$$0{,}59 = \frac{1{,}64}{1{,}96 \times \sqrt{2}}$$

11 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) the temperature of incubation;

e) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

Annex A
(normative)

Confidence limits for the estimation of small numbers of colonies

The confidence limits at the 95 % level for the estimation of small numbers, when the number of colonies on dishes retained is less than 15, are given in Table A.1.

Table A.1

Number of colonies	Confidence limits at the 95 % level	
	lower	upper
1	< 1	2
2	< 1	4
3	< 1	5
4	1	6
5	2	9
6	2	10
7	2	12
8	3	13
9	4	14
10	4	16
11	5	18
12	6	19
13	7	20
14	7	21
15	8	23

Annex B
(informative)

Results of interlaboratory test

An international collaborative test involving 20 laboratories in 17 countries was carried out on cheese, meat, dried potato powder and a reference material. The food samples were each tested at three different levels of contamination. The test was organized in October 1997 by the National Institute of Public Health (RIVM) as part of the European project SMT4 CT-96 2098 funded by the European Commission.

The method submitted to the interlaboratory trial was that of ISO 7932:1993 including the following confirmation tests: MYP agar medium, glucose agar medium, VP medium and nitrate medium.

In accordance with ISO 5725-1:1994 [5], the following parameters were calculated to give the precision data shown in Tables B.1 to B.4.

Table B.1 — Results of data analysis obtained with dried potato samples

Parameters	Sample (contamination level)		
	Dried potato powder (low level)	Dried potato powder (medium level)	Dried potato powder (high level)
Number of samples	2	2	2
Number of laboratories retained after eliminating outliers	18	18	18
Number of outliers	0	0	0
Number of accepted samples	36	35	36
Mean value $\sum a$ ($\log_{10}$ cfu/g)	3,3	4,7	6,1
Repeatability standard deviation s_r ($\log_{10}$ cfu/g)	0,09	0,05	0,10
Repeatability relative standard deviation (%)	2,63	1,16	1,60
Repeatability limit r:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,24	0,15	0,27
as ratio on normal scale (cfu/g)	1,7	1,4	1,9
Reproducibility standard deviation s_R ($\log_{10}$ cfu/g)	0,11	0,09	0,10
Reproducibility relative standard deviation (%)	3,24	1,98	1,71
Reproducibility limit R:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,30	0,26	0,29
as ratio on normal scale (cfu/g)	2,0	1,8	2,0

Table B.2 — Results of data analysis obtained with minced meat samples

Parameters	Sample (contamination level)		
	Minced meat (low level)	Minced meat (medium level)	Minced meat (high level)
Number of samples	2	2	2
Number of laboratories retained after eliminating outliers	18	18	18
Number of outliers	0	0	0
Number of accepted samples	36	36	36
Mean value $\sum a$ ($\log_{10}$ cfu/g)	3,1	3,9	4,9
Repeatability standard deviation s_r ($\log_{10}$ cfu/g)	0,13	0,14	0,08
Repeatability relative standard deviation (%)	4,15	3,49	1,56
Repeatability limit r:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,36	0,38	0,21
as ratio on normal scale (cfu/g)	2,3	2,4	1,6
Reproducibility standard deviation s_R ($\log_{10}$ cfu/g)	0,15	0,14	0,11
Reproducibility relative standard deviation (%)	4,72	3,49	2,30
Reproducibility limit R:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,41	0,38	0,31
as ratio on normal scale (cfu/g)	2,5	2,4	2,1

Table B.3 — Results of data analysis obtained with fresh cheese samples

Parameters	Sample (contamination level)		
	Fresh cheese (low level)	Fresh cheese (medium level)	Fresh cheese (high level)
Number of samples	2	2	2
Number of laboratories retained after eliminating outliers	12	12	12
Number of outliers	0	0	0
Number of accepted samples	23	23	23
Mean value $\sum a$ ($\log_{10}$ cfu/g)	3,4	4,1	6,2
Repeatability standard deviation s_r ($\log_{10}$ cfu/g)	0,05	0,06	0,12
Repeatability relative standard deviation (%)	1,50	1,57	1,95
Repeatability limit r:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,14	0,18	0,33
as ratio on normal scale (cfu/g)	1,4	1,5	2,2
Reproducibility standard deviation s_R ($\log_{10}$ cfu/g)	0,08	0,10	0,17
Reproducibility relative standard deviation (%)	2,28	2,38	2,78
Reproducibility limit R:			
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,22	0,27	0,48
as ratio on normal scale (cfu/g)	1,6	1,9	3,0

Table B.4 — Results of data analysis obtained with reference material

Parameters	Reference material
Number of samples	2
Number of laboratories retained after eliminating outliers	18
Number of outliers	0
Number of accepted samples	36
Mean value $\sum a$ ($\log_{10}$ cfu/capsule)	3,9
Repeatability standard deviation s_r ($\log_{10}$ cfu/capsule)	0,04
Repeatability relative standard deviation (%)	1,12
Repeatability limit r:	
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/capsule)	0,12
as ratio on normal scale (cfu/capsule)	1,3
Reproducibility standard deviation s_R ($\log_{10}$ cfu/capsule)	0,08
Reproducibility relative standard deviation (%)	2,16
Reproducibility limit R:	
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/capsule)	0,23
as ratio on normal scale (cfu/capsule)	1,7

Bibliography

[1] MOSSEL, D.A.A., KOOPMAN, M.J. and JONGERIUS, E. *Appl. Microbiol.*, **15**, 1967, pp. 650-653

[2] ICMSF. *Microorganisms in foods*, Vol. 1, 2nd edn., University of Toronto Press, 1978

[3] COWELL and MORISETTI *J. Sci. Fd. Agric.*, **20**, 1969, p. 573

[4] IN 'T VELD, P.H., SOENTORO, P.S.S. and NOTERMANS, S.H.W. *I. J. Food Microbiol.*, **20**, 1993, pp. 23-36

[5] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[6] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[7] SCHULTEN, S.M., VAN de LUSTGRAAF, B.E.B., NAGELKERKE, N.J.D. and IN 'T VELD, P.H. *Report No. 286555001*, National Institute of Public Health and the Environment, Bilthoven, The Netherlands, 1998

[8] SCHULTEN, S.M., IN 'T VELD, P.H., NAGELKERKE, N.J.D., SCOTTER, S., DE BUYSER, M.L., ROLLIER, P. and LAHELLEC, C. *I. J. Food Microbiology*, **57**, 2000, pp. 53-61

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