SLOVENSKI STANDARD
SIST-TS CEN ISO/TS 17919:2014

01-januar-2014

Mikrobiologija v prehranski verigi - Polimerazna verižna reakcija (PCR) za ugotavljanje prisotnosti patogenih mikroorganizmov v živilih - Ugotavljanje prisotnosti klostridijev, ki tvorijo botulinusne nevrotoksine tipov A, B, E in F (ISO/TS 17919:2013)

Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Detection of botulinum type A, B, E and F neurotoxin-producing clostridia (ISO/TS 17919:2013)

Mikrobiologie von Lebensmitteln, Futtermitteln und Umgebungsproben - Polymerase-Kettenreaktion (PCR) zum Nachweis von pathogenen Mikroorganismen in Lebensmitteln - Nachweis von Botulinum-Neurotoxin-Typ A, B, E und F produzierenden Clostridien (ISO/TS 17919:2013)

Microbiologie de la chaîne alimentaire - Réaction de polymérisation en chaîne (PCR) pour la détection de micro-organismes pathogènes dans les aliments - Détection des clostridies productrices de neurotoxine botulique de type A, B, E et F (ISO/TS 17919:2013)

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Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Detection of botulinum type A, B, E and F neurotoxin-producing clostridia (ISO/TS 17919:2013)

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Foreword

This document (CEN ISO/TS 17919:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN.

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The text of ISO/TS 17919:2013 has been approved by CEN as CEN ISO/TS 17919:2013 without any modification.

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Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Detection of botulinum type A, B, E and F neurotoxin-producing clostridia

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Foreword

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ISO/TS 17919 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275 *Food analysis — Horizontal methods*, in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

Botulinum neurotoxin-producing clostridia are ubiquitous in the environment. Botulism is a severe neuroparalytic disease resulting from the action of botulinum neurotoxins (BoNTs). Seven different serotypes of BoNTs (type A to G) and a number of subtypes have been identified to date.

BoNT type A (BoNT/A), type B (BoNT/B), type E (BoNT/E) and type F (BoNT/F) are mainly responsible for botulism in humans and the genes encoding these toxins are the targets of this Technical Specification. BoNT type A, B, E, and F-producing clostridia exist in four physiologically distinct groups (Group I *Clostridium botulinum*, Group II *C. botulinum*, *C. baratii*, *C. butyricum*).

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Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Detection of botulinum type A, B, E and F neurotoxin-producing clostridia

1 Scope

This Technical Specification specifies a horizontal method for the molecular detection of clostridia carrying botulinum neurotoxin A, B, E, and F genes by a PCR method. This method detects the genes and not the toxins, therefore a positive result does not necessarily mean the presence of these toxins in the sample investigated. This Technical Specification is applicable to products for human consumption, animal feed, and environmental samples.

The PCR assays for detection of genetic sequences encoding specific toxin types are described in Annexes B and C.

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 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*

ISO 20837, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for sample preparation for qualitative detection*

ISO 20838:2006, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods*

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Terms and definitions

For the purpose of this document, the terms and definitions given in ISO 22174 apply.

4 Symbols and abbreviated terms

4.1 Symbols

c substance concentration

ρ mass concentration

φ volume fraction

w mass fraction

4.2 Abbreviated terms

BoNT botulinum neurotoxin

5 Principle

5.1 General

The method comprises the following consecutive steps:

a) microbial enrichment (see 5.2);

b) nucleic acid extraction (see 5.3);

c) amplification (see 5.4);

d) detection of PCR products (see 5.5);

e) confirmation (see 5.6).

NOTE Real-time-PCR combines steps c) to e).

5.2 Microbial enrichment

The number of BoNT-producing clostridia (spores or vegetative cells) to be detected is increased by encouraging their germination and growth in non-selective liquid nutrient medium tryptone–peptose–glucose–yeast extract broth under anaerobic conditions.

5.3 Nucleic acid extraction

Bacterial cells are separated from the nutrient medium, lysed and the nucleic acids are extracted for use in the PCR reaction.

5.4 Amplification by PCR

The extracted nucleic acid is transferred to the PCR mix and the amplification is carried out in a thermal cycler.

5.5 Detection of PCR products

PCR products are detected by gel electrophoresis or an appropriate alternative.

5.6 Confirmation

The identity of the PCR products shall be confirmed by any appropriate method, e.g. sequencing, hybridization or restriction analysis.

6 Reagents

6.1 General

For all stages 5.1 b) to e), use only reagents of recognized analytical grade and consumables suitable for molecular biology applications as specified in ISO 20837 and ISO 20838.

Reagent requirements specified in ISO 20838:2006, Clause 5, apply.

6.2 Culture media

6.2.1 General

Follow ISO 11133 for the preparation, production and performance testing of culture media.

6.2.2 Diluent

Follow ISO 6887-1 and the relevant part of ISO 6887[9]–[13] dealing with the product to be examined.

6.2.3 Non–selective enrichment culture medium, tryptone–peptone–glucose–yeast extract broth (TPGY broth) (Reference [7])

6.2.3.1 General

Other approved non-selective enrichment culture media can be used provided equivalent performance is shown.

6.2.3.2 Composition and pH

Tryptone	50 g
Peptone	5 g
Yeast extract	20 g
D-Glucose	4 g
Sodium thioglycolate, $HSCH_2COONa$	1 g
Water	to 1 000 ml

pH 7,0 ± 0,2

6.2.3.3 Preparation

Dissolve the components in the water by boiling. After sterilization, adjust to pH 7,0 ± 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 4 weeks after preparation.

6.2.4 TPGY broth buffered — for acidic and acidifying foodstuffs only

6.2.4.1 Stock solution (phosphate buffer)

6.2.4.1.1 Solution 1