

TOIDUAHELA MIKROBIOLOOGIA.
HORISONTAALMEETOD KOAGULAASPOSITIIVSETE
STAFÜLOKOKKIDE (STAPHYLOCOCCUS AUREUS JA
TEISED LIIGID) LOENDAMISEKS. OSA 2:
KÜÜLIKUPLASMA-FIBRINOGEENAGARSÖÖTME
KASUTAMISE MEETOD

Microbiology of the food chain - Horizontal method for
the enumeration of coagulase-positive staphylococci
(Staphylococcus aureus and other species) - Part 2:
Method using rabbit plasma fibrinogen agar medium
(ISO 6888-2:2021)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 6888-2:2021 sisaldab Euroopa standardi EN ISO 6888-2:2021 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 6888-2:2021 consists of the English text of the European standard EN ISO 6888-2:2021.
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 08.09.2021.	Date of Availability of the European standard is 08.09.2021.
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 07.100.30

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

Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 2: Method using rabbit plasma fibrinogen agar medium (ISO 6888-2:2021)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (Staphylococcus aureus et autres espèces) - Partie 2 : Méthode utilisant le milieu gélosé au plasma de lapin et au fibrinogène (ISO 6888-2:2021)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren für die Zählung von koagulase-positiven Staphylokokken (Staphylococcus aureus und andere Spezies) - Teil 2: Verfahren mit Kaninchenplasma/Fibrinogen-Agar (ISO 6888-2:2021)

This European Standard was approved by CEN on 27 April 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

European foreword

This document (EN ISO 6888-2:2021) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 "Microbiology of the food chain" the secretariat of which is held by AFNOR.

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 2022, and conflicting national standards shall be withdrawn at the latest by March 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 ISO 6888-2:1999.

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

According to the CEN-CENELEC Internal Regulations, the national standards organizations 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.

Endorsement notice

The text of ISO 6888-2:2021 has been approved by CEN as EN ISO 6888-2:2021 without any modification.

Contents

	Page
Foreword.....	iv
Introduction.....	vi
1 Scope.....	1
2 Normative references.....	2
3 Terms and definitions.....	2
4 Principle.....	2
4.1 General.....	2
4.2 Incubation.....	2
4.3 Enumeration.....	3
5 Culture media and reagents.....	3
6 Equipment and consumables.....	3
7 Sampling.....	4
8 Preparation of the test sample.....	4
9 Procedure (see Figure A.1).....	4
9.1 Test portion, initial suspension and dilutions.....	4
9.2 Inoculation and incubation.....	4
9.3 Counting of colonies.....	5
9.3.1 General description of colonies growing on RPFA medium.....	5
9.3.2 Colony counting procedure.....	5
10 Expression of results.....	5
11 Performance characteristics of the method.....	5
11.1 Interlaboratory study.....	5
11.2 Repeatability limit.....	5
11.3 Reproducibility limit.....	6
12 Test report.....	7
13 Quality assurance.....	7
Annex A (normative) Flow diagram of the procedure.....	8
Annex B (normative) Culture media and reagents.....	9
Annex C (informative) Results of the interlaboratory study.....	12
Bibliography.....	14

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 6888-2:1999), which has been technically revised. It also incorporates the Amendment ISO 6888-2:1999/Amd 1:2003. The main changes compared with the previous edition are as follows:

- the title has been changed to relate to the “food chain”;
- the status of ISO 6888-1 and this document has been clarified;
- the document has been aligned with ISO 7218:2007, i.e. and pour molten agar medium at 44 °C to 47 °C;
- all occurrences, when appropriate, have been changed from “35 °C or 37 °C” to “34 °C to 38 °C”;
- all occurrences of incubation time, when appropriate, have been changed from “18 h to 24 h” to “24 h ± 2 h”;
- requirements have been added to use ISO 11133;
- all available standards related to sampling techniques have been updated;
- flow diagram procedure in [Annex A](#) has been updated;
- culture media and reagents with performance testing have been added and moved to [Annex B](#);
- performance testing for rabbit plasma fibrinogen agar (RPFA) medium has been added;
- results of the interlaboratory study (from ISO 6888-2:1999/Amd 1:2003 Precision data) have been updated;

— the Bibliography has been updated.

A list of all parts in the ISO 6888 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

ISO 6888-1, this document and ISO 6888-3 describe three horizontal methods for the detection and enumeration of coagulase-positive staphylococci among which enterotoxinogenic strains are encountered. It is mainly concerned with *Staphylococcus aureus*, but also with *S. intermedius* and certain strains of *S. hyicus*.

For the purposes of this document, the characterization of staphylococci is based on a positive coagulase reaction, but it is recognized that some strains of *Staphylococcus aureus* give weakly positive coagulase reactions. These latter strains can be confused with other bacteria but they can be distinguished by the use of additional tests not included in this document, such as tests for sensitivity to lysostaphin, and for production of haemolysin, thermostable nuclease and acid from mannitol (see ISO 7218 and Reference [13]).

The main technical changes listed in the Foreword, introduced in this document compared with the previous edition, are considered as minor (see ISO 17468). They have a minor impact on the performance characteristics of the method.

The results of the interlaboratory study and samples tested are described in [Annex C](#).

Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) —

Part 2: Method using rabbit plasma fibrinogen agar medium

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for enumerating staphylococci are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document specifies a horizontal method for the enumeration of coagulase-positive staphylococci by counting the colonies obtained on a solid medium (rabbit plasma fibrinogen agar medium) after aerobic incubation at 34 °C to 38 °C (see Reference [10]).

This document is applicable to:

- products intended for human consumption;
- products intended for animal feeding;
- environmental samples in the area of food and feed production and handling;
- samples from the primary production stage.

This horizontal method was originally developed for the examination of all samples belonging to the food chain.

Because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.

Based on the information available at the time of publication of this document, this method is not considered to be (fully) suited to the examination of fermented products or other products containing technological flora based on *Staphylococcus* spp. (e.g. *S. xylosus*) (such as cheeses made from raw milk and certain raw meat products) likely to be contaminated by:

- staphylococci forming atypical colonies on a Baird-Parker agar medium;
- background flora that can obscure the colonies being sought.

Nevertheless, both ISO 6888-1 and this document are given equivalent status.

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.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

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 <http://www.electropedia.org/>

3.1
coagulase-positive staphylococci
bacteria that form typical colonies in a selective culture medium (rabbit plasma fibrinogen agar medium)

Note 1 to entry: The typical colonies are described in 9.3.

3.2
enumeration of coagulase-positive staphylococci
determination of the number of *coagulase-positive staphylococci* (3.1) per gram, per millilitre, per square centimetre or per sampling device/sampled area.

Note 1 to entry: A sampled area is an area not defined by a numerical size, for example, a hot tap, a door handle.

4 Principle

4.1 General

Preparation of poured plate of the rabbit plasma fibrinogen agar medium, with a specified quantity of the test sample if the product is liquid or with a specified quantity of the initial suspension in the case of other products.

Inoculation, under the same conditions, using decimal dilutions of the test sample, follow the procedure(s) in accordance with ISO 7218.

NOTE The volume of rabbit plasma fibrinogen agar medium to be added to the inoculum is a critical point for the coagulase method and reaction.

4.2 Incubation

Aerobic incubation of the plates at 34 °C to 38 °C and examination after 24 h and, if necessary, after 48 h.