

TOIDUAHELA MIKROBIOLOOGIA.
HORISONTAALMEETOD KOAGULAASPOSITIIVSETE
STAFÜLOKOKKIDE (STAPHYLOCOCCUS AUREUS JA
TEISED LIIGID) LOENDAMISEKS. OSA 1:
BAIRD-PARKERI AGARSÖÖTME KASUTAMISE MEETOD

Microbiology of the food chain - Horizontal method for
the enumeration of coagulase-positive staphylococci
(Staphylococcus aureus and other species) - Part 1:
Method using Baird-Parker agar medium (ISO
6888-1:2021)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 6888-1:2021 sisaldab Euroopa standardi EN ISO 6888-1:2021 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 6888-1:2021 consists of the English text of the European standard EN ISO 6888-1:2021.
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.
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English Version

Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Method using Baird-Parker agar medium (ISO 6888-1:2021)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (Staphylococcus aureus et autres espèces) - Partie 1: Méthode utilisant le milieu gélosé de Baird-Parker (ISO 6888-1:2021)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren für die Zählung von koagulase-positiven Staphylokokken (Staphylococcus aureus und andere Spezies) - Teil 1: Verfahren mit Baird-Parker-Agar (ISO 6888-1:2021)

This European Standard was approved by CEN on 27 April 2021.

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COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN ISO 6888-1:2021) 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of an identical text or by endorsement, at the latest by March 2022, and conflicting national standards shall be withdrawn at the latest by March 2022.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 6888-1:1999.

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 6888-1:2021 has been approved by CEN as EN ISO 6888-1:2021 without any modification.

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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).

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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 6888-1:1999), which has been technically revised. It also incorporates the amendments ISO 6888-1:1999/Amd 1:2003 and ISO 6888-1:1999/Amd 2:2018. 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 this document and ISO 6888-2 has been clarified;
- the document has been aligned with ISO 7218:2007, i.e. 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;
- a description of typical and atypical colonies on Baird-Parker agar (BPA) medium has been updated;
- the rabbit plasma fibrinogen agar (RPFA) medium has been added as an alternative to the coagulase test for confirmation;
- the flow diagram procedure in [Annex A](#) has been updated;
- culture media and reagents with performance testing in [Annex B](#) have been added;

- results of the interlaboratory study (from ISO 6888-1:1999/Amd 1:2003, Precision data) has 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

This document, ISO 6888-2 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 confirmation of typical and atypical colonies 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 production of haemolysin, thermostable nuclease and acid from mannitol (see ISO 7218 and Reference [15]).

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 this method.

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 1: Method using Baird-Parker agar medium

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting 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 (Baird-Parker medium)^[10] after aerobic incubation at 34 °C to 38 °C and coagulase confirmation.

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, handling, and
- 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 this document and ISO 6888-2 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 either typical or atypical colonies, or both, on the surface of a selective culture medium (Baird-Parker agar medium) and that show a positive coagulase reaction in a tube test or on rabbit plasma fibrinogen agar

Note 1 to entry: The typical and atypical colonies are described in [9.3.1](#).

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

Inoculation of the surface of a solid selective culture 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.

4.2 Incubation

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

4.3 Enumeration and confirmation

Calculation of the number of coagulase-positive staphylococci per gram, per millilitre, per square centimetre or per sampling device of sample from the number of either typical or atypical colonies, or both, obtained on plates at dilution levels chosen to give a significant result, and confirmed by a positive coagulase test result, within the counting limits of the method and in accordance with ISO 7218.

NOTE See [Annex A](#) for a flow diagram.