SLOVENSKI STANDARD
oSIST prEN ISO 6888-2:2020

01-maj-2020

Mikrobiologija v prehranski verigi - Horizontalna metoda za štetje koagulazno pozitivnih stafilokokov (Staphylococcus aureus in drugih vrst) - 2. del: Tehnika uporabe agarja z zajčjo plazmo iz fibrinogenov (ISO/DIS 6888-2:2020)

Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 2: Technique using rabbit plasma fibrinogen agar medium (ISO/DIS 6888-2:2020)

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/DIS 6888-2:2020)

Microbiologie des aliments - Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (Staphylococcus aureus et autres espèces) - Partie 2: Technique utilisant le milieu gélosé au plasma de lapin et au fibrinogène (ISO/DIS 6888-2:2020)

Ta slovenski standard je istoveten z: **prEN ISO 6888-2**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 6888-2:2020 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 6888-2

ISO/TC **34**/SC **9**

Secretariat: **AFNOR**

Voting begins on:
2020-03-09

Voting terminates on:
2020-06-01

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

Part 2:
Technique using rabbit plasma fibrinogen agar medium

Microbiologie des aliments — Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (Staphylococcus aureus et autres espèces) —

Partie 2: Technique utilisant le milieu gélosé au plasma de lapin et au fibrinogène

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 6888-2:2020(E)

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Published in Switzerland

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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).

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 on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food Products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 6888-2:1999), which has been technically revised.

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

— Title relates to “Food chain”;

— Precision on the status of ISO 6888 part 1 and part 2;

— Consistency with ISO 7218 (2007, Amdt 1 2013): one plate per dilution and pour molten agar medium at 44 °C to 47 °C;

— All occurrences when appropriate; “35 °C or 37 °C” to “34 °C to 38 °C”;

— Normative reference ISO 11133;

— All available standards related to sampling techniques;

— Precision data;

— Flow diagram procedure in Annex A;

— Culture media and reagent with performance testing in Annex B;

— Performance testing for RPFA;

— Results of the interlaboratory study;

— Updated bibliography.

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

Introduction

1.1 Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this part of ISO 6888 is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain group of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this part of ISO 6888 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

1.2 ISO 6888 describes three horizontal methods (part 1, part 2 and part 3) 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.*

“Both parts 1 and 2 of ISO 6888 are given equivalent status. Nevertheless, it is recommended to use the procedure described in ISO 6888-2 for the foods (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 which can obscure the colonies being sought.

1.3 For the purposes of this part of ISO 6888, the characterisation 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 may be confused with other bacteria but they may be distinguished from such other bacteria by the use of additional tests not included in this part of ISO 6888, such as the sensitivity to lysostaphin, the production of haemolysin, thermostable nuclease and acid from mannitol (see ISO 7218 and reference [1]).

The main technical changes listed in the Foreword, introduced in this document compared to ISO 6888-2 (1999), are considered as minor (see ISO 17468).

They have a minor impact on the performance characteristics of the method.

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

Part 2: Technique using rabbit plasma fibrinogen 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 of colonies obtained on a solid medium (rabbit plasma fibrinogen medium) after aerobic incubation at 34 °C to 38 °C (see reference [2]).

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.

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 — Rules for the preparation of the test sample, of initial suspension and of 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*

ISO 17468, *Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method*

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 http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
coagulase-positive staphylococci
bacteria which form **typical colonies** in or on a selective culture medium (rabbit plasma fibrinogen agar medium).

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

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

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 or of the initial suspension, with one plate per dilution.

4.2 Incubation

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

4.3 Enumeration

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 typical colonies obtained on plates at dilution levels chosen to give a significant result.

NOTE See Annex A for flow diagram

5 Culture media and reagents

Follow current laboratory practices in accordance with ISO 7218.

The composition of culture media and reagents and their preparation are specified in Annex B.

For performance testing of culture media, follow the procedures in accordance with ISO 11133 and Annex B.

For the diluent(s), see the relevant part of ISO 6887 series.

Commercially available media, in accordance with this document, can be used. Nevertheless, considering the known variability of manufactured lots of the supplement, it is recommended that each batch of bovine fibrinogen/rabbit plasma solution be tested before use, by running positive and negative controls.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) and wet sterilization (autoclave)

See ISO 7218

6.2 Incubator, capable for maintaining the inoculated media, within the temperature range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of media includes the use of incubators set at 35 °C ± 1 °C or 37 °C ± 1 °C.

6.3 Water bath, or similar apparatus, capable of being maintained at 44 °C to 47 °C.

6.4 Petri dishes, with a diameter of approximately 90 mm, sterile, made of glass or plastic.

6.5 Sterile graduated pipettes or automatic pipettes of nominal capacities 1 ml, 2 ml and 10 ml, graduated in 0,1 ml, 0,1 ml and 0,5 ml divisions, respectively.

Graduated pipettes and pipettor tips should be fitted with a non-absorbent cotton wool plug to prevent contamination when used to manipulate microbial cultures.

6.6 pH-meter, it shall be capable of being read to the nearest 0,01 pH unit, enabling measurements to be made with a tolerance of ±0,1 pH unit. The pH meter shall be equipped with either manual or automatic temperature compensation.

6.7 Refrigerator capable of operating at 5°C±3°C.

7 Sampling

Sampling is not part of the method specified in this document. Follow the specific International Standard dealing with the product concerned. If there is no specific International Standard dealing with the sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

Recommended sampling techniques are given in the following documents:

— ISO/TS 17728 for food and animal feed[3];

— ISO 707 for milk and milk products[4];

— ISO 6887-3 for fish and fishery products[5];

— ISO 13307 for primary production stage[6];

— ISO 17604 for carcasses[7];

— ISO 18593 for surfaces[8].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.