
**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**

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

*Partie 2: Méthode utilisant le milieu gélosé au plasma de lapin et au
fibrinogène*



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Contents

	Page
Foreword.....	iv
Introduction.....	vi
1 Scope	1
2 Normative references	2
3 Terms and definitions	2
4 Principle	2
4.1 General.....	2
4.2 Incubation.....	2
4.3 Enumeration.....	3
5 Culture media and reagents	3
6 Equipment and consumables	3
7 Sampling	4
8 Preparation of the test sample	4
9 Procedure (see Figure A.1)	4
9.1 Test portion, initial suspension and dilutions.....	4
9.2 Inoculation and incubation.....	4
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	5
11 Performance characteristics of the method	5
11.1 Interlaboratory study.....	5
11.2 Repeatability limit.....	5
11.3 Reproducibility limit.....	6
12 Test report	7
13 Quality assurance	7
Annex A (normative) Flow diagram of the procedure	8
Annex B (normative) Culture media and reagents	9
Annex C (informative) Results of the interlaboratory study	12
Bibliography	14

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/Amd 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.

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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](#).

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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

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

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 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.

4 Principle

4.1 General

Preparation of poured plate of the rabbit plasma fibrinogen agar medium, 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, follow the procedure(s) in accordance with ISO 7218.

NOTE The volume of rabbit plasma fibrinogen agar medium to be added to the inoculum is a critical point for the coagulase method and reaction.

4.2 Incubation

Aerobic incubation of the plates at 34 °C to 38 °C and examination after 24 h and, if necessary, after 48 h.

4.3 Enumeration

Calculation of the number of coagulase-positive staphylococci per gram of sample, per millilitre of sample, per square centimetre or per sampling device from the number of typical colonies obtained on plates at dilution levels chosen to give a significant result within the counting limits of the method and in accordance with ISO 7218.

NOTE See [Annex A](#) for a flow diagram.

5 Culture media and reagents

Follow current laboratory practices in accordance with ISO 7218.

The composition of culture media and reagents and their preparation are specified in [Annex B](#).

For performance testing of culture media and reagents, follow the procedures in accordance with either [Annex B](#) or ISO 11133, or both.

For diluent(s), see the relevant part of the ISO 6887 series.

6 Equipment and consumables

Disposable equipment 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, capable of maintaining the inoculated media, within the temperature range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.

6.3 Water bath, or similar apparatus, capable of being maintained at 44 °C to 47 °C.

6.4 Sterile Petri dishes, with a diameter of approximately 90 mm, made of glass or plastic.

6.5 Sterile graduated pipettes or automatic 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. See ISO 7218.

Graduated pipettes and pipettor tips should be fitted with a non-absorbent cotton wool plug to prevent contamination when used to manipulate microbial cultures.

6.6 pH-meter, capable of being read to the nearest 0,01 pH unit, enabling measurements to be made with a tolerance of ±0,1 pH unit. The pH meter shall be equipped with either manual or automatic temperature compensation. See ISO 7218.

6.7 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.8 Sterile tubes, bottles or flasks with caps, of appropriate capacity. Bottles or flasks with non-toxic metallic or plastic screw-caps may be used.

6.9 Membranes, with 0,2 µm pore size.

7 Sampling

Sampling is not part of the method specified in this document. Follow the specific International Standard dealing with the product concerned. If there is no specific International Standard dealing with the sampling of the product concerned, the parties concerned should come to an agreement on this subject.

Recommended sampling techniques are given in the following documents:

- ISO/TS 17728 for food and animal feed;
- ISO 707 for milk and milk products;
- ISO 6887-3 for fish and fishery products;
- ISO 13307 for the primary production stage;
- ISO 17604 for carcasses;
- ISO 18593 for surfaces.

It is important that the laboratory receive a sample that is representative. The sample should not have been damaged or changed during transport or storage.

8 Preparation of the test sample

Prepare the test sample from the laboratory sample in accordance with the specific International Standard dealing with the product concerned: follow the procedures specified in the ISO 6887 series and if necessary ISO 18593. If there is no specific International Standard available, the parties concerned should come to an agreement on this subject.

