DRAFT AMENDMENT ISO 7932:1993/DAM 1

ISO/TC **34**/SC **9** Secretariat: **AFNOR**

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INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

Microbiology — General guidance for the enumeration of *Bacillus cereus* — Colony-count technique at 30 °C

AMENDMENT 1: Inclusion of precision data and limitation of confirmatory tests

Microbiologie — Directives générales pour le dénombrement de Bacillus cereus *— Méthode par comptage des colonies à 30 °C*

AMENDEMENT 1: Inclusion de données de fidélité et limitation des essais de confirmation

ICS 07.100.01

ISO/CEN PARALLEL ENQUIRY

The CEN Secretary-General has advised the ISO Secretary-General that this ISO/DIS covers a subject of interest to European standardization. **In accordance with subclause 5.1 of the Vienna Agreement, consultation on this ISO/DIS has the same effect for CEN members as would a CEN enquiry on a draft European Standard.** Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month FDIS vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

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

Amendment 1 to International Standard ISO 7932:1993 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

Microbiology — General guidance for the enumeration of *Bacillus cereus* — Colony-count technique at 30 °C

AMENDMENT 1: Inclusion of precision data and limitation of confirmatory tests

Title

Change the title to include the word presumptive :

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

Page 1, change subclause 3.1 to

3.1
Presumptive *Bacillus cereus*
microorganism that forms colonies on the surface of a selective culture medium and which gives positive confirmation reactions under the conditions specified in this International Standard

Page 1, change subclause 4.3 to

Calculation of the number of presumptive *B. cereus* per gram or per millilitre of sample from the number of confirmed colonies obtained on plates at dilutions levels chosen as to give a significant result, and confirmation according to the tests specified.

Page 3, change subclause 5.4 to

5.4 Sheep blood agar

5.4.1 Base medium

5.4.1.1 Composition

Sheep blood agar base n° 2 :	
proteose peptone or equivalent peptone	15 g
liver hydrolysat	2,5 g
yeast extract	5 g
sodium chloride (NaCl)	5 g
agar	12 to 18 g[a]
water	1 000 ml
[a] Depending on the gel strength of the agar.	

5.4.1.2 Preparation

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

Adjust the pH, if necessary, so that after sterilisation it is 7,0 ± 0,2 at 25 °C.

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

5.4.2 Sheep blood without fibrin

5.4.3 Complete medium

5.4.3.1 Composition

Base medium (5.4.1)	100 ml
Sheep blood without fibrin	5 to 7 ml

5.4.3.2 Preparation

After cooling at 44°C to 47°C, add to the base medium (5.4.1) the sheep blood without fibrin. Mix.

Pour 12 ml to 20 ml portions of the complete medium (5.4.3) into sterile Petri dishes (6.8) and allow to solidify.

Pages 3 and 4, delete subclauses 5.5, 5.6, 5.7, 5.8 and 5.9

This subclause should be deleted since the confirmation tests are replace by haemolysis test on sheep blood agar.

Pages 4, delete subclauses 6.5, 6.7 and 6.11

Pages 6, delete subclauses 9.4.2, 9.4.3, 9.4.4 and change to

9.4.2 Haemolysis test on sheep blood agar

Streak the selected colonies (9.4.1) onto the surface of sheep blood agar (5.4) in a manner which allows well-separated colonies to develop.

Incubate at 30°C for 24h and read haemolysis reaction.

Pages 6, change subclause 9.4.5 to

9.4.3 Biochemical interpretation

See table 1.

Table 1 — Result of tests

Tests	Result confirming presumptive *Bacillus cereus*
MYP agar (9.4.1)	Formation of pink colonies surrounded by precipitate (see note in 9.3)
Haemolysis (9.4.2)	positive reaction

Pages 6 and 7, subclause 10

See ISO 7218/A1 for calculation.

B.cereus has to be change to "presumptive *B. cereus*".

Page 7, change subclause 10.3.1 to

10.3.1 Precision data

10.3.1.1 General

Details of the interlaboratory test of the method are published (see references [6] and [7] in annex B) and are summarized in annex C. Repeatability and reproducibility values were determined using three types of food contaminated at various levels and for reference materials. These values are presented in annex C. All values were calculated without the use of the Voges-Proskauer (VP) test results. (The values derived from the interlaboratory test may not be applicable to other analyte concentration ranges and matrices than given in annex C.)

10.3.1.2 Repeatablility

The absolute difference between two single ($\log_{10}$-transformed) test results (number of B. cereus per gram or ml) or the absolute ratio between two test results on the normal scale found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value r in not more than 5 % of the cases.

As a general indication of repeatability (r) the following values can 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 reference [4] in annex B) the following values can be used :

r = 0,11 (expressed as a difference between $\log_{10}$-transformed test results) or

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

For example : A first test result of 10 000 or 1,0 x 10^4 presumptive B. cereus per gram food product was observed. Under repeatability conditions the difference 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 x 2,0) presumptive B. cereus per gram.

10.3.1.3 Reproducibility

The absolute difference between two single ($\log_{10}$-transformed) test results (number of B. cereus per gram or ml) or the absolute ratio between two test results on the normal scale found on identical test material reported by two laboratories will exceed the repeatability value R in not more than 5 % of the cases.

As a general indication of reproducibility (R) the following values can 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 reference [4] in annex B) the following values can be used :

R = 0,22 (expressed as a difference between $\log_{10}$-transformed test results) or

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

For example : A first laboratories test result of 10 000 or 1,0 x 10^{+4} presumptive B. cereus per gram food product was observed. Under reproducibility conditions the difference between the first and second laboratories test result should not be greater than 2,6. So the second laboratories result should be between 3 800 (= 10 000/2,6) and 26 000 (10 000 x 2,6) presumptive B. cereus per gram.

Secondly a laboratory wants to know the maximum level it may find that is still in compliance with a pre-set limit (for example 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 x 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 x 1,78) do not indicate non-compliance with the limit.

Page 9, annex B

Add reference [4], [5], [6] and [7] :

[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] 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.

[7] Schulten, S.M., in 't Veld, P.H., Nagelkerke, N.J.D., Scotter, S., de Buyser, M.L. Rollier, P. and Lahellec, C. Submitted for publication to. I.J. Food Microbiol

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Add the following annex after annex B :

Annex C
(informative)

Results of interlaboratory tests

C.1 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 organised in October 1997 by National Institute of Public Health (RIVM) in the frame of the European project SMT4 CT-96 2098 coordinated by Dr C. Lahellec (AFSSA) and funded by the European Commission.

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

Table C.1 - Results of data analysis obtained with dried potato samples

Sample	dried potato powder (low level)	dried potato powder (medium level)	dried potato powder (high level)
Year of inter-laboratory test	1997	1997	1997
Number of laboratories with valid results	18	18	18
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 RSD_r	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 RSD_R	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