European Union Reference Laboratory for Parasites
Unit of Foodborne and Neglected Parasitic Diseases
Department of Infectious Diseases
ISTITUTO SUPERIORE DI SANITÀ

15th Proficiency Test on the detection of *Anisakis spp.* L3 larvae in fish fillets

Antonio Di Grazia, Marco Lalle

XXI Workshop of National Reference Laboratories for Parasites
28th and 29th october 2026
Istituto Superiore di Sanità



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets- 2026

✓ Identification of the presence of Anisakidae L3 larvae in fish fillets







✓ PT is accredited according to the ISO 17043

INTERNATIONAL STANDARD **ISO/IEC 17043**

First edition
2010-02-01

ACCREDIA
L'ENTE ITALIANO DI ACCREDITAMENTO

CERTIFICATO DI ACCREDITAMENTO
Accreditation Certificate

ACCREDITAMENTO N. / *ACCREDITATION N.* **0005P** REV. **05**

EMESSO DA / *ISSUED BY* **DIPARTIMENTO LABORATORI DI PROVA**

SI DICHIARA CHE / *WE DECLARE THAT* **Reparto di Parassitosi Alimentari e Neglette - Istituto Superiore di Sanità**
Appartenente all'ente/Belonging to the organization:
ISTITUTO SUPERIORE DI SANITA'
Sede/Headquarters:
- Viale Regina Elena 299 - 00161 Roma RM

✓ The PT has been organized following the NRL request

PT timing 2026



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

January 27th

https://www.iss.it/en/web/iss-en/eurlp-proficiency-testing

March 16th



March 23th





Istituto Superiore di Sanità
Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases
European Union Reference Laboratory for Parasites

Individual PT Report n. _____ **Laboratory Code _____**

PT "Detection of Anisakidae L3 larvae in fish fillets"

Name .....
Institution .....
Address .....
Tel. *Fax* *e-mail*

Criteria for the result evaluation

The PT result evaluation is expressed as "**correct**" (right identification of positives and negatives) or "**incorrect**" (false positive or false negative).

The final evaluation is "**positive**" if the results of all samples are correct. The final evaluation "**negative**" if at least one result is incorrect.

SAMPLE CODE	N° of spiked larvae	Result (N° of detected larvae)	Evaluation

NOTE:

FINAL EVALUATION:

Recommendations:

Date

Head of EURLP
Dr S.M. Cacciò

CONFIDENTIALITY: the report is sent, in the .pdf format, by e-mail to the participant laboratory **only**. The EURLP reserves itself the right to provide, on request, the present PT result to the competent authority.

End of the report

PT Provider
Unit of Foodborne and Neglected Parasitic Diseases
Istituto Superiore di Sanità, Rome, Italy

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MD/MAQ/11.3 rev. 3

page 1 of 1

Individual Report PT-04



May 29th

Istituto Superiore di Sanità
Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases
European Union Reference Laboratory for Parasites

Final report PT-04: An 1/2021

PT-04: "Detection of Anisakidae L3 larvae in fish fillets"

Design

Purpose	Evaluation of laboratories in charge of official control on food	
Scheme type	Single, simultaneous	
Participants	Public and private, European laboratories	
N. of participants	Depending on request	
Method	not regulated	
Test method	chosen by the participant	
PT items	Matrix	freshwater farmed fish fillet
	Item	Anisakidae live larvae
	N. of samples	3 for each participant
	Distribution	Immediate shipment after preparation
Subcontracted activities	NA	
Results evaluation	Quantitative	

Implementation

N. of participants	93	PT items	fish fillet sandwiches	93
Public laboratories	0			
Private laboratories	2		PT panel composition	3 fish fillet sandwiches: one spiked with 1 larva, two spiked with 3 larvae.
NRL	28		Shipping	DHL
Shipping dates	15/03/2021			

PT Provider
Unit of Foodborne and Neglected Parasitic Diseases
Istituto Superiore di Sanità

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle
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ACCREDIA
PTP N° 0005 P
Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
Signatory of EA, IAF and ILAC Mutual Recognition Agreements

