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Microbiology of food and animal feeding stuffs – Horizontal method for the determination of low numbers of presumptive Bacillus cereus – Most probable number technique and detection method (ISO 21871:2006)

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The European Standard EN ISO 21871:2006 has the status of a Swedish Standard. This document contains the official English version of EN ISO 21871:2006.

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English Version

Microbiology of food and animal feeding stuffs - Horizontal method for the determination of low numbers of presumptive Bacillus cereus - Most probable number technique and detection method (ISO 21871:2006)

Microbiologie des aliments - Méthode horizontale pour le dénombrement de Bacillus cereus présumés en petit nombre - Technique du nombre le plus probable et méthode de recherche (ISO 21871:2006)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zur Bestimmung niedriger Zahlen von präsumtivem Bacillus cereus - Verfahren der wahrscheinlichsten Keimzahl (MPN) und Nachweisverfahren (ISO 21871:2006)

This European Standard was approved by CEN on 28 November 2005.

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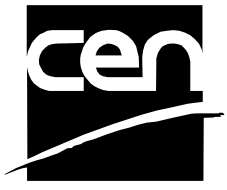

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Foreword

This document (EN ISO 21871:2006) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2006, and conflicting national standards shall be withdrawn at the latest by July 2006.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 21871:2006 has been approved by CEN as EN ISO 21871:2006 without any modifications.

Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method 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 this horizontal method. 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 this horizontal method will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the determination of low numbers of presumptive *Bacillus cereus* — Most probable number technique and detection method

1 Scope

This International Standard specifies a horizontal method for the detection or the enumeration of low numbers of viable presumptive *Bacillus cereus* by means of the most probable number technique. This International Standard 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.

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 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2, *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 or atypical colonies on the surface of selective culture media and which gives positive confirmation reactions under the conditions specified in this International Standard

NOTE For the purpose of a practical test method, this definition of presumptive *Bacillus cereus*, used as a basis for the procedure, does not exclusively describe strains of *Bacillus cereus*. In particular, the confirmatory test is inadequate to distinguish between *Bacillus cereus* and other closely related but less commonly encountered *Bacillus* species such as *Bacillus weihenstephanensis*, *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus pseudomycoides*.

4 Principle

4.1 Enumeration method

4.1.1 Inoculation of three tubes of double-strength liquid selective enrichment medium [5.3.1.1 a)] with a specified quantity of the primary dilution (initial suspension).

4.1.2 Inoculation of three tubes of single-strength liquid selective enrichment medium [5.3.1.1 b)] with a specified quantity of the primary dilution (initial suspension). Then, under the same conditions, inoculation of single-strength liquid selective enrichment medium [5.3.1.1 b)] with specified quantities of decimal dilutions of the primary dilution (initial suspension).

4.1.3 Incubation of the tubes of double and single-strength liquid selective enrichment medium (5.3) for 48 h at 30 °C.

4.1.4 Inoculation of the solid selective medium (5.4 or 5.5) from the liquid selective enrichment medium (5.3).

4.1.5 Incubation of the solid selective medium (5.4 or 5.5) for 18 h to 48 h at 37 °C (5.4) or 30 °C (5.5) and examination of the plates to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Bacillus cereus*.

4.1.6 Confirmation of suspected colonies by means of haemolysis (9.1.5.3) or by microscopic examination (9.1.5.4).

4.1.7 Calculation of the most probable number of presumptive *Bacillus cereus* per gram or per millilitre of sample from selected dilutions by reference to most probable number tables.

4.2 Detection method

4.2.1 Inoculation of a liquid selective enrichment medium (5.3) with a specified quantity of the initial suspension of the test sample.

4.2.2 Incubation of the tube for 48 h at 30 °C.

4.2.3 Inoculation of a solid selective medium (5.4 or 5.5) from the liquid selective enrichment medium (5.3).

4.2.4 Incubation of the solid selective medium (5.4 or 5.5) for 18 h to 48 h at 37 °C (5.4) or 30 °C (5.5) and examination of the plates to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Bacillus cereus*.

4.2.5 Confirmation of suspected colonies by means of haemolysis (9.1.5.3) or by microscopic examination (9.1.5.4).

4.2.6 The results are given as the “presence” or “absence” of presumptive *Bacillus cereus* in grams or millilitres of product.

5 Diluent, culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218, ISO/TS 11133-1 and ISO/TS 11133-2.

5.2 Diluent

See ISO 6887 (all parts), ISO 8261 and any specific standard dealing with the product to be examined.