

Microbiology of the food chain - Methods for the detection of Anisakidae L3 larvae in fish and fishery products - Part 1: UV-press method (ISO 23036-1:2021)

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

|   |  |
|---|--|
| See Eesti standard EVS-EN ISO 23036-1:2021 sisaldab Euroopa standardi EN ISO 23036-1:2021 ingliskeelset teksti.     | This Estonian standard EVS-EN ISO 23036-1:2021 consists of the English text of the European standard EN ISO 23036-1:2021.                            |
| Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas   | This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation. |
| Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 12.05.2021. | Date of Availability of the European standard is 12.05.2021.   |
| Standard on kättesaadav Eesti Standardimis- ja Akrediteerimiskeskusest.   | The standard is available from the Estonian Centre for Standardisation and Accreditation.  |

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile [standardiosakond@evs.ee](mailto:standardiosakond@evs.ee).

ICS 07.100.30

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardimis- ja Akrediteerimiskeskusele. Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardimis- ja Akrediteerimiskeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardimis- ja Akrediteerimiskeskusega: Koduleht [www.evs.ee](http://www.evs.ee); telefon 605 5050; e-post [info@evs.ee](mailto:info@evs.ee)

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation and Accreditation. No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation and Accreditation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation and Accreditation: Homepage [www.evs.ee](http://www.evs.ee); phone +372 605 5050; e-mail [info@evs.ee](mailto:info@evs.ee)

English Version

Microbiology of the food chain - Methods for the detection  
of Anisakidae L3 larvae in fish and fishery products - Part  
1: UV-press method (ISO 23036-1:2021)

Microbiologie de la chaîne alimentaire - Méthodes de  
recherche des larves L3 d'Anisakidae dans le poisson et  
les produits de la pêche - Partie 1: Méthode presse/UV  
(ISO 23036-1:2021)

Mikrobiologie der Lebensmittelkette - Verfahren zum  
Nachweis von Anisakidae L3-Larven in Fisch und  
Fischereierzeugnissen - Teil 1: UV-Pressverfahren (ISO  
23036-1:2021)

This European Standard was approved by CEN on 24 March 2021.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

## European foreword

This document (EN ISO 23036-1:2021) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 "Microbiology of the food chain" the secretariat of which is held by AFNOR.

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 November 2021, and conflicting national standards shall be withdrawn at the latest by November 2021.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## Endorsement notice

The text of ISO 23036-1:2021 has been approved by CEN as EN ISO 23036-1:2021 without any modification.

# Contents

|  | Page      |
|--|-----------|
| <b>Foreword</b> .....  | <b>iv</b> |
| <b>Introduction</b> .....  | <b>v</b>  |
| <b>1 Scope</b> .....   | <b>1</b>  |
| <b>2 Normative references</b> .....  | <b>1</b>  |
| <b>3 Terms and definitions</b> .....   | <b>1</b>  |
| <b>4 Principle</b> .....   | <b>2</b>  |
| <b>5 Equipment and consumables</b> .....   | <b>2</b>  |
| <b>6 Sampling</b> .....  | <b>2</b>  |
| <b>7 Procedure</b> .....   | <b>2</b>  |
| 7.1 Weighing the sample.....   | 2         |
| 7.2 Preparation of the sample.....   | 3         |
| 7.3 Pressing.....  | 3         |
| 7.4 Freezing.....  | 3         |
| 7.5 Thawing.....   | 3         |
| 7.6 Visual inspection.....   | 3         |
| <b>8 Expression of the results</b> .....   | <b>4</b>  |
| <b>9 Performance characteristics of the method</b> .....   | <b>4</b>  |
| <b>10 Test report</b> .....  | <b>4</b>  |
| <b>11 Quality assurance</b> .....  | <b>4</b>  |
| <b>Annex A (informative) Sample collection</b> .....   | <b>5</b>  |
| <b>Annex B (informative) Findings after UV-press method</b> .....  | <b>6</b>  |
| <b>Annex C (informative) Example of a laboratory worksheet for recording data when testing fish fillets with the UV-press method</b> ..... | <b>8</b>  |
| <b>Bibliography</b> .....  | <b>9</b>  |

## Foreword

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.

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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of 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 [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## 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 third-stage larvae (L3). This stage is infective to fish and squid, and larvae are transferred between fishes through predation, maintaining the L3 stage. Some larvae migrate from the abdominal cavity into muscle tissues. Humans are incidental hosts and can 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 is not only 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*, is also sometimes used). 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,<sup>[1],[2]</sup> 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<sup>[3]</sup> (see [Clause 9](#)).

# 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 document specifies a method for the detection of Anisakidae L3 larvae commonly found in marine and anadromous fishes. The method is applicable to fresh fish and/or frozen fish, as well as lightly processed fish products, such as marinated, salted or cold smoked.

This method is applicable to quantifying parasitic infections by estimating the number of parasites in the fish musculature.

This method does not apply to determining the 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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

#### 3.1

##### **Anisakidae L3 larvae**

third-stage 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.