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

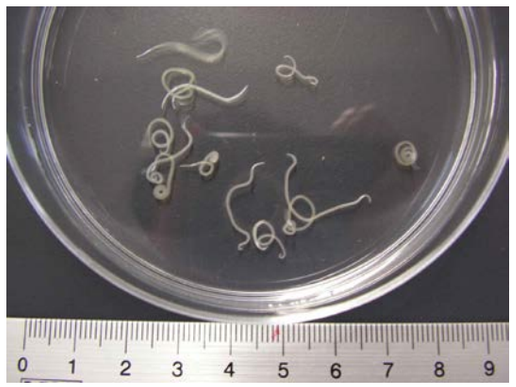
14th Proficiency Test on the detection of *Anisakis spp.* L3 larvae in fish fillets

Antonio Di Grazia, Marco Lalle

XX Workshop of National Reference Laboratories for Parasites
28th and 29th october 2025
Istituto Superiore di Sanità

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets- 2025

- ✓ 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
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Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
Signatory of EA, IAF and ILAC Mutual Recognition Agreements

ilac-MRA

DL0005P/005

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 2025

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

February 3rt

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

March 17th

March 24th

March 28th

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 is "**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

PT Coordinator
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Viale Regina Elena, 299 – 00161 Rome, Italy

MO/MAQ/11.3 rev. 3

page 1 of 1

Individual Report PT-04

May 19th

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	No	
Results evaluation	qualitative	

Implementation

N. of participants	30	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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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: An 1/2024

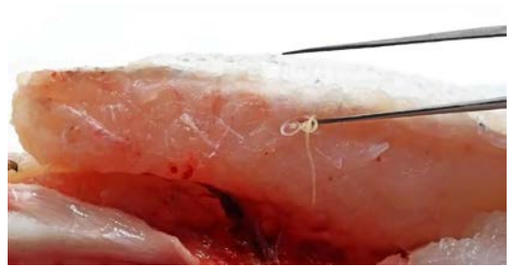
Test material

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

- ✓ 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

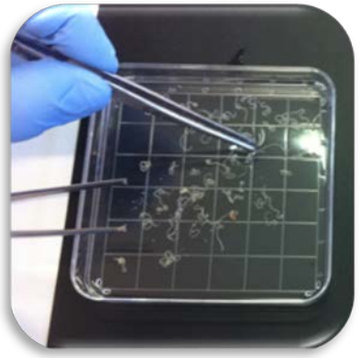
- ✓ Anisakidae L3 larvae were recovered from the body cavity of a heavily parasitized European hake

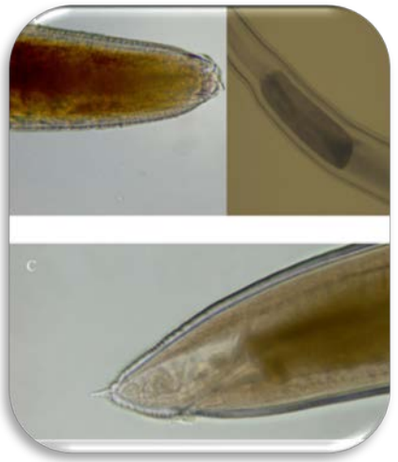
European hake
(Merlucius merlucius)

Test material

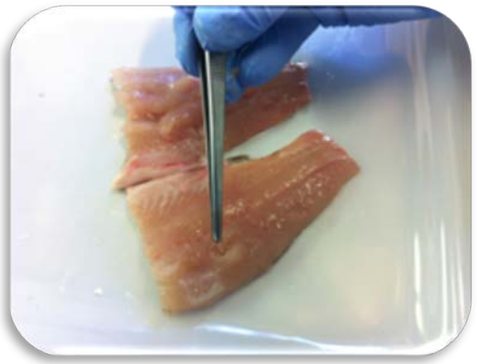
PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

