

**Loomasööt. Eeldatava Bacillus spp. isoleerimine ja loendamine**

**Animal feeding stuffs - Isolation and enumeration of presumptive Bacillus spp.**

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EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

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English Version

**Animal feeding stuffs - Isolation and enumeration of presumptive  
Bacillus spp.**

Aliments des animaux - Isolement et dénombrement des  
souches probiotiques de *Bacillus* spp.

Futtermittel - Keimzählung von *Bacillus* spp.

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## Foreword

This document (EN 15784:2009) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.

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## Introduction

This methodology has been developed to enumerate and differentiate probiotic bacilli spores capable of germinating, to enable the European Commission to control proper labelling of animal feeding products (EU project SMT4-CT98-2235 - “Methods for the official control of probiotics (microorganisms) used in animals feeds”) [1]. Vegetative cells are not taken into account in this method, as all approved *Bacillus* species products at present are spores.

Spores of *Bacillus* species survive a heat-treatment at 80 °C for 10 minutes and the *Bacillus* species characteristic colony morphology of the individually authorised strains is examined using the proposed method [2].

This method is not selective for probiotic bacilli but can be applied to enumerate bacilli in additives, premixtures and feeding stuffs assuming that the probiotic bacilli are present in far higher numbers than any other bacilli.

If the feeding stuffs are “contaminated” with a high level of non-probiotic *Bacillus* species it can be recommended to use a procedure based on antibiotics for more specific selective counting, taking the antibiotic resistance profile of the different *Bacillus* strains into account.

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## 1 Scope

This European Standard defines general rules for the enumeration of probiotic bacilli in feeds containing bacilli (*Bacillus* species) as a single microorganism, component or mixed with other microorganisms. This method is not applicable to mineral feeds which are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40% crude ash (Council Directive 79/373/EEC) [3].

There are different categories of feed samples:

- a) Additives containing about  $10^{10}$  colony forming units (CFU)/g;
- b) Premixtures containing about  $10^8$  CFU/g;
- c) Feeds, meal or pellets, which contain about  $10^6$  CFU/g and include complete feeding stuffs, and milk replacers.

The detection limits are 500 ( $5 \times 10^2$ ) colony forming units per gram (CFU/g). The limits of determination are  $2 \times 10^4$  CFU/g.

## 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.

EN 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* (ISO 6887-1:1999)

EN ISO 7218, *Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations* (ISO 7218:2007)

ISO 6498, *Animal feeding stuffs – Preparation of test samples*

## 3 Terms and definitions

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

### 3.1

#### **bacilli (described by their characteristics as used for this standard)**

bacteria of the genus *Bacillus* which form colonies fitting the descriptions of these species, on the surface of Tryptone Soy Agar (TSA) after heat treatment and incubation at 37°C for 16 h to 24 h under aerobic conditions

Morphology of colonies on TSA of four *Bacillus* species:

- a) *Bacillus subtilis*: 3 mm to 8 mm in diameter, round, surface dull, opaque, wrinkled and cream or brown coloured;
- b) *Bacillus cereus* and *Bacillus coagulans*: 3 mm to 8 mm in diameter, dull or of frosted glass appearance and undulate shaped;