INTERNATIONAL STANDARD

ISO 6888-1

First edition
1999-02-15

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

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

Partie 1: Technique utilisant le milieu gélosé de Baird-Parker

Contents

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

Printed in Switzerland

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 3.

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.

International Standard ISO 6888-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 6888-1, together with ISO 6888-2, cancels and replaces ISO 6888:1983, which has been technically revised.

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*

0 Introduction

0.1 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.

0.2 ISO 6888 describes two horizontal methods (part 1 and part 2) for the 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.*

In the general case, use part 1 of ISO 6888. However, it is preferable to use the procedure described in part 2 (see reference [1]) only for foodstuffs (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.

0.3 For the purposes of this part of ISO 6888, the confirmation of staphylococci is based on a positive coagulase reaction, but it is reconized 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, of thermostable nuclease and of acid from mannitol (see reference [2]).

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

1 Scope

This part of ISO 6888 specifies a horizontal method for the enumeration of coagulase-positive staphylococci in products intended for human consumption or feeding of animals, by counting of colonies obtained on a solid medium (Baird-Parker medium) after aerobic incubation at 35 °C or 37 °C.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 6888. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 6888 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Rules for the preparation of the test sample, of initial suspension and of decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and of decimal dilutions*.

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

3 Terms and definitions

For the purposes of this part of ISO 6888, 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 when the test is performed following the method specified in this part of ISO 6888

3.2
enumeration of the coagulase-positive staphylococci
determination of the number of coagulase-postive 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 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, with two plates per dilution.

4.2 Aerobic incubation of the plates at 35 °C or 37 °C[1)] and examination after both 24 h and 48 h.

4.3 Calculation of the number of coagulase-positive staphylococci 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 a positive coagulase test result.

5 Diluent and culture media

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Diluent

See ISO 6887-1 and the specific standard dealing with the product to be examined.

5.3 Baird-Parker agar medium[2)]

NOTE Commercially available media may be used. In such cases, the manufacturer's instructions should be followed carefully.

5.3.1 Base medium

5.3.1.1 Composition

Pancreatic digest of casein	10,0 g
Yeast extract	1,0 g
Meat extract	5,0 g
Sodium pyruvate	10,0 g
L-Glycine	12,0 g
Lithium chloride	5,0 g
Agar	12 g to 22 g [1)]
Water, to a final volume of	1 000 ml

1) Depending on the gel strength of the agar.

5.3.1.2 Preparation

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

If necessary, adjust the pH so that after sterilization it is 7,2 ± 0,2 at 25 °C.

---

1) The temperature is agreed between the interested parties and is indicated in the test report.

2) The agar medium is that of Baird-Parker (see reference [3]) with the addition of sulfamezathine (see reference [4]) if the presence of *Proteus* is suspected.

Transfer the medium in quantities of 100 ml to flasks or bottles (6.5) of appropriate capacity.

Sterilize the medium for 15 min at 121 °C.

5.3.2 Solutions

5.3.2.1 Potassium tellurite solution

5.3.2.1.1 Composition

Potassium tellurite [1] (K_2TeO_3)	1,0 g
Water	100 ml

1) It is recommended to ensure beforehand that the potassium tellurite available is suitable for this test (see 5.3.2.1.2).

5.3.2.1.2 Preparation

Dissolve the potassium tellurite completely in the water with minimal heating.

The solid should be readily soluble. If a white insoluble material is present in the water, discard the powder.

Sterilize by filtration using 0,22 µm pore size membranes.

The solution may be stored at the maximum for one month at +3 °C ± 2 °C.

Discard the solution if a white precipitate forms.

5.3.2.2 Egg yolk emulsion (concentration approximately 20 % or according to the manufacturer's instructions)

NOTE If a commercial preparation is available, it should be used.

Use fresh hen eggs with intact shells. Clean the eggs with a brush using a liquid detergent. Rinse them under running water, then disinfect the shells either by immersing them in ethanol (70 % volume fraction) for 30 s and allowing them to dry in the air, or by spraying them with alcohol followed by flame sterilization.

Proceeding under aseptic conditions, break each egg and separate the yolk from its white by repeated transfer of the yolk from one half of the shell to the other. Place the yolks in a sterile flask (6.5) and add four times their volume of sterile water. Mix thoroughly. Heat the mixture in the water bath (6.4) set at 47 °C for 2 h and leave for 18 h to 24 h at +3 °C ± 2 °C to allow a precipitate to form. Aseptically collect the supernatant liquid into a fresh sterile flask for use.