- ✓ 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

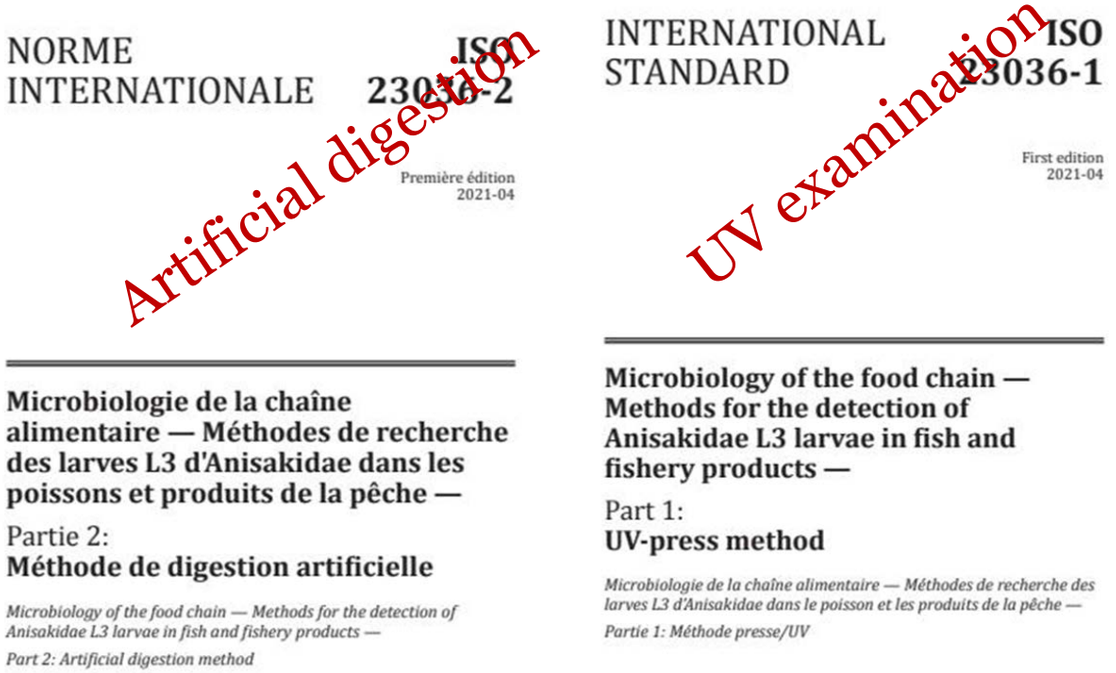
- ✓ The parcel were sent to participants by international courier

Instructions and Detection Methods

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

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 —

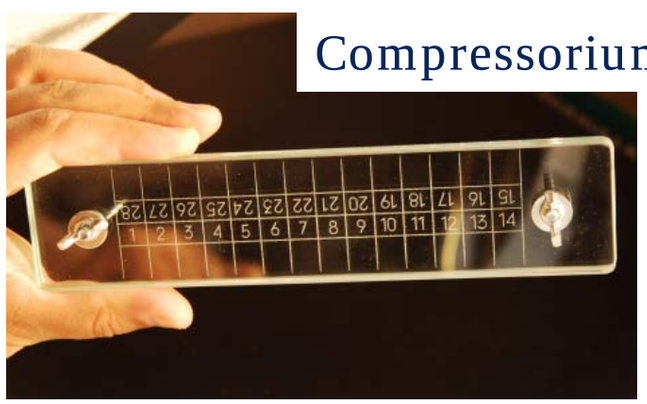
Part 1:
UV-press method

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

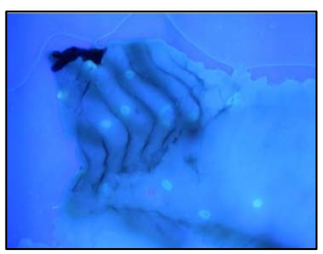
Partie 1: Méthode presse/UV

UV examination

Candling



Compressorium



PT Evaluation criteria

The PT evaluation is qualitative (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** **2** **0**	**2** **2** **0**	correct correct correct	Positive
AXX	**2** **2** **0**	0 **1** **1**	incorrect correct incorrect	Negative