9 Procedure (see [Figure A.1](#))

9.1 Test portion, initial suspension and dilutions

See the relevant part of the ISO 6887 series.

9.2 Inoculation and incubation

Transfer, by means of a sterile pipette ([6.5](#)), 1 ml of the test sample if liquid, or 1 ml of the initial suspension (10^{-1} dilution) in the case of other products, to a Petri dish (see [Annex B](#)). For enumeration techniques in microbiology of the food chain, the number of Petri dishes to be used, according to the tested dilutions, is stated in ISO 7218. Repeat the procedure for further decimal dilutions if necessary.

Into each Petri dish ([6.4](#)), immediately pour 18 ml to 20 ml freshly prepared complete medium ([B.2.3](#)) to obtain a depth of at least 3 mm.

Carefully mix the inoculum with the culture medium and leave to solidify by placing the Petri dishes on a horizontal surface.

After complete solidification, invert the dishes prepared above and place them in the incubator ([6.2](#)) set at 34 °C to 38 °C. After incubation for 24 h ± 2 h, mark on the bottom of the plates the positions of any typical colonies present. If no colonies or no typical colonies are obtained at 24 h ± 2 h, re-incubate all plates at 34 °C to 38 °C for a further 24 h ± 2 h (to a total of 48 h ± 4 h), and mark any typical colonies.

NOTE Even if there is an inhibitor of trypsin in the RPFA medium, colonies with typical appearance after 24 h ± 2 h incubation can lose typical appearance after 48 h ± 4 h incubation, due to enzymatic processes (trypsin) or due to overgrowth.^[1] Counting only at 48 h ± 4 h can lead to low counts or no counts.

9.3 Counting of colonies

9.3.1 General description of colonies growing on RPFA medium

Typical colonies are black or grey or even white, small and are surrounded by an opacity halo of precipitation, indicating coagulase activity. *Proteus* colonies can appear to look like those of coagulase-positive staphylococci early on in the incubation. However, they can be distinguished from staphylococci after $24 \text{ h} \pm 2 \text{ h}$ and $48 \text{ h} \pm 4 \text{ h}$ of incubation, as their colonies become more or less brownish and start to spread.

9.3.2 Colony counting procedure

At the end of the incubation period (see 9.2), count the typical colonies in each dish.

At reading the plates after 24 h, mark on the bottom of the plates the positions of any typical colonies present before further incubation.

If plates are re-incubated at $34 \text{ }^\circ\text{C}$ to $38 \text{ }^\circ\text{C}$ for $24 \text{ h} \pm 2 \text{ h}$, mark any new typical colonies.

For enumeration, only retain plates containing a maximum of 300 colonies, with 100 typical colonies.

One of the plates shall contain at least 10 colonies.

NOTE As the rabbit plasma fibrinogen agar medium is based on a coagulase reaction, it is not necessary to confirm this activity.

When the number of cfu is expected to be at or near the limit of determination, the use of duplicate plates is preferable. If duplicate plates are used the minimum for the sum of colonies should be 10. In this case, the level of contamination is expected to be higher than 5 cfu/ml for liquid samples or higher than 50 cfu/g for solid samples.

10 Expression of results

For calculation of the results, follow the procedure(s) in accordance with ISO 7218. Calculate and report the results as the number of coagulase-positive staphylococci in cfu (colony forming unit) per gram, millilitre, per square centimetre or per sampling device.

In cases where no colonies of the target microorganism have been detected, follow ISO 7218 for the expression of results for special cases.

11 Performance characteristics of the method

11.1 Interlaboratory study

Results of the interlaboratory study to determine the precision of the method are summarized in [Annex C](#). Repeatability and reproducibility limits were determined using four sample types (cheese, meat, egg powder and reference materials) contaminated at various levels.

The values derived from the interlaboratory study may not be applicable to concentration ranges and sample types other than those given in [Annex C](#).

11.2 Repeatability limit

The absolute difference between two single (\log_{10} -transformed) test results (number of coagulase-positive staphylococci per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within the shortest feasible time interval will, in no more than 5 % of cases, exceed the repeatability limit r .

