INTERNATIONAL STANDARD

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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

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

Partie 3: Recherche et méthode NPP pour les faibles nombres

Reference number
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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 6888-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 6888 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus *and other species)*:

— *Part 1: Technique using Baird-Parker agar medium*

— *Part 2: Technique using rabbit plasma fibrinogen agar medium*

— *Part 3: Detection and MPN technique for low numbers*

This corrected version of ISO 6888-3:2003 incorporates the following correction:

Subclause 9.1.1

The second paragraph has been amended to resolve any ambiguity.

Introduction

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 groups 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.

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

1 Scope

This part of ISO 6888 specifies a horizontal method for the enumeration and detection of coagulase-positive staphylococci, using the most probable number (MPN) technique. It is applicable to

— products intended for human consumption and the feeding of animals, and

— environmental samples in the area of food production and food handling.

This method is recommended for products where staphylococci are expected to be stressed and in low numbers as, for example, in dried products. Coagulase-positive staphylococci will primarily be *Staphylococcus aureus* but *Staphylococcus intermedius* and some strains of *Staphylococcus hyicus* also produce coagulase.

2 Normative references

The following referenced documents are indispensable for the application 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 food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO 6888-1:1999, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus *and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2:1999, *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus *and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

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

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:—[1], *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing culture media*

3 Terms and definitions

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

3.1
coagulase-positive staphylococci
bacteria which form typical and/or atypical colonies on the surface of a selective culture medium and which show a positive coagulase reaction or a specific rabbit plasma reaction on rabbit plasma fibrinogen agar

NOTE For the purpose of this part of ISO 6888, the confirmation of coagulase-positive staphylococci is based on a strongly positive coagulase reaction, but it is recognized that some strains of coagulase-positive staphylococci give weakly positive coagulase reactions. These latter strains can be confused with other bacteria but they can be distinguished from such other bacteria by the use of additional tests such as the production of thermonuclease (for details, see IDF 83).

3.2
enumeration of the coagulase-positive staphylococci
determination of the number of coagulase-positive staphylococci found per millilitre or per gram of sample when the test is carried out according to the method specified in this part of ISO 6888

4 Principle

4.1 Detection method

4.1.1 A selective culture medium is inoculated with a specified quantity of the test sample if the initial product is liquid, or a specified quantity of an initial suspension in the case of other products.

4.1.2 The tubes are incubated at 37 °C, anaerobically, for 24 h and 48 h. The presence of presumptive coagulase-positive staphylococci is indicated by the reduction of potassium tellurite.

NOTE In this part of ISO 6888, anaerobiosis is obtained by pouring a plug of agar or paraffin into each tube, but an alternative procedure is to incubate the tubes in a jar or an incubator under anaerobic conditions.

4.1.3 The surface of solid selective Baird-Parker medium is inoculated from presumptive positive tubes (4.1.2) after 24 h, and all the remaining tubes after 48 h.

4.1.4 The plates are inoculated at 37 °C for 24 h and 48 h. The presence of presumptive coagulase-positive staphylococci is indicated by the reduction of potassium tellurite and an egg yolk reaction.

4.1.5 Typical and/or atypical colonies are confirmed by a coagulase reaction.

4.1.6 Alternatively, the surface of rabbit plasma fibrinogen agar may be inoculated and, after appropriate incubation, the presence of coagulase-positive staphylococci is indicated by colonies showing a specific rabbit plasma fibrinogen reaction.

4.1.7 The results are given as the “presence” or “absence” of coagulase-positive staphylococci in x g or x ml of product.

4.2 Enumeration method

4.2.1 Serial dilutions of product are inoculated into liquid selective culture medium.

1) To be published.

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4.2.6 Alternatively, the surface of rabbit plasma fibrinogen agar may be inoculated and, after appropriate incubation, the presence of coagulase-positive staphylococci is indicated by colonies showing a specific rabbit plasma fibrinogen reaction.

4.2.7 The most probable number of coagulase-positive staphylococci per gram or per millilitre of sample is calculated by reference to most probable number tables for confirmed dilutions (4.2.5 or 4.2.6).

5 Diluents and culture media

For current laboratory practice, see ISO 7218.

The chemical products used for the preparation of the culture media and reagents shall be of recognized analytical quality.

5.1 Diluents

Refer to the relevant part of ISO 6887 or to ISO 8261, or the specific standard dealing with the product to be examined.

5.2 Modified Giolitti and Cantoni broth

5.2.1 Base medium

5.2.1.1 Composition

	Double-strength medium	Single-strength medium
Enzymatic digest of casein	20,0 g	10,0 g
Meat extract	10,0 g	5,0 g
Yeast extract	10,0 g	5,0 g
Lithium chloride	10,0 g	5,0 g
Mannitol	40,0 g	20,0 g
Sodium chloride	10,0 g	5,0 g
Glycine	2,4 g	1,2 g
Sodium pyruvate	6,0 g	3,0 g
Polyoxyethylene sorbitan mono-oleate (Tween 80)	2,0 g	1,0 g
Water	1 000 ml	1 000 ml