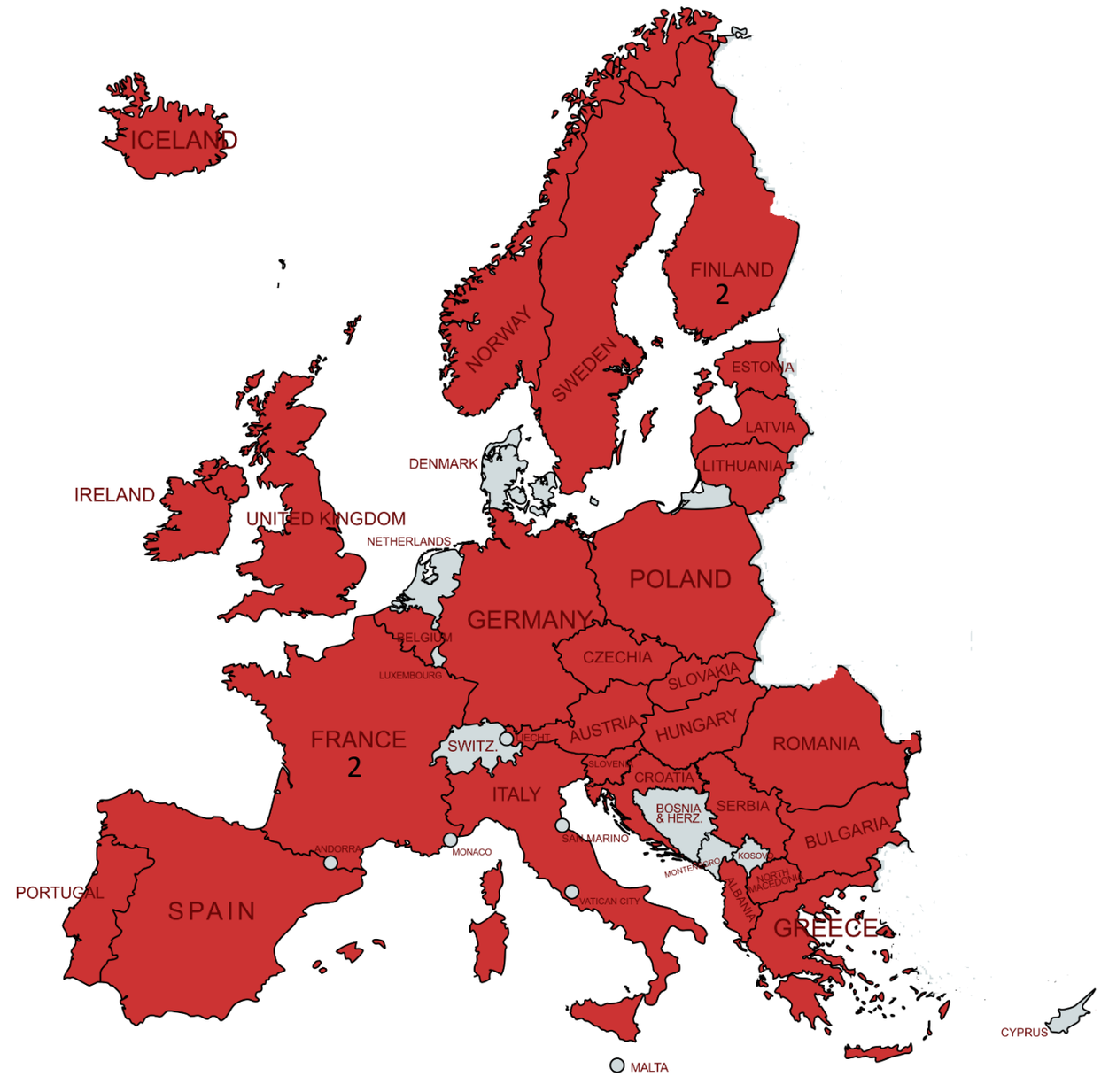
The PT is considered “POSITIVE” if “correct” results were obtained
The PT is considered “NEGATIVE” if at least one “incorrect” result was obtained

