
International Standard



6888

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

Microbiology — General guidance for enumeration of *Staphylococcus aureus* — Colony count technique

Microbiologie — Directives générales pour le dénombrement de Staphylococcus aureus — Méthode par comptage des colonies

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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 developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been authorized 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 6888 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in November 1981.

It has been approved by the member bodies of the following countries:

Australia	India	Sri Lanka
Brazil	Israel	Tanzania
Canada	Italy	Thailand
Chile	Mexico	Turkey
Czechoslovakia	Netherlands	United Kingdom
Egypt, Arab Rep. of	New Zealand	USA
Ethiopia	Poland	USSR
France	Romania	Venezuela
Germany, F.R.	South Africa, Rep. of	Yugoslavia
Hungary	Spain	

The member body of the following country expressed disapproval of the document on technical grounds:

Portugal

Microbiology — General guidance for enumeration of *Staphylococcus aureus* — Colony count technique

0 Introduction

0.1 This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International Standards and for the consideration of bodies preparing reference microbiological methods of test for application to food products or to animal feeding stuffs. In view of the large variety of products within this field of application, these guidelines may not be appropriate for some products in every detail, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that, in all cases, every attempt will be made to apply these guidelines as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons which necessitated deviation from them 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, that do not comply with these guidelines, may already exist. In cases where International Standards already exist for the product to be tested, they should be followed, but it is hoped that, when they are reviewed, they will be aligned with this International Standard so that, eventually, the only remaining departures from these guidelines will be those necessary for well established technical reasons.

0.2 For the purpose of this International Standard, the confirmation of *Staphylococcus aureus* is based on a strongly 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 the International Standard, such as the production of thermonuclease, of acid from mannitol, the production of haemolysin and sensitivity to lysostaphin¹⁾.

0.3 For statistical reasons, the lowest limit for the number of colonies counted per dish has been set at 15, but, for practical purposes, a count of lower numbers of *Staphylococcus aureus* is often required. The confidence limits of such determinations (estimated counts) are given in the annex.

1 Scope and field of application

This International Standard gives general guidance for the enumeration of *Staphylococcus aureus* in products intended for human consumption or feeding of animals.

2 Reference

ISO 6887, *Microbiology — General guidance for the preparation of dilutions for microbiological examination*.

3 Definition

For the purpose of this International Standard, the following definition applies.

Staphylococcus aureus: Micro-organisms which form typical and/or atypical colonies on the surface of a selective culture medium and which show a strongly positive coagulase reaction.

4 Principle

4.1 Inoculation of the surface of a solid selective culture medium, using duplicate plates, 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.

1) See Devriese *et al* (1978) *Staphylococcus hyicus* (Sompolinsky 1953) comb. Nov. and *Staphylococcus hyicus* ssp. chromogens ssp. Nov. *International Journal of Systematic Bacteriology* **28**, pp. 482-490.

Bacteriological Analytical Manual, 5th ed. 1978.
US Food and Drug Administration p. XI-4 + 5.

4.2 Incubation of the plates at 35 °C or 37 °C for 24 to 48 h.

4.3 Calculation of the number of *Staphylococcus aureus* per millilitre, or per gram, of sample from the number of typical and/or atypical colonies obtained on plates at dilution levels chosen so as to give a significant result, and confirmed by the coagulase test.

5 Diluent and culture media

5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluent and culture media, dehydrated basic components or complete dehydrated media and, for the egg yolk emulsion, a commercially available preparation, be used. The manufacturer's instructions shall be rigorously followed.

Chemical products shall be of recognized analytical quality.

The water used shall be distilled or deionized water, and shall be free from substances that might inhibit growth of *Staphylococcus aureus* under the test conditions.

If the diluent and the culture media are not used immediately, they shall be kept in the dark at a temperature between 0 and +5 °C, in conditions that prevent any change in their composition.

5.2 Diluent

See ISO 6887 and the specific standard dealing with the product to be examined.

5.3 Agar medium¹⁾

5.3.1 Base medium

Composition

tryptone	10,0 g
yeast extract	1,0 g
meat extract	5,0 g
glycine	12,0 g
lithium chloride	5,0 g
agar	12 to 20,0 g ²⁾
water	1 000 ml
and, if necessary:	
sulphamezathine solution (5.3.2.2)	27,5 ml

Preparation

Dissolve the components or the complete agar base in the water by boiling.

Add, if necessary, the 27,5 ml of the sulphamezathine (sulphadimidine, INN) solution (5.3.2.2).

Adjust the pH so that after sterilization it is 7,2 at 25 °C.

Transfer the medium in quantities of 90 ml to flasks or bottles of capacity not more than 300 ml.

Sterilize the medium for 15 min at 121 °C.

The medium may be stored for 1 month between 0 and +5 °C.

5.3.2 Solutions

5.3.2.1 Tellurite solution

Composition

potassium tellurite ³⁾ (dipotassium trioxotellurate)	1,0 g
water	100 ml

Preparation

Dissolve the potassium tellurite in the water with minimal heating.

Sterilize by filtration.

The solution may be stored for several months between 0 and +5 °C.

5.3.2.2 Sulphamezathine (sulphadimidine, INN) solution (only if the presence of *Proteus* is suspected)

Composition

sulphamezathine	0,2 g
sodium hydroxide solution, c(NaOH) = 0,1 mol/l	10 ml
water, to a final volume of	100 ml

Preparation

Dissolve the sulphamezathine in the sodium hydroxide solution.

Make up to a final volume of 100 ml with the water.

5.3.2.3 Sodium pyruvate solution

Composition

sodium pyruvate	20,0 g
water	100 ml

Preparation

Dissolve the sodium pyruvate in part of the water.

1) The agar medium is that of Baird-Parker, *J. Appl. Bacteriol.* **25** (1962) 12, with the addition of sulphamezathine [see Smith and Baird-Parker, *ibid.* **27** (1964) 78] if the presence of *Proteus* is suspected.

2) According to the manufacturer's instructions.

3) It is recommended to ensure beforehand that the potassium tellurite available is suitable for this test.