SLOVENSKI STANDARD
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Mikrobiologija v prehranski verigi - Metode ugotavljanja prisotnosti Anisakidae L3 larv v ribah in ribjih proizvodih - 2. del: Umetna digestivna metoda (ISO/DIS 23036-1:2019)

Microbiology of the food chain - Methods for the detection of Anisakidae L3 larvae in fish and fishery products - Part 2: Artificial digestion method (ISO/DIS 23036-2:2019)

Mikrobiologie der Lebensmittelkette - Verfahren zum Nachweis von Anisakidae L3-Larven in Fisch und Fischereierzeugnissen - Teil 2: Verfahren der künstlichen Verdauung (ISO/DIS 23036-2:2019)

Microbiologie de la chaîne alimentaire - Méthodes de recherche des larves L3 d'Anisakidae dans les poissons et produits de la pêche - Partie 2: Méthode de digestion artificielle (ISO/DIS 23036-2:2019)

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Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —

Part 2:
Artificial digestion method

Microbiologie de la chaîne alimentaire — Méthodes de recherche des larves L3 d'Anisakidae dans les poissons et produits de la pêche —

Partie 2: Méthode de digestion artificielle

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

A list of all parts in the ISO 23036 series can be found on the ISO website.

Introduction

Nematodes of the Anisakidae family, have a complex life cycle involving a high number of hosts. Adult stages of Anisakidae reside in the stomach of marine mammals, where they are embedded in the mucosa. Unembryonated eggs produced by adult females are released with the faeces of marine mammals and become embryonated in seawater, where first-stage larvae (L1) develop in the eggs. The larvae moult to become free-swimming second stage larvae (L2) and if ingested by crustaceans, mature into L3 stage. This stage is infective to fish and squid, and larvae are transferred between fishes through predation, maintaining the L3 stage. Upon the host's death, some larvae migrate from the abdominal cavity into muscle tissues. Humans are incidental hosts and may be infected after ingesting raw or undercooked infected fish or cephalopods containing viable L3.

Nematodes of the family Anisakidae, are the causative agents of human anisakidosis, a disease that not only is a public health hazard affecting humans, but also represents an economic problem in fishery and food safety (the term anisakiasis, designating the disease caused by members of the genus *Anisakis*, has been used by some authors as well). Worldwide, marine and wild anadromous fishes are intermediate hosts of Anisakidae, whereas marine mammals are the definitive hosts.

Visual inspection procedures for the detection of Anisakidae larvae in fish are employed to minimize the risk that contaminated fish will reach the consumer, thus preventing human anisakidosis.

The UV-press and the artificial digestion of the fish muscular tissue are the methods specifically designed to detect nematode larvae in fish and to evaluate the infestation level of a batch, and have been validated and tested in multicenter collaborative studies (see clause 11).

Alternative methods can be used for analysis, provided their equivalence with the methods described in this standard are demonstrated.

Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —

Part 2: Artificial digestion method

1 Scope

This part of ISO 23036 specifies a method that is applicable for the detection of Anisakidae L3 larvae commonly found in marine and anadromous fishes. The method can be applied to fresh fish and/or frozen fish, as well as lightly processed fish products, such as marinated, salted or smoked. It is also suitable for visceral organs as confirmatory method for visual inspection scheme.

The artificial digestion method allows quantifying parasitic infections by estimating the number of parasites in the fish musculature and, when applied to fresh fish or lightly processed fish products (never frozen before processing), determining the viability of Anisakidae L3, which may be present.

This method doesn't allow determining species or genotype of detected parasites. Final identification is made by morphological and/or molecular methods.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

CODEX STAN 244, 2004, Standard for salted Atlantic herring and salted sprat. CODEX STAN 244-2004.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
Anisakidae L3 larvae
Stage 3 larvae (L3) belonging to the Anisakidae family, in particular to *Anisakis*, *Contracaecum* and *Pseudoterranova* genera

Note 1 to entry: for practical purposes, the genus *Hysterothylacium* belonging to Raphidascarididae family and already classified as Anisakidae, can be also included.

3.2
Artificial digestion assay
Method to detect Anisakidae larvae in fish muscle tissue by an enzymatic (pepsin-HCl) digestion step to release larvae from muscle tissues followed by a filtration step and detection of larvae by microscopy. If applied to fresh fish, it allows checking of the viability of larvae.

4 Principle

4.1 General

The artificial digestion method relies on enzymatic degradation of muscle fibers in a fluid composed of pepsin and hydrochloric acid followed by filtration and washing steps.

The procedure allows a differentiation between dead and viable anisakid larvae if the temperature of the digestion solution does not exceed 37 °C (with the exception of *Hysterothylacium* sp. larvae, which are killed at 37 °C), and assuming the fish was never frozen.

There are no internal quality controls that can be used while performing the method.

4.2 Sample size

For inspection of fishery products for public health purposes, the sample size shall be risk-based, as determined by the competent authority.

4.3 Sample preparation

To increase the surface area for enzymatic degradation, samples are gently eased apart taking care not to disrupt larvae by checking for them. Alternatively, a smasher/stomacher, that facilitates the digestion but does not damage the nematode larvae, can be used.

Note Blending or grinding procedure should be avoided as they can damage or disrupt larvae.

4.4 Digestion of the sample

Viable Anisakidae larvae are resistant to the pepsin-HCl digest fluid and therefore can be recovered free from muscle tissues.

To facilitate an efficient and rapid digestion, a maximum ratio of 1:20, meat to digest fluid, and a temperature of 37 °C ± 1 °C, shall be maintained throughout the process. The time required for digestion shall be 15 min to 30 min, but in case of muscle samples which are less digestible, the digestion time should be increased but, unless otherwise validated for a particular sample matrix, shall not exceed 45 min.

4.5 Filtration of the digest fluid

Following digestion, the digest fluid shall be filtered through a sieve with specific mesh (6.11), and retained larvae shall be rinsed with tap water.

4.6 Verification of findings

If positive or doubtful findings occur, confirmation and identification at the species level should be performed by a qualified reference laboratory, by means of morphological and/or molecular methods.

5 Reagents

5.1 Tap water.