The emulsion may be stored at +3 °C ± 2 °C for a maximum of 72 h.

5.3.2.3 Sulfamezathine (sulfamethazine, sulfadimidine) solution

NOTE This is to be used only if *Proteus* species are suspected in the test sample.

5.3.2.3.1 Composition

Sulfamezathine	0,2 g
Sodium hydroxide solution, c(NaOH) = 0,1 mol/l	10 ml
Water	90 ml

5.3.2.3.2 Preparation

Dissolve the sulfamezathine in the sodium hydroxide solution.

Dilute to 100 ml with the water.

Sterilize by filtration using 0,22 µm pore size membranes.

The solution may be stored at the maximum for one month at +3 °C ± 2 °C.

5.3.3 Complete medium

5.3.3.1 Composition

Base medium (5.3.1)	100 ml
Potassium tellurite solution (5.3.2.1)	1,0 ml
Egg-yolk emulsion (5.3.2.2)	5,0 ml
Sulfamezathine solution (5.3.2.3) (if necessary)	2,5 ml

5.3.3.2 Preparation

Melt the base medium, then cool it to approximately 47 °C by means of the water bath (6.4).

Add, under aseptic conditons, the two other solutions (5.3.2.1 and 5.3.2.2) and if necessary (if *Proteus* species are suspected in the test sample) the sulfamezathine solution (5.3.2.3), each solution being previously warmed in a water bath at 47 °C, mixing well after each addition.

5.3.4 Preparation of agar plates

Place the appropriate quantity of the complete medium (5.3.3) into sterile Petri dishes in order to obtain an agar thickness of about 4 mm, and allow to solidify.

The plates may be stored, prior to drying, for up to 24 h at +3 °C ± 2 °C.

NOTE The manufacturer's instructions should be followed concerning the storage period for industrially prepared plates.

Before use, dry the plates, preferably with the lids off and the agar surface downwards, in an oven set at a temperature between 25 °C and 50 °C, until the droplets have disappeared from the surface of the medium.

5.4 Brain-heart infusion broth

5.4.1 Composition

Enzymatic digest of animal tissues	10,0 g
Dehydrated calf brain infusion	12,5 g
Dehydrated beef heart infusion	5,0 g
Glucose 2,0 g	
Sodium chloride	5,0 g
Disodium hydrogenphosphate, anhydrous (Na_2HPO_4)	2,5 g
Water	1 000 ml

5.4.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, heating if necessary.

Adjust the pH so that after sterilization it is 7,4 ± 0,2 at 25 °C.

Transfer the culture medium in quantities of 5 ml to 10 ml to tubes or bottles (6.5) of appropriate capacity.

Sterilize the medium for 15 min at 121 °C.

5.5 Rabbit plasma

Use commercially available dehydrated rabbit plasma and rehydrate it according to the manufacturer's instructions.

If dehydrated rabbit plasma is not available, dilute one volume of fresh sterile rabbit plasma with three volumes of sterile water.

Add EDTA (ethylenediaminetetraacetic acid) solution to give 0,1 % EDTA in the rehydrated or diluted plasma, if potassium citrate or sodium citrate has been used as the plasma anticoagulant [3].

Unless stated by the manufacturer, the rehydrated or diluted plasma shall be used immediately.

Before use, test each batch of plasma with coagulase-positive strains of staphylococci and strains of coagulase-negative staphylococci.

6 Apparatus and glassware

NOTE Disposable apparatus 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, for maintaining the inoculated media, plates and tubes within the temperature range 35 °C ± 1 °C or 37 °C ± 1 °C.

6.3 Drying cabinet or **incubator**, capable of being maintained at between 25 °C ± 1 °C and 50 °C ± 1 °C.

6.4 Water bath, or similiar apparatus, capable of being maintained at 47 °C ± 2 °C.

6.5 Test tubes, flasks or **bottles with screw caps**, of appropriate capacity, for sterilization and storage of culture media and incubation of liquid media; in particular, sterile haemolysis tubes, or round-bottom bottles of approximate dimensions 10 mm × 75 mm.

6.6 Petri dishes, sterile, made of glass or plastic.

6.7 Straight wire (see ISO 7218) and **Pasteur pipette**.

6.8 Total-delivery graduated 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.

---

3) Oxalated or heparinized plasma does not require EDTA (see reference [5]).