Final Report PT-04

Test material



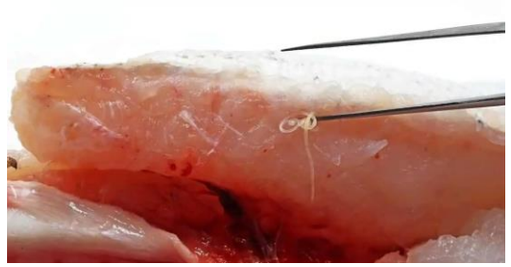
PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

- ✓ A panel of 3 items (fish fillet sandwiches) spiked or not with larvae has been prepared

Fish fillet sandwiches



Spiked with a single larva



- ✓ Fillets of farmed rainbow trout were freshly prepared and used to guarantee an Anisakidae-free matrix

Rainbow trout



- ✓ **Viable motile** Anisakidae L3 larvae were recovered from the body cavity of a heavily parasitized European hake

European hake
(Merlucius merlucius)

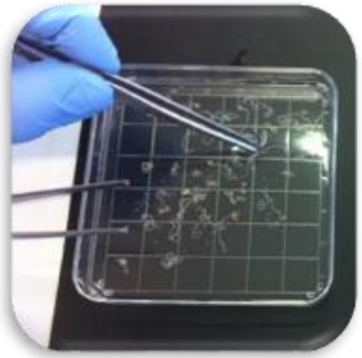


Test material

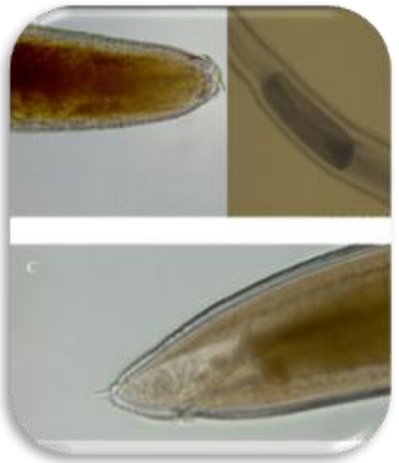


PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

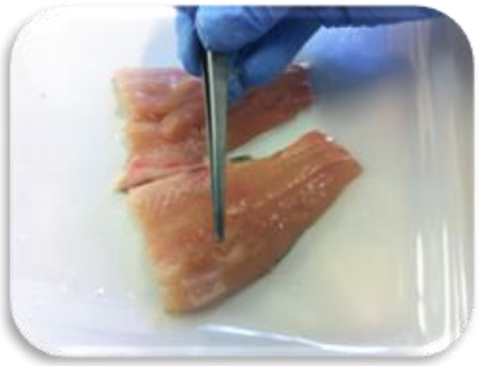
- ✓ The L3 identification at genus level was assessed by microscopic examination







- ✓ The correct number of larvae was transferred in the pockets by tweezers



- ✓ Fish sandwiches were sealed individually in a plastic bag under vacuum



- ✓ The parcel were sent to participants by international courier



Instructions and Detection Methods



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

The laboratories were allowed to use one (or a combination) of the following methods

- ✓ **Artificial digestion (ISO Standard)**
- ✓ **UV on squeezed and frozen (ISO Standard)**
- ✓ **Candling by lighting (Guidance document on the implementation of certain provisions of European regulation (EC) No 853/2004)**
- ✓ Compression system ★

