SLOVENSKI STANDARD
SIST EN 13401:1999

01-december-1999

Mikrobiologija živil in krmil - Horizontalna metoda za štetje Clostridium perfringens - Tehnika štetja kolonij (ISO 7937:1997, spremenjen)

Microbiology of food and animal feeding stuffs - Horizontal method for enumeration of Clostridium perfringens - Colony-count technique (ISO 7937:1997 modified)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zur Zählung von Clostridium perfringens - Koloniezählverfahren (ISO 7937:1997 modifiziert)

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Microbiologie des aliments - Méthode horizontale pour le dénombrement de Clostridium perfringens - Méthode par comptage des colonies (ISO 7937:1997 modifiée)

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Ta slovenski standard je istoveten z: **EN 13401:1999**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST EN 13401:1999 **en**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 13401

April 1999

ICS 07.100.30

English version

Microbiology of food and animal feeding stuffs - Horizontal method for enumeration of Clostridium perfringens - Colony-count technique (ISO 7937:1997 modified)

Microbiologie des aliments - Méthode horizontale pour le dénombrement de Clostridium perfringens - Méthode par comptage des colonies (ISO 7937:1997 modifiée)

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This European Standard was approved by CEN on 1 April 1999.

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Foreword

This European Standard has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 1999, and conflicting national standards shall be withdrawn at the latest by October 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

In view of the Voting results, the Technical Board of CEN decided not to take over ISO 7937:1997 unchanged but to publish this European Standard EN 13401 allowing, for the step of confirmation of the colonies, the use of the procedure as described in ISO 7937:1997 (using LS medium), or the procedure as described in the former version of ISO 7937:1985 (using the motility-nitrate medium and the lactose-gelatine medium).

The text of the International Standard ISO 7937:1997 was approved by CEN as a European Standard with agreed common modifications as indicated by a vertical line in the left margin of the text.

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

1 Scope

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

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2 Normative references

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This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

ISO 6887	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

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3 Definitions

For the purposes of this European 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 either of two techniques specified in this European 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 European Standard.

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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.

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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.

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.

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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 [a)]	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.

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5.3.1.2 Preparation

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5.3.1.2.1 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 + 3 °C ± 2 °C.

Discard unused medium 2 weeks after preparation.

5.3.1.2.2 In some cases (see 9.4.3.1), it may be necessary to prepare dishes of SC agar base medium for confirmation with the motility-nitrate medium (5.6) and the lactose-gelatine medium (5.7).

To this purpose, transfer portions of about 15 ml of the base, melted and cooled to approximately 47 °C (with a water bath (6.10)) into Petri dishes and allow to solidify.

Immediately before use, dry the plates with a drying cabinet, or an oven (6.13).

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

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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).

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5.4 Fluid thioglycollate medium

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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.

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5.5 Lactose sulfite medium (LS) (if necessary)

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	10,0 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.

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

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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	1,0 g
Water	100 ml

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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 base 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 base 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).

5.6 Motility-nitrate medium (if necessary)

5.6.1 Composition

Peptone	5,0 g
Beef extract	3,0 g
Galactose	5,0 g
Glycerol	5,0 g
Potassium nitrate (KNO_3)	1,0 g
Disodium hydrogenorthophosphate (Na_2HPO_4)	2,5 g
Agar	1,0 g to 5,0 g [1)]
Water	1 000 ml
1) Depending on the gel-strength of the agar.	

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5.6.2 Preparation

Dissolve the components in the water by boiling.

Adjust the pH so that it will be 7,3 at 25 °C after sterilization.

Transfer the medium to culture tubes in 10 ml quantities and sterilize at 121 °C for 15 min.

If not used the same day, store in a refrigerator at (4 ± 2) °C ; just prior to use, heat in boiling water or flowing steam for 15 min and then cool rapidly to the incubation temperature.

Discard unused medium 4 weeks after preparation.