SLOVENSKI STANDARD
SIST EN 15788:2009

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Krma - Izolacija in štetje domnevno prisotnih Enterococcus (E. faecium) spp

Animal feeding stuffs - Isolation and enumeration of Enterococcus (E. faecium) spp

Futtermittel - Keimzählung von Enterococcus (E. faecium) spp

Aliments des animaux - Isolement et dénombrement de l'Entérocoque (E. faecium) spp

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English Version

Animal feeding stuffs - Isolation and enumeration of Enterococcus (E. faecium) spp.

Aliments des animaux - Isolement et dénombrement de l'Entérocoque (E. faecium) spp.

Futtermittel - Keimzählung von Enterococcus spp. (E. faecium)

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Foreword

This document (EN 15788:2009) 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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.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 enterococci (*E. faecium*) 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 as animal feeds”) [1]. The method is based on an extensive screening of 12 pre-selected, commercially available media for the detection and enumeration of enterococci. The described methodology was validated in an interlaboratory study [2].

This method is not selective for probiotic enterococci (*E. faecium*) but can be applied to enumerate enterococci in additives, premixtures and feeding stuffs assuming that the probiotic enterococci (*E. faecium*) is present in far higher numbers than any other enterococci.

1 Scope

This European Standard defines general rules for the enumeration of enterococci in feed samples (additives, premixtures and feeding stuffs) that contain enterococci (*E. faecium*) as a single microorganism component or in a mixture with other microorganisms. This standard is not applicable to mineral feeds which are defined as complementary feedingstuffs 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 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 limit is as defined in EN ISO 7218.

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
enterococcus faecium **(described by their characteristics as used for this standard)**

enterococcus faecium is charaterised as a bacterium which forms colonies fitting the description of the species on the specified selective medium after incubation of 24 h at a temperature of 37 °C under aerobic conditions:

a) morphology of colonies on selective medium;

b) circular;

c) convex to dome-shaped;

d) entire;

e) white;

f) glistening surface;

g) opaque.

Colony size varies between 1 mm and 2 mm in diameter

The medium surrounding the colonies shows a dark brown to black coloration, due to the hydrolysis of esculin

Phase contrast microscopical examination of selected colonies typically shows spherical cells arranged in pairs.

4 Principle

An initial suspension of the sample is prepared in a diluent with suitable buffer capacity using a suitable homogeniser. Dilutions of the initial suspension have to be immediately prepared before the suspension settles. Spread plates (Bile Esculin Azide Agar) are inoculated with the chosen dilutions. The plates are incubated aerobically for 24 h ± 2 h at 37 °C ± 1 °C.

Presumptive enterococci (*E. faecium*) colonies are counted and the number of colony forming units per g or kg is calculated.

NOTE To verify the colony count, a phenotypic characterisation and confirmation of a selection of colonies may be done by means of an identification kit.

5 Diluents, selective medium and test kit for phenotypic characterisation

5.1 Diluents

5.1.1 Diluent for initial suspension of premixtures, additives and feeding stuffs

This diluent is used to decimally dilute the sample to prepare an initial decimally diluted sample suspension (10^{-1}) in appropriate containers (e.g. universals, bottles or flasks).

Phosphate buffered saline (PBS):

Dissolve 8 g sodium chloride, 0,2 g potassium chloride, 1,15 g disodium hydrogen phosphate, 0,2 g potassium dihydrogen phosphate, pH 7,3 ± 0,2 in 1 l of distilled water. Aliquote this saline into appropriate containers (e.g. universals, bottles or flasks). Autoclave all capped containers with the initial diluent at 121 °C ± 1 °C for 10 min. To avoid loss during autoclaving, screw cap bottles are recommended.

Bring the diluent to room temperature before use.

Measure the pH of the diluent to ensure the suitable buffer capacity.

5.1.2 Diluent for serial dilutions

This diluent is used to decimally dilute the initial sample suspension and subsequent dilutions.

Peptone salt solution:

A peptone salt solution is made complying with EN ISO 6887-1.

Compose the solution of enzymatic digest of 1 g casein such as pancreatic peptone of casein (or peptone of same quality) and 8,5 g sodium chloride) per liter (l) distilled water. Dissolve the ingredients in water. Adjust the pH to 7,0 ± 0,2 at 25 °C ± 1 °C. For decimal dilutions, prepare test tubes containing 9,0 ml ± 0,1 ml after sterilisation or use screw cap bottles to avoid weight loss during autoclaving.

Sterilise in the autoclave for 15 min at 121 °C ± 1 °C. Bring the diluent to room temperature before use.

5.2 Selective medium

Bile Esculin Azide Agar[1)] is used as a selective medium.

Composition in g/l final medium:

a)	Peptone 1 (pancreatic digest of casein)	17,0 g
b)	Peptone 2 (peptic digest of meat)	3,0 g
c)	Yeast extract	5,0 g
d)	Ox bile (dehydrated)	10,0 g
e)	Sodium chloride	5,0 g
f)	Esculin	1,0 g
g)	Ferric ammonium citrate	0,5 g
h)	Sodium azide	0,25 g
i)	Agar	13,5 g

Final pH 7,1 ± 0,2.

5.3 Phenotypic characterization and confirmation

Check selected colonies microscopically for enterococci-like morphology.

NOTE A phenotypic kit may be used for phenotypic characterisation of isolated colonies if required.

6 Apparatus and glassware

Usual microbiological laboratory equipment and, in particular, the following:

6.1 Equipment for dry sterilisation (oven) and wet sterilisation (autoclave)

According to EN ISO 7218.

6.2 Incubator

Capable of maintaining a temperature of 37 °C ± 1 °C.

1) the agar is commercially available from various suppliers.