NORME INTERNATIONALE **ISO 23036-2**

Première édition 2021-04

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

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

Part 2: Artificial digestion method

Artificial digestion

INTERNATIONAL STANDARD **ISO 23036-1**

First edition 2021-04

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

Part 1:
UV-press method

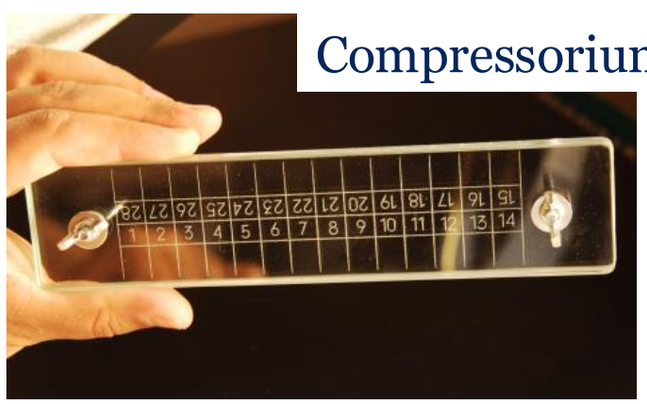
Microbiologie de la chaîne alimentaire — Méthodes de recherche des larves L3 d'Anisakidae dans le poisson et les produits de la pêche —

Partie 1: Méthode presse/UV

UV examination

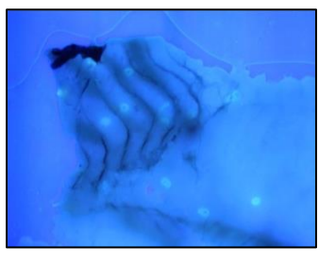


Candling



Compressorium





★ Only for not accredited labs or in combination with offical methods

PT Evaluation criteria

The PT evaluation is <u>qualitative</u> (presence or absence of larvae)

The result is “correct” if the laboratory detected all positive and all negative samples
The result is “incorrect” if the laboratory did not detect any larva in the spiked samples or detect larva in negative sample

Lab code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
AX	**2** **1** **0**	**2** **1** **0**	correct correct correct	Positive
AXX	**2** **1** **0**	0 **1** **1**	incorrect correct incorrect	Negative

The PT is considered “POSITIVE” if “correct” results were obtained
The PT is considered “NEGATIVE” if at least one “incorrect” result was obtained

PT Participants



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

Country
Albania
Austria
Belgium
Bulgaria
Cyprus
Croatia
Czech Republic
Estonia
Finland
Finland
France
France
Germany
Greece
Hungary
Iceland
Ireland
Italy
Latvia
Lithuania
North Macedonia
Norway
Poland
Portugal
Romania
Serbia
Slovak Rep.
Slovenia
Spain
Swedwn
Uk

31 Participants



PT Results



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

Laboratory code	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	**2026**
AF01	NP	NP	NP	NP	POS	NEG	POS	POS	NEG	POS	POS	POS	POS
AF02	NP	NP	NP	NP	NP	POS	NP	POS	POS	POS	POS	POS	POS
AF03	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	NP	NEG	POS	POS
AF04	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	NEG
AF05	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF06	NP	NP	NP	NP	POS	NP	NP	NEG	NEG	POS	POS	POS	POS
AF07	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	POS	NEG
AF08	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF09	NP	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF10	POS	POS	NEG	NEG	NP	NP	NP	NP	POS	POS	POS	POS	POS
AF11	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS
AF12	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
AF13	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF14	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS	POS
AF15	NP	POS	POS	POS	POS	POS	NP	NEG	POS	POS	POS	POS	POS
AF16	NEG	POS	POS	POS	POS	POS	NP	POS	NEG	POS	POS	POS	POS
AF17	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF18	NP	NP	NP	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF19	NP	NP	POS	NP	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF20	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
AF21	POS	POS	POS	POS	POS	POS	NP	POS	POS	POS	POS	POS	POS
AF22	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF23	POS	NP	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS
AF24	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS	NEG	POS	POS
AF25	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
AF26	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF27	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG
AF28	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS
AF29	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG
AF30	POS	NEG	POS	NEG	POS	POS	NEG	POS	NP	NP	NP	NP	POS
AF31	POS	NP	POS	NEG	POS	NP	POS	POS	POS	POS	POS	POS	POS

27 labs of 31 passed the PT, all four labs that failed the test reporting a false negative in the item spiked with 1 larva