PT Participants

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

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

30Participants



PT Results

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

Laboratory code	N° of samples correctly identified	N° of samples NOT correctly identified	Method applied	Final evaluation
AF01	3	0	Artificial Digestion	**POSITIVE**
AF02	3	0	Artificial Digestion	**POSITIVE**
AF03	3	0	UV examination after freezing (UV-Press)	**POSITIVE**
AF04	3	0	UV examination after freezing (UV-Press)	**POSITIVE**
AF05	3	0	Artificial digestion	**POSITIVE**
AF06	3	0	Artificial digestion	**POSITIVE**
AF07	3	0	Artificial digestion	**POSITIVE**
AF08	3	0	Artificial digestion	**POSITIVE**
AF09	3	0	Artificial digestion	**POSITIVE**
AF10	3	0	UV examination after freezing (UV-Press)	**POSITIVE**
AF11	3	0	Artificial digestion	**POSITIVE**
AF12	3	0	Artificial digestion;	**POSITIVE**
AF13	3	0	Artificial digestion	**POSITIVE**
AF14	**1**	**2**	**UV examination after freezing (UV-Press)**	**NEGATIVE**
AF15	3	0	Artificial digestion	**POSITIVE**
AF16	3	0	Artificial digestion	**POSITIVE**
AF17	3	0	Artificial digestion	**POSITIVE**
AF18	3	0	Candling;UV examination after freezing (UV-Press); Artificial digestion	**POSITIVE**
AF19	3	0	Artificial digestion	**POSITIVE**
AF20	3	0	Candling; Compressorium; Artificial digestion	**POSITIVE**
AF21	3	0	Artificial digestion	**POSITIVE**
AF22	3	0	Artificial digestion	**POSITIVE**
AF23	3	0	Candling;Artificial digestion	**POSITIVE**
AF24	3	0	Candling; UV examination after freezing (UV-Press); Artificial digestion;	**POSITIVE**
AF25	3	0	Artificial digestion	**POSITIVE**
AF26	3	0	Artificial digestion	**POSITIVE**
AF27	3	0	Artificial digestion	**POSITIVE**
AF28	3	0	Artificial digestion	**POSITIVE**
AF29	3	0	Artificial digestion	**POSITIVE**
AF30	3	0	Artificial digestion	**POSITIVE**