As a general indication of the repeatability limit r , the following values (medians of values per matrix and contamination level, see [Annex C](#)) may be used when testing food samples in general:

- $r = 0,22$, range [0,13; 0,33] (expressed as an absolute difference between \log_{10} -transformed test results); or
- $r = 1,70$, range [1,35; 2,14] (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

EXAMPLE A first test result of 10 000 or $1,0 \times 10^4$ or $\log_{10} 4,00$ of coagulase-positive staphylococci per gram of food was observed in a given laboratory. Under repeatability conditions, the difference between \log_{10} -transformed results is not intended to be greater than $\pm 0,22 \log_{10}$ units. So the result from a second test result of the same sample is expected to be between 3,78 ($4,00 - 0,22$) \log_{10} units and 4,22 ($4,00 + 0,22$) \log_{10} units. For non-log-transformed results, the ratio between the first test result and the second test result from the same sample is not intended to be greater than 1,70. So the second test result is expected to be between 5 882 (= $10\ 000/1,70$) cfu per gram and 17 000 (= $100\ 000 \times 1,70$) cfu per gram. For reference materials (capsules containing approximately 5 000 cfu, see [Annex C](#)), the following values may be used:

- $r = 0,17$ (expressed as an absolute difference between \log_{10} -transformed test results); or
- $r = 1,48$ (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

11.3 Reproducibility limit

The absolute difference between two single (\log_{10} -transformed) test results (number of coagulase-positive staphylococci per gram or per millilitre) or the ratio of the higher to the lower of the two test results on the normal scale, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will, in no more than 5 % of cases, exceed the reproducibility limit R .

As a general indication of the reproducibility limit R , the following overall values (medians of values per matrix and contamination level, see [Annex C](#)) can be used when testing food samples in general:

- $R = 0,33$, range [0,27; 0,39] (expressed as a difference between \log_{10} -transformed test results); or
- $R = 2,20$, range [1,86; 2,45] (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

EXAMPLE 1 A test result of 10 000 or $1,0 \times 10^4$ or $\log_{10} 4,00$ coagulase-positive staphylococci per gram of food product was obtained in a first laboratory. Under reproducibility conditions, the difference between \log_{10} -transformed results is not intended to be greater than $\pm 0,33 \log_{10}$ units. So the results from a second laboratory are expected to be between 3,67 ($4,00 - 0,33$) \log_{10} units and 4,33 ($4,00 + 0,33$) \log_{10} units. For non-log-transformed results, the ratio between the test results from this first laboratory and a second laboratory is not intended to be greater than 2,20. So the result from the second laboratory is expected to be between 4 545 (= $10\ 000/2,20$) cfu per gram and 22 000 (= $10\ 000 \times 2,20$) cfu per gram.

EXAMPLE 2 A laboratory wants to know the maximum level it can find, which is still in compliance with a pre-set limit (e.g. a limit of 10^5 or $\log_{10} 5$). For this, the R value (on the log scale) is multiplied by a factor of 0,59¹⁾. The maximum value is 0,19 ($0,33 \times 0,59$) as a difference between \log_{10} -transformed test results or 1,55 ($10^{0,19}$) as a ratio between test results. So, results up to $\log_{10} 5,19$ ($\log_{10} 5 + \log_{10} 0,19$) or 15 500 (= $10\ 000 \times 1,55$) indicate compliance with the limit.

For reference materials (capsules containing approximately 5 000 cfu, see [Annex C](#)), the following values may be used:

- $R = 0,31$ (expressed as a difference between \log_{10} -transformed test results); or
- $R = 2,04$ (expressed as a ratio of the higher to the lower of the two test results on the normal scale).

1) The factor 0,59 reflects the fact that a test with a one-sided 95 % interval is used to test whether the limit is exceeded; it is obtained from the following formula: $0,59 = 1,64 / (1,96 \times \sqrt{2})$.

12 Test report

The test report shall specify, at least the following information/aspects:

- the test method used, with reference to this document, i.e. ISO 6888-2:2021;
- the sampling method used, if known;
- all operating conditions not specified in this document, or regarded as optional, together with details of any incidents that could have influenced the test result(s);
- any deviations from this document;
- all information necessary for the complete identification of the sample;
- the test result(s) obtained after 24 h \pm 2 h or after 48 h \pm 4 h;
- measurement uncertainty
- the date of the test.