PT Results



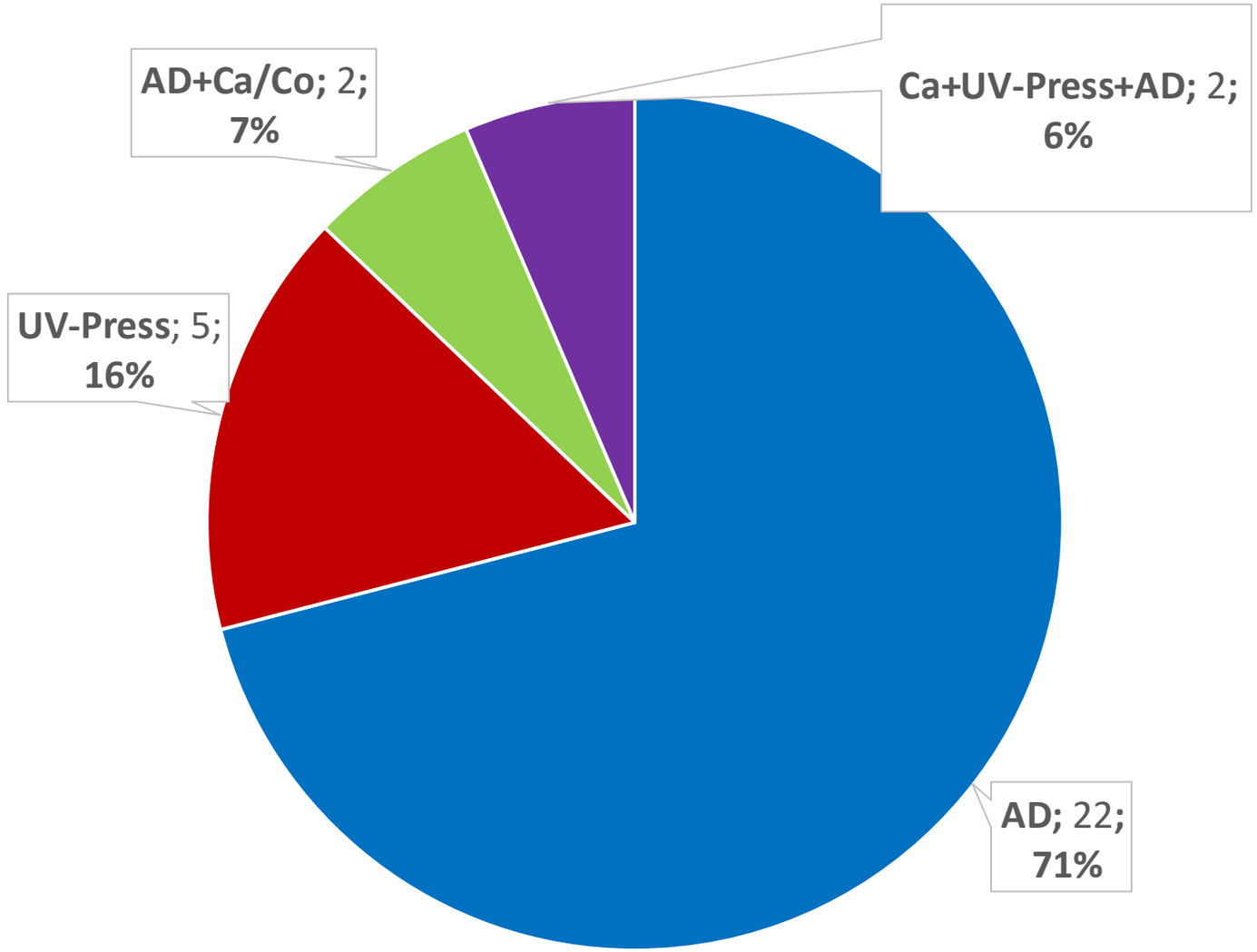
PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

Participation

31/31 labs sent the results

Methods

- 22 Artificial digestion (AD) (71%)
- 5 UV-Press (UV) (16%)
- 2 Candling (Ca) +UV + AD (6%)
- 2 AD + Ca + Compression system (Co) (7%)



Detection

- 27 labs of 31 passed the PT
- Amonge four laboratories that did not pass the test, three used the AD and one used the UV