PT Results

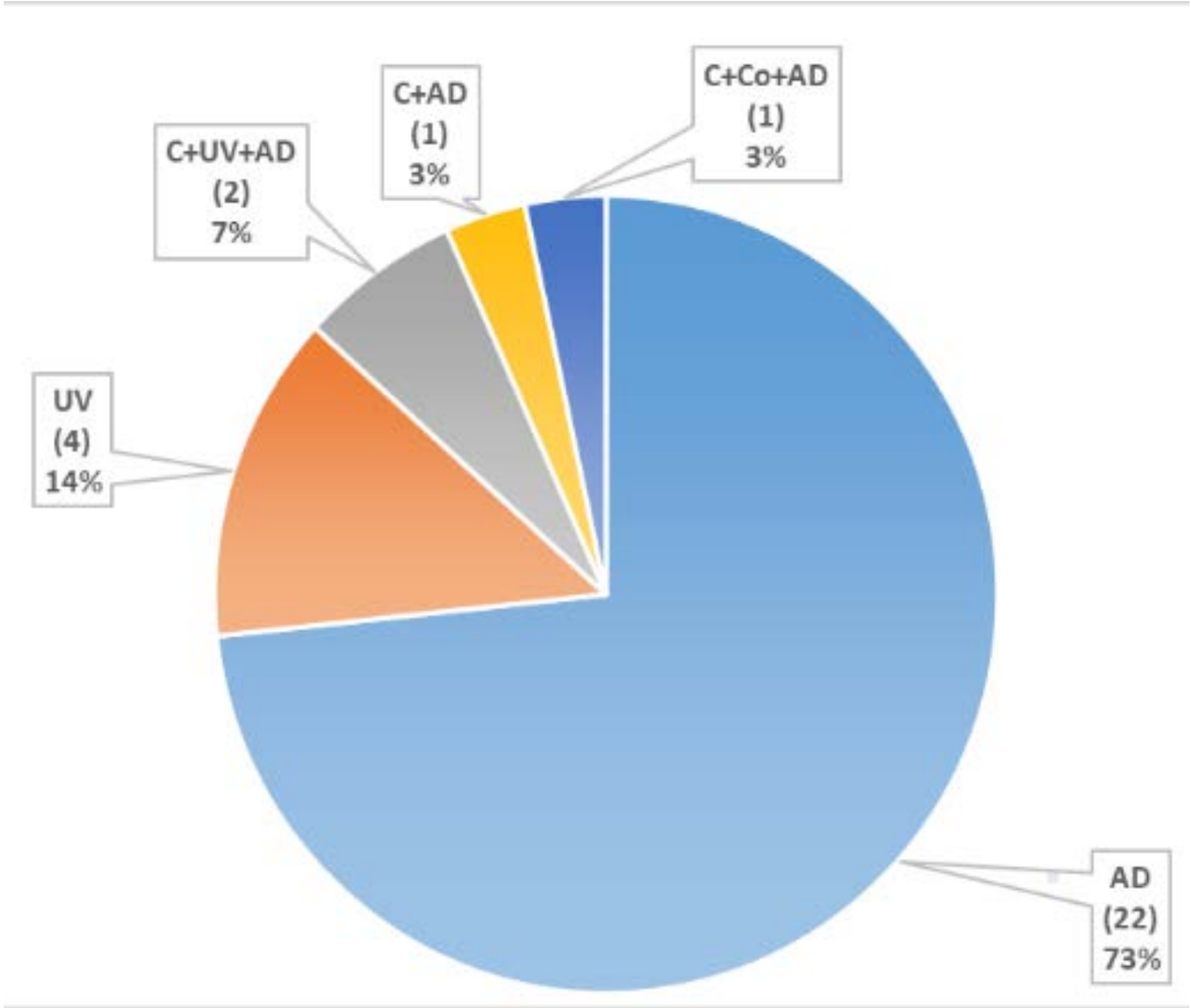
PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

Participation

30/30 labs sent the results

Methods

- 22 Artificial digestion (AD) (73%)
- 4 UV-Press (UV) (14%)
- 2 Candling (C) +UV + AD (7%)
- 1 C + Compression system (Co) + AD (7%)
- 1 C + AD



Detection

- 29 labs of 30 passed the PT
- 1 labs reported one false negative and one false positive

