INTERNATIONAL STANDARD

ISO 7937

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

Microbiologie des aliments — Méthode horizontale pour le dénombrement de Clostridium perfringens — Technique par comptage des colonies

Reference number
ISO 7937:1997(E)

Forword

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.

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

This second edition cancels and replaces the first edition (ISO 7937:1985), which has been technically revised.

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Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products for which it may be necessary to use different methods. Nevertheless, in all cases, every attempt should be made to apply this horizontal method as far as possible and deviations from this should 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 this horizontal method has been followed and the reasons for deviations from it 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.

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

1 Scope

This International Standard describes a horizontal method for the enumeration of viable *Clostridium perfringens* in products intended for human consumption or the feeding of animals.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 ***Clostridium perfringens***: Bacteria that form characteristic colonies (surrounded by a black halo) in the specified selective medium and which give positive confirmatory reactions when the test is carried out by the method specified in this International Standard.

NOTE — For practical reasons, this definition of *Clostridium perfringens* does not exclusively describe strains of *C. perfringens*. In particular, the confirmatory tests are inadequate to distinguish between *C. perfringens* and other closely related but less commonly encountered *Clostridium* species such as *C. paraperfringens* and *C. absonum*.

3.2 enumeration of *C. perfringens*: Determination of the number of viable 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 Inoculation of Petri dishes 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.

Inoculation, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

Mixing with a selective medium (poured-plate technique) and adding an overlay of the same medium.

4.2 Anaerobic incubation of the plates at 35 °C or 37 °C for 20 h. The temperature shall be agreed between the parties concerned and recorded in the test report.

4.3 Enumeration of the characteristic colonies.

4.4 Confirmation of the number of characteristic colonies and calculation of the number of *C. perfringens* per millilitre or per gram of sample.

5 Diluent, culture media and reagents

5.1 General

See ISO 7218.

5.2 Diluent

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

5.3 Egg-yolk-free tryptose-sulfite-cycloserine agar (SC) [1)]

5.3.1 Base

5.3.1.1 Composition

Tryptose [a)]	15,0 g
Soytone [b)]	5,0 g
Yeast extract	5,0 g
Disodium disulfite ($Na_2S_2O_5$), anhydrous	1,0 g
Ammonium iron(III) citrate [b)]	1,0 g
Agar	9,0 g to 18,0 g [c)]
Water	1 000 ml

a) The names tryptose and soytone are used at present only by certain producers of media. Any other pancreatic casein or soybean digest giving comparable results may be used.

b) This reagent should contain at least 15 % (*m*/*m*) of iron.

c) Depending on the gel strength of the agar.

1) This was originally designated EY-free TSC (Hauschild and Hilsheimer, *Appl. Microbiol.*, **27**, 1974, pp. 78-82).

5.3.1.2 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.

5.3.2 D-Cycloserine solution

5.3.2.1 Composition

D-Cycloserine [a]	4,0 g
Water	100 ml
[a] Use white crystalline powder only.	

5.3.2.2 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.

5.3.3 Complete medium

Immediately before use in the pour-plate method (see 9.2) add, to each 100 ml of sterile molten base (5.3.1) cooled to 47 °C ± 2 °C, 1 ml of D-cycloserine solution (5.3.2).

5.4 Fluid thioglycollate medium

5.4.1 Composition

Enzymatic digest of casein	15,0 g
L-Cysteine	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.	

5.4.2 Preparation

Dissolve the components in the water by boiling.

Adjust the pH so that after sterilization it is 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.

5.5 Lactose sulfite medium (LS)

5.5.1 Base medium

5.5.1.1 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

5.5.1.2 Preparation

Dissolve the components in the water by boiling (if necessary).

Adjust the pH so that after sterilization it is 7,1 ± 0,2 at 25 °C.

Dispense 8 ml portions into test tubes or bottles 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.

5.5.2 Disodium disulfite, anhydrous solution

5.5.2.1 Composition

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

5.5.2.2 Preparation

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

Use the solution within a day.

5.5.3 Ammonium iron(III) citrate solution

5.5.3.1 Composition

Ammonium iron(III) citrate	1 g
Water	100 ml

5.5.3.2 Preparation

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

Use the solution within a day.

5.5.4 Complete medium

If the medium is not used on the day of the 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 (5.5.2) and 0,5 ml of the ammonium iron(III) citrate solution (5.5.3) to each 8 ml of base (5.5.1).

6 Apparatus and glassware

Usual microbiological equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)**

See ISO 7218.

6.2 Incubator, capable of being maintained at 35 °C ± 1 °C or 37 °C ± 1 °C, depending on the temperature agreed.

6.3 Anaerobic jars or any other apparatus appropriate for anaerobic culture.

6.4 pH-meter, capable of being read to the nearest ± 0,01 pH unit at 25 °C, enabling measurements to be made which are accurate to 0,1 pH unit.

6.5 Loops, of platinium-iridium or nickel-chromium, of diameter approximately 3 mm, and **stab-inoculation needle** of the same material.

6.6 Filtration apparatus, for sterilization of solutions.