PT04 Trend

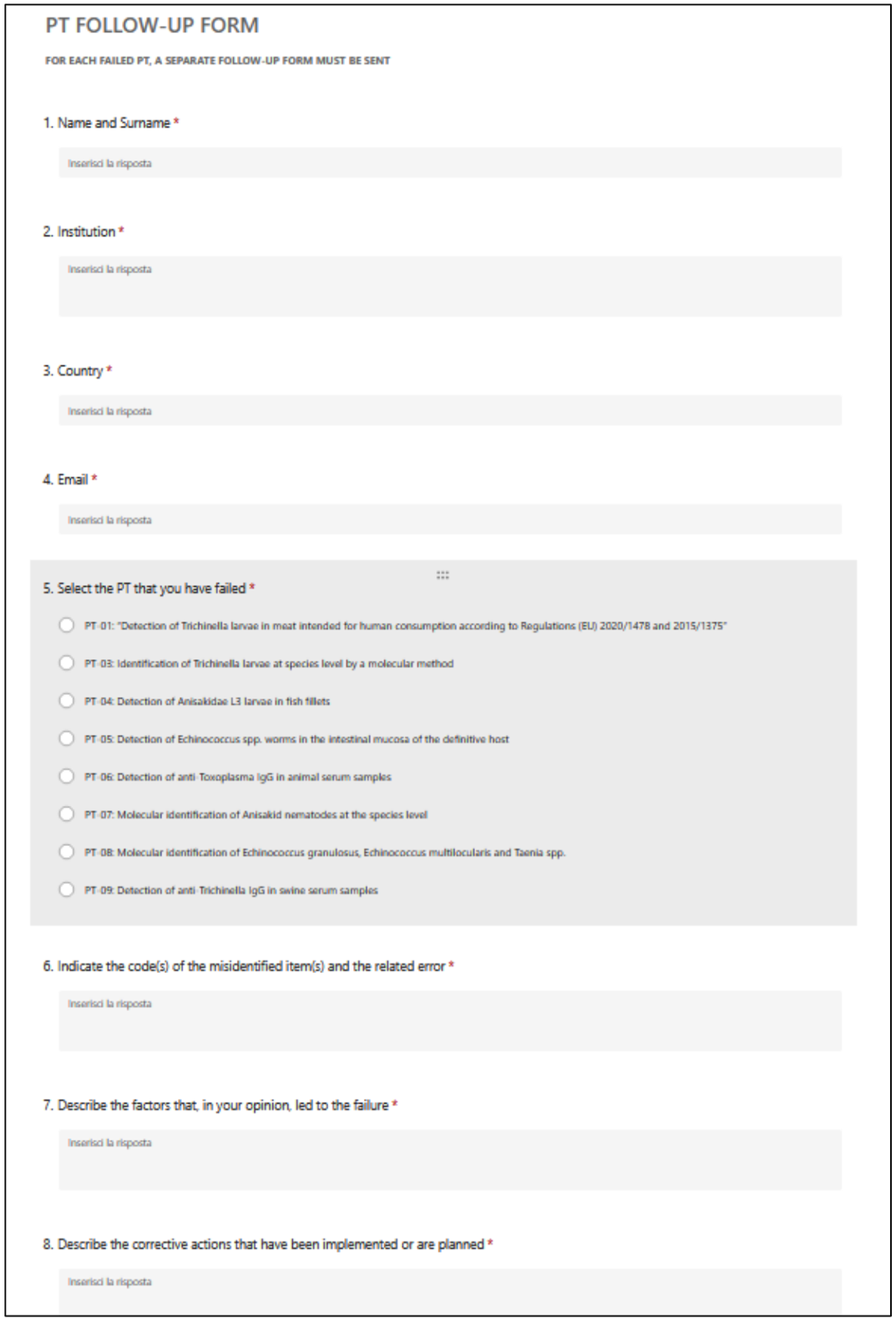


PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**

Follow Up

Two laboratories that failed the test did not describe the possible cause by online follow-up form

- 1 reported objective sensitivity limitation of the Artificial Digestion (AD) method
- 1 reported low expertise with AD method applied to Anisakidae detection in fish



