AS 5013.12.2:2026

Australian STANDARD®

STANDARDS Australia

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

Part 12.2: Method using rabbit plasma fibrinogen agar medium (ISO 6888-2:2021, MOD)



AS 5013.12.2:2026

This Australian Standard® was prepared by FT-035, Food Microbiology. It was approved on behalf of Standards Australia's Standards Development and Accreditation Committee on 16 June 2026.

This Standard was published on 26 June 2026.

The following are represented on Committee FT-035:

ACT Health Directorate
Australian Society for Microbiology
CSIRO
Dairy Industry Association of Australia
Department of Agriculture, Fisheries and Forestry
Department of Health (TAS)
National Association of Testing Authorities Australia
National Measurement Institute
PathWest Laboratory Medicine WA
Queensland Health Forensic and Scientific Services
The University of Melbourne

This Standard was issued for comment as DR AS 5013.12.2:2025

Status of this document

Standards Australia publications are periodically reviewed to ensure their ongoing relevance and accuracy. This document may be withdrawn, revised, or superseded at any time. To verify this document's current status, visit:

www.standards.org.au

ISBN: 978 1 76175 871 3

Australian Standard®

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

Part 12.2: Method using rabbit plasma fibrinogen agar medium (ISO 6888-2:2021, MOD)

First published as AS 1095.3.2-1976 and AS 1766.2-1976.
Previous edition AS 5013.12.2-2004.
This document AS 5013.12.2:2026.

© ISO 2026 — All rights reserved
© Standards Australia Limited 2026

All rights are reserved. No part of this work may be reproduced or copied in any form or by any means, electronic or mechanical, including photocopying, without the written permission of the publisher, unless otherwise permitted under the Copyright Act 1968 (Cth).

Users must not use the content in this publication in combination with artificial intelligence (AI) tools for any purpose, unless they have the express prior written consent from Standards Australia.

How to read this Standard

This page explains the meaning of the language and structure of this Standard.

Refer to Standards Australia's Standardisation Guide 006 for more details about drafting rules.

Australian and Australian/New Zealand Standards are voluntary unless they are referenced in legislation or called up in contracts.

Requirements

To conform to a Standard, all requirements in the Standard need to be met.

A requirement is any statement in the Standard which uses the word "shall".

Recommendations, permissions and possibilities

The following words are commonly used in Standards, but statements using them do not have to be followed to conform to the Standard:

(a) "should" means that something is recommended.

(b) "may" means that something is permitted.

(c) "can" means that something is possible.

Structure of Standards

A Standard always has the following parts:

(i) The Preface states who developed the Standard, what the Standard is aiming to do, and how it relates to other documents.

(ii) The Scope states what the Standard is about, what it covers and what it does not cover.

(iii) The Normative references clause lists other documents that are referenced in the Standard as part of requirements.

(iv) The Terms and definitions clause defines important terms to help with understanding the Standard.

A Standard may also include other parts, such as the following:

(1) A normative appendix sets additional requirements that need to be conformed to.

(2) An informative appendix provides additional information or guidance. They usually do not contain requirements. If an informative appendix does contain requirements, the Standard will explain when those requirements apply.

(3) A Bibliography lists documents referenced in the Standard but not as part of requirements.

Many Standards include notes. Notes provide recommendations and/or guidance only. They never contain requirements.

This Standard is a modified adoption of an International Standard. It makes changes to the international text.

The changes to the international text are shown in boxes in the text. These boxes have the heading "National Variations".

To use this Standard in Australia/New Zealand, the changes in the national variation boxes need to be followed.

Preface

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS 5013.12.2—2004, *Food microbiology, Method 12.2: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus *and other species)—Technique using rabbit plasma fibrinogen agar medium*.

The objective of this document is to specify 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.

This document is an adoption with national modifications and has been reproduced from ISO 6888-2:2021, *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* and its Amendment 1 (2023) which has been added at the end of the source text. The modifications are set out in national variations boxes which give instructions where the ISO text is to be modified for use in Australia. Due to ISO copyright policy, it is not possible to directly modify the ISO content.

As this document has been reproduced from an international document, a full point substitutes for a comma when referring to a decimal marker.

Australian or Australian/New Zealand Standards that are identical adoptions of international normative references may be used interchangeably. Refer to the online catalogue for information on specific Standards.

© Standards Australia Limited 2026

Contents

Page

Preface ..... **v**
Foreword ..... **vii**
Introduction ..... **ix**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **2**
4 Principle **3**
4.1 General 3
4.2 Incubation 3
4.3 Enumeration 3
5 Culture media and reagents **3**
6 Equipment and consumables **4**
7 Sampling **4**
8 Preparation of the test sample **5**
9 Procedure (see Figure A.1) **5**
9.1 Test portion, initial suspension and dilutions 5
9.2 Inoculation and incubation 5
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 **6**
11 Performance characteristics of the method **6**
11.1 Interlaboratory study 6
11.2 Repeatability limit 6
11.3 Reproducibility limit 7
12 Test report **7**
13 Quality assurance **8**
Annex A (normative) **Flow diagram of the procedure** **9**
Annex B (normative) **Culture media and reagents** **10**
Annex C (informative) **Results of the interlaboratory study** **13**
Bibliography **15**
Amendment 1 ..... **16**

© Standards Australia Limited 2026

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/Amendment 1:2003 Precision data) have been updated;

© Standards Australia Limited 2026

— 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.

© Standards Australia Limited 2026

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.

© Standards Australia Limited 2026

Australian Standard®

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

Part 12.2: Method using rabbit plasma fibrinogen agar medium (ISO 6888-2:2021, MOD)

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

NATIONAL VARIATIONS

1. After the first paragraph, *add* the following:

© Standards Australia Limited 2026