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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 1: Technique using Baird-Parker agar medium

AMENDMENT 2: Inclusion of an alternative confirmation test using RPFA stab method

*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

AMENDEMENT 2: Ajout d'un essai alternatif de confirmation utilisant la méthode de piqûre sur RPFA

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Foreword

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This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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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 1: Technique using Baird-Parker agar medium

AMENDMENT 2: Inclusion of an alternative confirmation test using RPFA stab method

Clause 2

Add the following normative reference.

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

Clause 5

Add the following text.

5.6 Rabbit plasma fibrinogen agar (RPFA) medium

5.6.1 General

Commercially available media, in accordance with this document, can be used. Nevertheless, considering the established variability of manufactured lots of the supplement, it is recommended that each batch of bovine fibrinogen/rabbit plasma solution be tested before use, by running positive and negative controls.

5.6.2 Base medium

Prepare the base medium as stated in 5.3.1, with the exception of the distribution of the base medium, in quantities of 90 ml per flask or bottle.

5.6.3 Solutions

5.6.3.1 Potassium tellurite solution

Prepare the potassium tellurite solution as indicated in 5.3.2.1.

5.6.3.2 Bovine fibrinogen solution

5.6.3.2.1 Composition

Bovine fibrinogen	5 g to 7 g[a]
Sterile water	100 ml

[a] Depending on the purity of the bovine fibrinogen.

5.6.3.2.2 Preparation

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

5.6.3.3 Rabbit plasma and trypsin inhibitor solution

5.6.3.3.1 Composition

Rabbit plasma with EDTA for coagulase (EDTA coagulase plasma)	30 ml
Trypsin inhibitor	30 mg

5.6.3.3.2 Preparation

Operating under aseptic conditions, dissolve the components in the water, just prior to use.

5.6.4 Complete medium

5.6.4.1 Composition

Base medium (5.6.2)	90 ml
Potassium tellurite solution (5.6.3.1)	0,25 ml
Bovine fibrinogen solution (5.6.3.2)	7,5 ml
Rabbit plasma and trypsin inhibitor solution (5.6.3.3)	2,5 ml

5.6.4.2 Preparation

Melt the base medium, then let it cool down to 47 °C to 50 °C in a water bath (6.4) because at a lower temperature, this medium solidifies.

Under aseptic conditions, add the three solutions previously warmed to 48 °C ± 1 °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.

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.

5.6.5 Preparation of RPFA plates

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