PT FOLLOW-UP FORM

FOR EACH FAILED PT, A SEPARATE FOLLOW-UP FORM MUST BE SENT

1. Name and Surname *

Inserisci la risposta

2. Institution *

Inserisci la risposta

3. Country *

Inserisci la risposta

4. Email *

Inserisci la risposta

Inserisci la risposta

5. Select the PT that you have failed *

- PT-01: "Detection of Trichinella larvae in meat intended for human consumption according to Regulations (EU) 2020/1478 and 2015/1375"
- PT-03: Identification of Trichinella larvae at species level by a molecular method
- PT-04: Detection of Anisakidae L3 larvae in fish fillets
- PT-05: Detection of Echinococcus spp. worms in the intestinal mucosa of the definitive host
- PT-06: Detection of anti-Toxoplasma IgG in animal serum samples
- PT-07: Molecular identification of Anisakid nematodes at the species level
- PT-08: Molecular identification of Echinococcus granulosus, Echinococcus multilocularis and Taenia spp.
- PT-09: Detection of anti-Trichinella IgG in swine serum samples

6. Indicate the code(s) of the misidentified item(s) and the related error *

Inserisci la risposta

7. Describe the factors that, in your opinion, led to the failure *

Inserisci la risposta

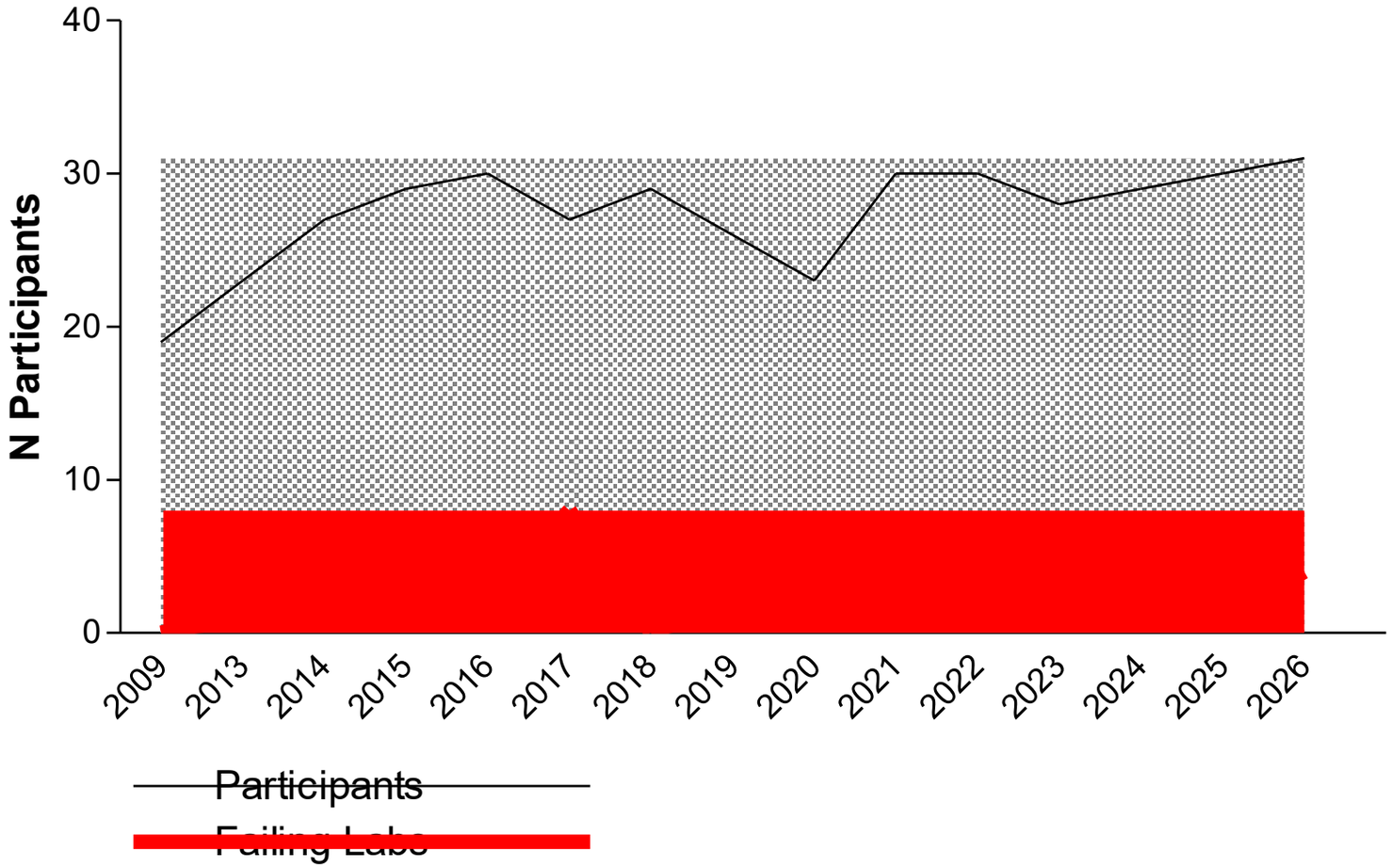
8. Describe the corrective actions that have been implemented or are planned *

