RESOLUTION OIV-OENO 6620-2023

HORIZONTAL METHOD FOR THE ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCI

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Methods of Analysis" Sub-Commission on the development of methods of analysis for grape juices, concentrated grape juices, reconstituted grape juices and grape nectars,

CONSIDERING the ISO standard ISO 6888-2:2021 regarding the enumeration of coagulase-positive staphylococci which can be access through the ISO website [1]

CONSIDERING the work of the OIV "Microbiology" Expert Group and the favourable opinion of the Scientific and Technical committee of the OIV to make reference to this ISO standard knowing that some of the elements of this ISO standard may be the subject of copyright protection,

AT THE PROPOSAL OF the Commission Oenology,

DECIDES to adopt the following microbiological method of analysis for grape juices, concentrated grape juices, reconstituted grape juices and grape nectars:

HORIZONTAL METHOD FOR THE ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCI

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

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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;

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- 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/Amendment 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.

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

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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

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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.

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Bibliography

- [1] ISO 707, Milk and milk products — Guidance on sampling
- [2] ISO 6888-1, Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium
- [3] ISO 6888-3, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers
- [4] ISO 13307, Microbiology of food and animal feed — Primary production stage — Sampling techniques
- [5] ISO 16140:2003, Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative method
- [6] ISO 17604, Microbiology of the food chain — Carcass sampling for microbiological analysis
- [7] ISO 17468, Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method
- [8] ISO/TS 17728, Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples
- [9] ISO 18593, Microbiology of the food chain — Horizontal methods for surface sampling
- [10] IDF 145A:1997, Milk and milk products — Enumeration of coagulase-positive staphylococci — Colony-count technique
- [11] BOOTHBY J., GENIGEORGIS C. and FANELLI M.J. Tandem Coagulase / Thermonuclease Agar Method for the Detection of Staphylococcus aureus. Appl. and Environmental microbiology. 1979, Vol.37, pp. 298-302.
- [12] De Buyser M.L., Lombard B., Schulten S.M., In't Veld P.H., Scotter S.L., Rollier P., Lahellec C., Validation of EN ISO standard methods 6888 part 1 and part 2:1999, Enumeration of coagulase-positive staphylococci in foods. Int. J. Food Microbiol. 2003, 83(2), pp. 185–194

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- [13] Kloos W.E., Systematics and the natural history of staphylococci. In: Staphylococci, J. Appl. Bacteriol. Symp. Suppl., 1990, 69, pp. 25 s–37 s; and Bergey's Manual of Systematic Bacteriology 2nd Edition, Vol 3, 2009, pp 372-421

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[1] https://www.iso.org/obp/ui/#iso:std:iso:16649:-2:ed-1:v1:en

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