SLOVENSKI STANDARD
oSIST prEN ISO 23036-1:2020

01-februar-2020

Mikrobiologija v prehranski verigi - Metode ugotavljanja prisotnosti Anisakidae L3 larv v ribah in ribjih proizvodih - 1. del: UV metoda s stiskanjem (ISO/DIS 23036-1:2019)

Microbiology of the food chain - Methods for the detection of Anisakidae L3 larvae in fish and fishery products - Part 1: UV-press method (ISO/DIS 23036-1:2019)

Mikrobiologie der Lebensmittelkette - Verfahren zum Nachweis von Anisakidae L3-Larven in Fisch und Fischereierzeugnissen - Teil 1: UV-Pressverfahren (ISO/DIS 23036-1:2019)

Microbiologie de la chaîne alimentaire - Méthodes de détection des larves L3 d'Anisakidae dans le poisson et les produits de la pêche - Partie 1: Méthode presse/UV (ISO/DIS 23036-1:2019)

Ta slovenski standard je istoveten z: **prEN ISO 23036-1**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology
67.120.30	Ribe in ribji proizvodi	Fish and fishery products

oSIST prEN ISO 23036-1:2020 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 23036-1

ISO/TC **34**/SC **9**

Secretariat: **AFNOR**

Voting begins on:
2019-11-04

Voting terminates on:
2020-01-27

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

Part 1:
UV-press method

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

Partie 1: Méthode avec presse et lumière UV

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 23036-1:2019(E)

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Published in Switzerland

Contents

Page

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 fish muscle tissues 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 multicentre collaborative studies (see clause 10).

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 1: UV-press 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 cold smoked.

This method allows quantifying parasitic infections by estimating the number of parasites in the fish musculature.

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*

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
UV- press method
Method to detect Anisakidae larvae in fish muscle tissue by UV examination after pressing and freezing

Note 1 to entry: Under UV light, L3 appear as brightly fluorescent spots of different colours, partially depending on the anisakid species.

4 Principle

The UV-press method relies on the peculiar feature of frozen Anisakidae larvae, which show fluorescence under UV light, due to the presence of the pigment ("lipochrome") Lipofuscin. Nematode detection is based on screening under UV-light of flattened and frozen fillets, of either fresh or thawed fish. The method may also be used for parasite inspection of larger fish such as farmed Atlantic salmon (*Salmo salar*), cod (*Gadus* spp.) or halibut (*Hippoglossus hippoglossus*). In these cases, each fillet or fish side has to be cut into smaller pieces which are then processed and examined separately. Samples are placed in clear plastic bags, pressed to a 1 mm to 2 mm thin layer and then frozen. After freezing and subsequent thawing of the fillets, visual inspection is carried out by examining each bag containing fillet under a 366 nm UV-light source. Any anisakid nematode larva will appear as a brightly fluorescent spot, so that it can be easily recorded and approximate site of infection may be determined.

Empirical evidence suggests that Anisakidae larvae killed by cooking, salting, and pickling, show brilliant fluorescence without prior freezing, therefore the method can be used to detect nematodes in such products.

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

5 Equipment and consumables

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

5.1 Press-vacuum system, an automatic or manually operated hydraulic pressing device at 7 bar to 8 bar.

5.2 Transparent plastic bags of the appropriate thickness and size (e.g., 300 x 700 x 0,075 mm LDPE), the size of the plastic bag depends on the size of both sample and pressing device (e.g., for herring fillets bags of 700 mm length and 300 mm width can be used).

5.3 UV-light transilluminator (366 nm).

5.4 – 20 °C freezer.

6 Sampling

Sampling is not part of the method specified in this International Standard, but is indicated in Annex A. If there are no specific International Standards dealing with sampling of fish, it is recommended that the interested parties come to an agreement on this subject.

7 Procedure

Warnings General safety measures should be addressed when working with UV (e.g., avoid prolonged skin contact); eye protection or a UV protecting glass sheet installed in the viewing chamber, appropriate for the type of UV lamp, should be used. Specific attention should be observed when handling pressing devices, in order to avoid any harm to the personnel performing the test.

7.1 Weighing the sample.

Each sample shall be weighed and its weight shall be recorded.