Inserisci la risposta

PT04 Trend



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2026**



2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	**2026**
4%	18,5%	7%	7%	30%	0%	7,5%	22%	13%	16%	4%	24%	3%	**12%**

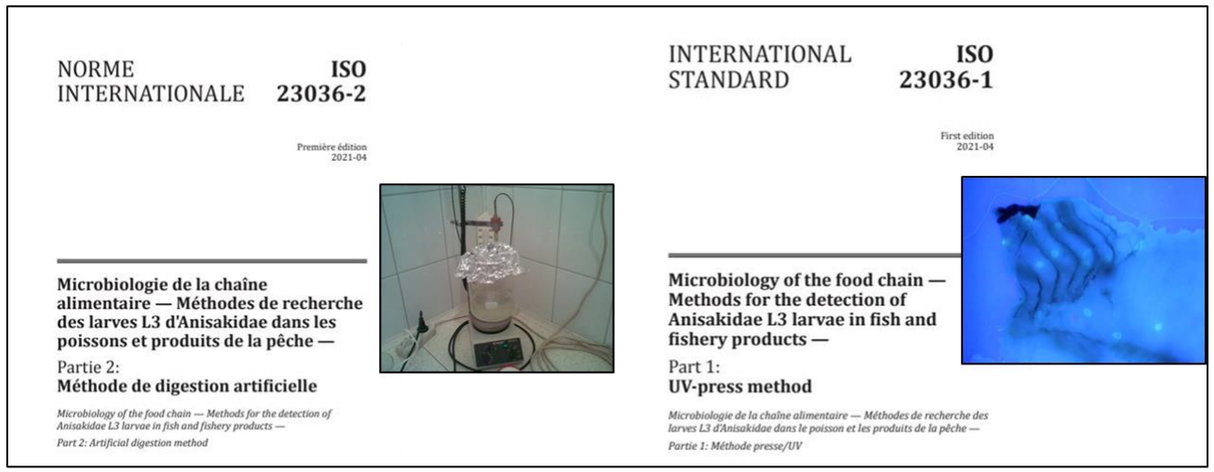
Percentage of participants failing the PT overtime



PT-04 Detection of Anisakidae L3 larvae in Fish Fillets- 2026

Conclusions

- ✓ A stable number of PT participants was recorded in 2026 compared to previous years
- ✓ Four participants failed the PT, all reporting a false negative in the item spiked with 1 larva. Three of the four used the artificial digestion method (AD) and one the UV examination after freezing (UV-Press)
- ✓ All other labs that passed the PT reported the exact number of larvae
- ✓ Among the methods adopted the most widespread is artificial digestion followed by UV examination and candling used in combination with artificial digestion



Thanks for your attention

ACKNOWLEDGMENTS

Marco Lalle
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Federica Santolamazza
Irene Tartarelli

EURLP