13 Quality assurance

The laboratory should have a clearly defined quality control system to ensure that the equipment, reagents and techniques are suitable for the method. The use of positive controls, negative controls and blanks are part of the method. Performance testing of culture media and reagents is specified in [Annex B](#) and described in ISO 11133.

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Annex A (normative)

Flow diagram of the procedure

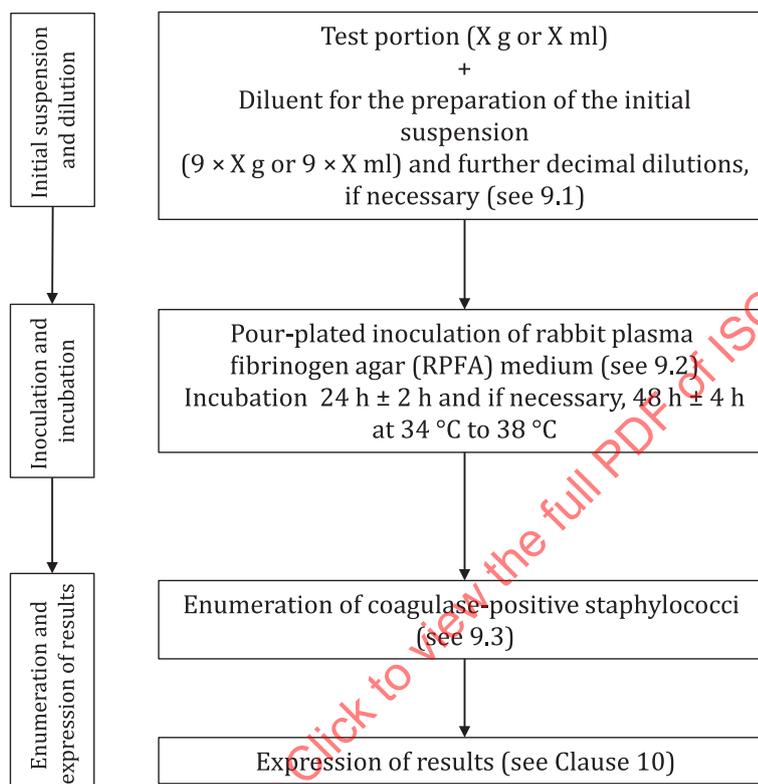


Figure A.1 — Flow diagram of procedure for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) using RPFa medium

Annex B (normative)

Culture media and reagents

B.1 General

The general specifications of ISO 11133 are applicable to the preparation and performance testing of the culture media described in this annex. If culture media or reagents are prepared from dehydrated complete media/reagents or if ready-to-use media/reagents are used, follow the manufacturer's instructions regarding preparation, storage conditions, expiry date and use.

The shelf life of the media and reagents indicated in this annex has been determined in some studies. The user shall verify these under their own storage conditions (in accordance with ISO 11133).

Performance testing of culture media is described in [B.3](#).

B.2 RPFA medium

B.2.1 Base medium

B.2.1.1 Composition

Enzymatic digest of casein	10,0 g
Yeast extract	1,0 g
Meat extract	5,0 g
Sodium pyruvate (CAS Number 113-24-6)	10,0 g
Glycine (CAS Number 56-40-6)	12,0 g
Lithium chloride (CAS Number 7447-41-8)	5,0 g
Agar	12 g to 22 g ^a
Water, to a final volume of	1 000 ml
^a	Depending on the gel strength of the agar.

B.2.1.2 Preparation

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

Adjust the pH ([6.6](#)), if necessary, so that after sterilization it is $7,2 \pm 0,2$ at 25 °C.

Dispense the medium in quantities of 90 ml into flasks or bottles ([6.8](#)) of appropriate capacity.

Sterilize for 15 min in the autoclave ([6.1](#)) set at 121 °C.

Store the medium in closed containers at 5 °C ([6.7](#)) for up to six months.