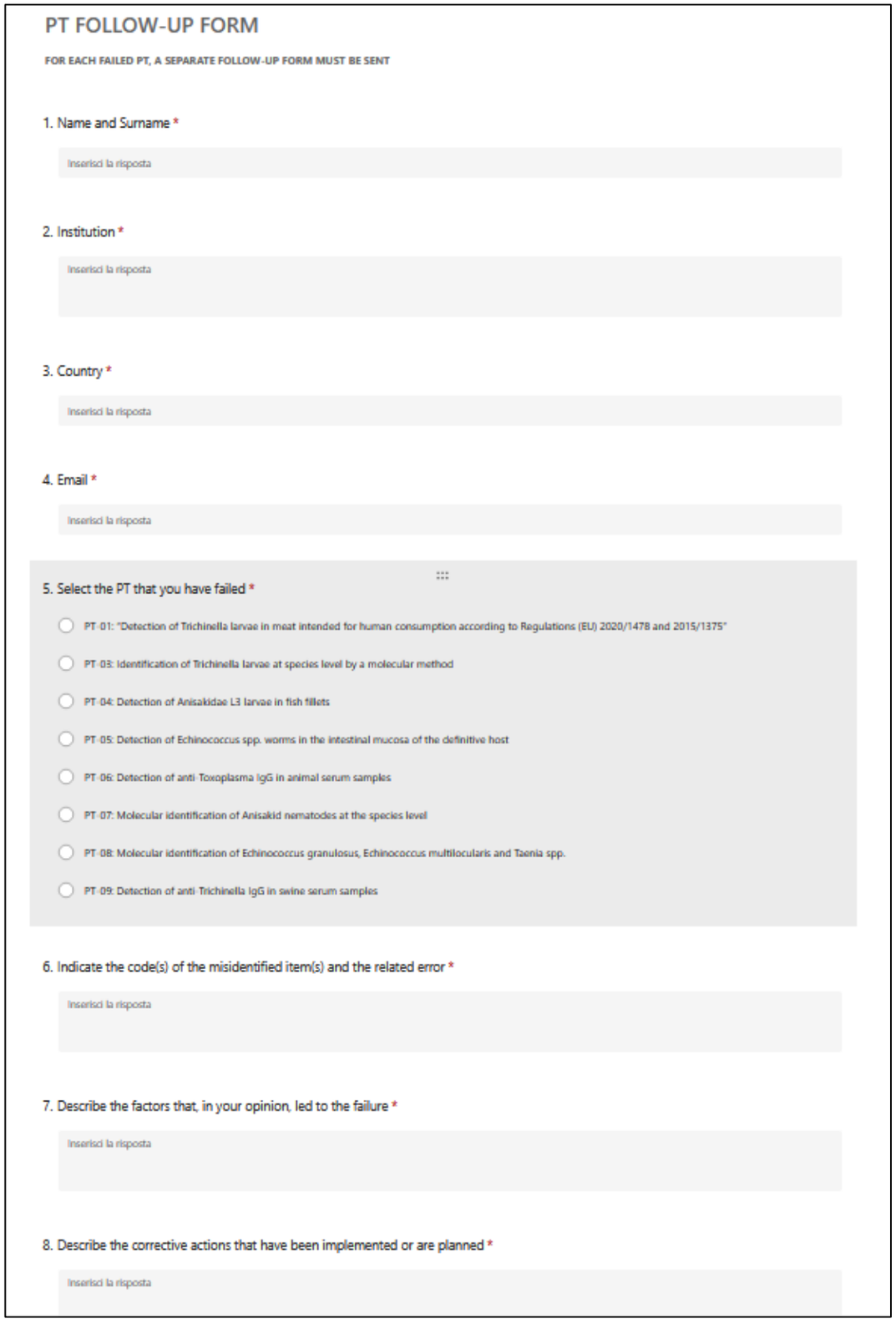
PT04 Trend

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-**2025**

Follow Up

No analysis of the cause of failure was provided by the lab using the online follow-up form.

The EURLP provided a possible explanation to the laboratory:
«...since the laboratory used UV-Press method, exchange of PVI items during analysis or failure to recognize the larvae by the operator are the most potential reasons of reported results”.



