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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of *Clostridium perfringens* — Colony-count technique

Microbiologie des aliments — Méthode horizontale pour le dénombrement de Clostridium perfringens *— Technique 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 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 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 7937 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition cancels and replaces the second edition (ISO 7937:1997, EN 13401:1999), which has been technically revised.

Introduction

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 that 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 International Standard 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 groups 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 international Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of *Clostridium perfringens* — Colony-count technique

1 Scope

This International Standard describes a horizontal method for the enumeration of viable *Clostridium perfringens.* It is applicable to

— products intended for human consumption and the feeding of animals, and

— environmental samples in the area of food production and food handling.

2 Normative references

The following referenced documents are indispensable for the application 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 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products meat and meat products, and fish and fishery products*

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

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1 : General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
Clostridium perfringens
C. perfringens
bacteria that form characteristic colonies (black precipitate, caused by the reduction of sulfite to sulfide, which colours the colonies black) in the specified selective medium, and which give positive confirmatory reactions when the test is carried out by either of two techniques specified in this International Standard

3.2
enumeration of *C. perfringens*
determination of the number of culturable and confirmed *Clostridium perfringens* bacteria per millilitre or per gram of sample when the test is carried out by the method specified in this International Standard

4 Principle

4.1 Petri dishes are inoculated with a specified quantity of the test sample if the initial product is liquid, or a specified quantity of the initial suspension in the case of other products.

Further Petri dishes are inoculated, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

A selective medium is added (poured-plate technique) and then an overlay of the same medium.

4.2 The plates are incubated anaerobically at 37 °C for 20 h ± 2 h.

4.3 The characteristic colonies are enumerated.

4.4 The numbers of characteristic colonies are confirmed and the number of *C. perfringens* per millilitre or per gram of sample is calculated.

5 Diluent, culture media and reagents

See ISO 7218, ISO/TS 11133-1 and ISO/TS 11133-2 for the preparation, production and performance testing of culture media.

5.1 Diluent

See the relevant part of ISO 6887 or ISO 8261.

5.2 Sulfite-cycloserine agar (SC)

NOTE This was originally designated “egg-yolk-free TSC” (see [1]).

5.2.1 Base

5.2.1.1 Composition

Enzymatic digest of protein	15,0 g
Enzymatic digest of soya	5,0 g
Yeast extract	5,0 g
Disodium disulfite ($Na_2S_2O_5$), anhydrous	1,0 g
Ammonium iron(III) citrate[a]	1,0 g
Agar	9,0 g to 18,0 g[b]
Water	1 000 ml

[a] This reagent should contain at least 15 % (mass fraction) of iron.
[b] Depending on the gel strength of the agar.

5.2.1.2 Preparation

Dissolve the components in the water by boiling. Adjust the pH so that after sterilization it will be 7,6 ± 0,2 at 25 °C. Dispense the base into flasks or bottles of appropriate capacity. Sterilize for 15 min at 121 °C. Store in a refrigerator at 5 °C ± 3 °C. Discard unused medium 2 weeks after preparation.

In some cases (see 9.4.3.1), it may be necessary to prepare dishes of SC agar base medium for confirmation with the nitrate motility medium (5.5) and the lactose-gelatin medium (5.8). For this purpose, transfer portions of about 15 ml of the base [melted and cooled to approximately 44 °C to 47 °C using a water bath (6.10)] into Petri dishes and allow to solidify. Immediately before use, dry the plates (see ISO 7218).

5.2.2 D-Cycloserine solution

5.2.2.1 Composition

D-Cycloserine[a]	4,0 g
Water	100 ml

[a] Use white crystalline powder only.

5.2.2.2 Preparation

Dissolve the D-cycloserine in the water and sterilize the solution by filtration.

Store in a refrigerator at 3 °C ± 2 °C.

Discard unused solution 4 weeks after preparation.

5.2.3 Complete medium

Immediately before use in the pour-plate method (see 9.2), to each 100 ml of sterile molten base (5.2.1) cooled to 44 °C to 47 °C, add 1 ml of D-cycloserine solution (5.2.2).

5.2.4 Performance testing for the quality assurance of SC medium

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. To check the performance, refer to ISO/TS 11133-2:2003, Table B.1 [see TS(C)].

5.3 Fluid thioglycollate medium

5.3.1 Composition

Enzymatic digest of casein	15,0 g
L-Cystine	0,5 g
D-Glucose	5,5 g
Yeast extract	5,0 g
Sodium chloride	2,5 g
Sodium thioglycollate (mercaptoacetate)	0,5 g
Agar	0,5 g to 2,0 g[a]
Resazurin	0,001 g
Water	1 000 ml

[a] Depending on the gel strength of the agar.

5.3.2 Preparation

Dissolve the components in the water by boiling. Adjust the pH so that after sterilization it will be 7,1 ± 0,2 at 25 °C.

Dispense 10 ml portions into tubes and sterilize at 121 °C for 15 min.

Before use, this medium shall be de-aerated.

5.3.3 Performance testing for the quality assurance of thioglycollate broth

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. To check the performance, refer to ISO/TS 11133-2:2003, Table B.4.

5.4 Lactose sulfite medium (LS) (optional)

5.4.1 Base medium

5.4.1.1 Composition

Enzymatic digest of casein	5,0 g
Yeast extract	2,5 g
Sodium chloride	2,5 g
Lactose	10 g
L-Cysteine hydrochloride	0,3 g
Water	1 000 ml

5.4.1.2 Preparation

Dissolve the components in the water by boiling (if necessary). Adjust the pH so that after sterilization it will be 7,1 ± 0,2 at 25 °C.

Dispense 8 ml portions into test tubes with inverted Durham tubes (6.7) and sterilize at 121 °C for 15 min.

The medium may be stored at 3 °C ± 2 °C for up to 4 weeks.

5.4.2 Disodium disulfite solution

5.4.2.1 Composition

Disodium disulfite ($Na_2S_2O_5$), anhydrous	1,2 g
Water	100 ml

5.4.2.2 Preparation

Dissolve the disodium disulfite in the water and sterilize the solution by filtration.

Use the solution within a day.

5.4.3 Ammonium iron(III) citrate solution

5.4.3.1 Composition

Ammonium iron(III) citrate	1 g
Water	100 ml

5.4.3.2 Preparation

Dissolve the ammonium iron(III) citrate in the water and sterilize the solution by filtration.

Use the solution within a day.

5.4.4 Complete medium

If the medium is not used on the day of preparation, just prior to completion de-aerate the medium by heating and then cool rapidly. If the medium is in screw-cap bottles, loosen the caps before heating and tighten them before cooling.

Then add 0,5 ml of the disodium disulfite solution (5.4.2) and 0,5 ml of the ammonium iron(III) citrate solution (5.4.3) to each 8 ml of base (5.4.1).

Use the complete medium within a day.

5.5 Nitrate motility medium (optional)

5.5.1 Composition

Enzymatic digest of casein	5,0 g
Meat extract	3,0 g
Galactose	5,0 g
Glycerol	5,0 g
Potassium nitrate (KNO_3)	1,0 g
Disodium hydrogen orthophosphate (Na_2HPO_4)	2,5 g
Agar	1,0 g to 5,0 g[a]
Water	1 000 ml

[a] Depending on the gel strength of the agar.