B.2.2 Solutions for RPFA medium

B.2.2.1 Potassium tellurite solution

B.2.2.1.1 Composition

Potassium tellurite ^a (K ₂ TeO ₃) (CAS Number 7790-58-1)	1,0 g
Water	100 ml
^a It should be ensured beforehand that the potassium tellurite available is suitable for this test (see B.2.2.1.2).	

B.2.2.1.2 Preparation

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

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

Sterilize by filtration using 0,2 µm pore size membranes ([6.9](#)).

The solution may be stored at 5 °C ([6.7](#)) for up to one month.

Discard the solution if a white precipitate forms.

B.2.2.2 Bovine fibrinogen solution

B.2.2.2.1 Composition

Bovine fibrinogen	5 g to 7 g ^a
Sterile water	100 ml
^a Depending on the purity of the bovine fibrinogen.	

B.2.2.2.2 Preparation

Under aseptic conditions, dissolve the bovine fibrinogen in the water just prior to use.

B.2.2.3 Rabbit plasma and trypsin inhibitor solution

B.2.2.3.1 Composition

Rabbit plasma with ethylene diamine tetra acetic acid (EDTA) for coagulase (EDTA coagulase plasma)	30 ml
Trypsin inhibitor	30 mg

B.2.2.3.2 Preparation

Under aseptic conditions, dissolve the trypsin inhibitor in the rabbit plasma, just prior to use.

B.2.3 Complete RPFA medium

B.2.3.1 Composition

Base medium (see B.2.1)	90 ml
Potassium tellurite solution (see B.2.2.1)	0,25 ml
Bovine fibrinogen solution (see B.2.2.2)	7,5 ml
Rabbit plasma and trypsin inhibitor solution (see B.2.2.3)	2,5 ml

B.2.3.2 Preparation

Melt the base medium, then let it cool down to 44 °C to 47 °C in a water bath ([6.3](#)).

Under aseptic conditions, add the three solutions (see [B.2.2.1](#), [B.2.2.2](#) and [B.2.2.3](#)) previously warmed to 44 °C to 47 °C in a water bath. Mix thoroughly after each addition by rotation to minimize foaming.

Use the complete medium immediately after its preparation, in order to avoid any precipitation of the plasma. Hold the molten media for as short a time as possible (see ISO 11133).

WARNING — If a commercially available solution of bovine fibrinogen/rabbit plasma is used, follow with great care the manufacturer's instructions for the preparation of this solution and of the complete medium (in particular the temperature of the base medium). Otherwise, the medium can completely lose its activity.

B.3 Performance testing

The definitions of productivity, selectivity and specificity is specified in ISO 11133. In general, follow the procedures for performance testing described in ISO 11133. Performance testing details are given in [Table B.1](#).

Table B.1 — Performance testing for quality assurance of culture media and reagents

Medium	Type ^e	Function	Incubation	Control strain	WDCM number ^a	Reference media	Method of control	Criteria ^c	Characteristic reaction
RPFA	S	Productivity	(24 ± 2) h / 34 °C to 38 °C	<i>Staphylococcus aureus</i>	00034 ^b 00032	Tryptone soya agar (TSA)	Quantitative	$P_R \geq 0,5$	Black or grey colonies with opacity halo
		Selectivity	(48 ± 4) h / 34 °C to 38 °C	<i>Escherichia coli</i> ^d	00012 or 00013	—	Qualitative	Total inhibition (0)	—
		Specificity	(24 ± 2) h to (48 ± 4) h / 34 °C to 38 °C	<i>Staphylococcus saprophyticus</i> or <i>Staphylococcus epidermidis</i>	00159 ^b 00036	—	Qualitative	1 (weak growth) or 2 (good growth)	If growth, black or grey colonies without opacity halo

^a Refer to the reference strain catalogue on at <http://www.wfcc.info> for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

^b Strain to be used as a minimum by the user laboratory.

^c Growth is categorized as: 0: no growth; 1: weak growth (partial inhibition); 2: good growth; P_R is the productivity ratio (see ISO 11133).

^d Strain free of choice, one of the strains has to be used as a minimum.

^e S: solid medium.