

Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of Enterococcus (*E. faecium*) spp. used as feed additive

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN 15788:2021 sisaldab Euroopa standardi EN 15788:2021 ingliskeelset teksti.	This Estonian standard EVS-EN 15788:2021 consists of the English text of the European standard EN 15788:2021.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 24.11.2021.	Date of Availability of the European standard is 24.11.2021.
Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 65.120

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardimis- ja Akrediteerimiskeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardimis- ja Akrediteerimiskeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autoriõiguse kaitse kohta, võtke palun ühendust Eesti Standardimis- ja Akrediteerimiskeskusega: Koduleht www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation and Accreditation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation and Accreditation.

If you have any questions about standards copyright protection, please contact the Estonian Centre for Standardisation and Accreditation: Homepage www.evs.ee; phone +372 605 5050; e-mail info@evs.ee

English Version

Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of Enterococcus (*E. faecium*) spp. used as feed additive

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Détection et dénombrement des souches de *Enterococcus* (*E. faecium*) spp. utilisées comme additifs pour l'alimentation animale

Futtermittel: Probenahme- und Untersuchungsverfahren - Nachweis und Zählung von *Enterococcus* spp. (*E. faecium*) als Futtermittelzusatzstoff

This European Standard was approved by CEN on 2 August 2021.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Contents	Page
European foreword	3
Introduction	5
1 Scope	6
2 Normative references	6
3 Terms and definitions	6
4 Principle	6
5 Diluents and culture media	7
5.1 Diluents	7
5.2 Culture media	8
6 Apparatus	10
7 Sampling	11
8 Preparation of the test sample	11
9 Procedure	11
9.1 Preparation of poured agar plates for spread plate method	11
9.2 Preparation of culture media for pour plate method	12
9.3 Preparation of the initial suspension and decimal dilutions	12
9.4 Inoculation and incubation of the plates	13
9.5 Enumeration of colonies	14
9.6 Confirmation	14
10 Expression of results	15
11 Precision	15
11.1 General	15
11.2 Interlaboratory studies	15
11.3 Repeatability	16
11.4 Reproducibility	16
12 Test report	16
Annex A (informative) Notes on the procedure	17
A.1 General	17
A.2 Critical copper concentration	17
Annex B (informative) Results of the interlaboratory studies	18
B.1 Interlaboratory study based on bile aesculin azide agar	18
B.2 Interlaboratory study based on Slanetz and Bartley agar	18
Bibliography	20

European foreword

This document (EN 15788:2021) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, 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 May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 15788:2009.

The main changes compared to the previous edition are as follows:

- Amendment of the title;
- Extension of the scope of application to all *Enterococci* used as feed additive;
- Updating of normative cross references;
- Supplement of phosphate buffered saline with Tween® 80;
- Addition of the option to use Tween® 80 supplemented phosphate buffered saline for the preparation of the initial suspension as well as diluent for serial dilutions;
- Addition of Slanetz and Bartley agar as selective detection medium;
- Replacement of the required laboratory mixer with a rotation speed of 18 000 min⁻¹ to 22 000 min⁻¹ by homogenization devices, for example according to EN ISO 7218, with a maximal requested rotation speed of 10 000 min⁻¹;
- Unification of the homogenization time for the preparation of initial suspensions to five minutes for all feed matrices;
- Addition of the option to use a spiral plater for plating;
- Preparation of initial suspensions generally conducted with tempered tPBS;
- Addition of the pour plate method as an alternative cultivation technique;
- Addition of a procedure for the investigation of feeding stuffs containing high amounts of copper in the informative Annex A;
- Addition of validation data derived from VDLUFA ring trials of different feeding stuff matrices using Slanetz and Bartley agar as enumeration media;
- Adjustment of the range of accepted colony numbers for counting from '≥ 30 to ≤ 350' to '≥ 10 to ≤ 200' colonies per plate.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

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

This document is a preview generated by EVS

Introduction

This methodology has been developed to enumerate enterococci (*E. faecium*) as feed additives to enable the European Commission to control proper labelling of animal feeding products. It was compiled first during the EU project SMT4-CT98-2235 “Methods for the official control of probiotics used as feed additives” [1]. It was amended with a second medium from VDLUFA method 28.2.3 “Enumeration of *Enterococcus faecium*” [2]. The method is based on an extensive screening of 12 pre-selected, commercially available media for the detection and enumeration of enterococci. The specified methodology was validated in interlaboratory studies for *E. faecium* ([1], [2], [3]). It can be assumed that the method is also suitable for other *Enterococcus* spp.

This method is not selective for enterococci (*E. faecium*) used as feed additives, but can be applied to enumerate *Enterococcus* spp. in additives, premixtures and compound feeds assuming that the added enterococci (*E. faecium*) are present in far higher numbers than any other enterococci.

This method is not applicable for the detection of any ubiquitous or faecal contaminants of *Enterococcus* spp. in food and animal feeding stuffs.

1 Scope

This document specifies general rules for the enumeration of enterococci (*E. faecium*) in feeding stuffs (additives, premixtures and compound feeds excluding mineral feeds) that contain enterococci as a single microorganism component or in a mixture with other microorganisms. Applying the method to premixtures and compound feeds with critical amounts of copper demands a special procedure (see A.2). The document is not applicable to mineral feeds which are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40 % crude ash (Regulation (EC) 767/2009) [4].

There are different categories of feed samples:

- a) Additives containing about 10^{10} colony forming units (CFU)/g;
- b) Premixtures containing 10^{11} CFU/kg;
- c) Compound feeds, meal or pellets which contain about 10^9 CFU/kg.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

Enterococci

Gram-positive, catalase negative cocci, which usually occurs in pairs or short chains

Note 1 to entry: This description is based on their characteristics as used for this document.

Note 2 to entry: *Enterococci* are classified as aerotolerant anaerobes with the ability to reduce 2,3,5-triphenyl tetrazolium chloride to formazan and capable of hydrolyzing aesculin at $44\text{ °C} \pm 0,5\text{ °C}$. *Enterococci* form colonies fitting the description of this species on the specified culture media after incubation at a temperature of 37 °C under aerobic conditions for 24 h resp. 48 h (see 9.6).

4 Principle

- a) Preparation of sterile and dry poured agar plates or preparation of sterile liquid culture media tempered at 44 °C to 47 °C ;
- b) Drawing a representative test sample under aseptic conditions;
- c) Preparation of the initial suspension with a tempered diluent to obtain a homogeneous distribution of bacterial cells from the test portion;