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

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 *

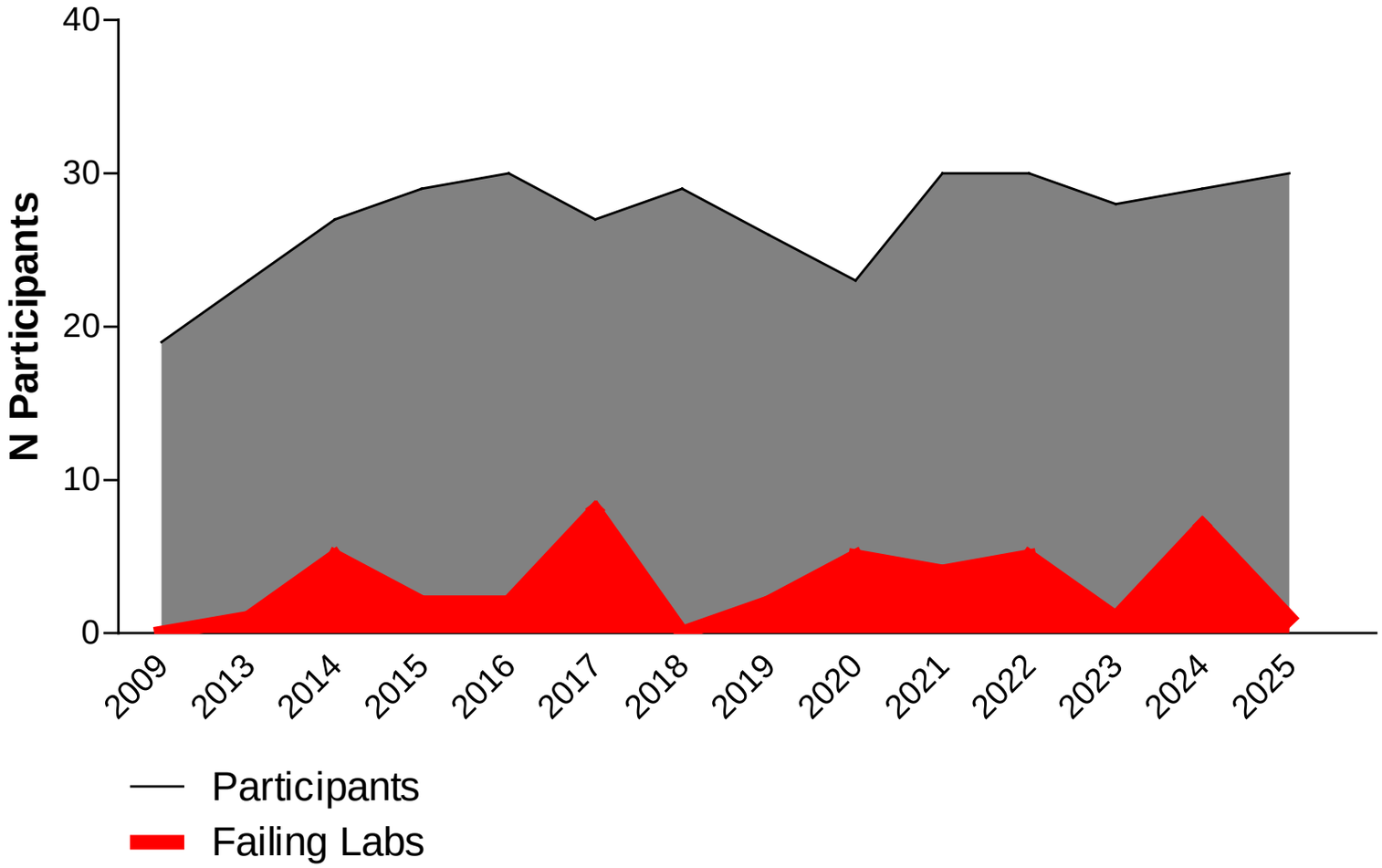
Inserisci la risposta

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-**2025**



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

Percentage of participants failing the PT overtime

Conclusions

- ✓ A stable number of PT participants was recorded in 2025 compared to previous years
- ✓ Only one laboratory failed the PT reporting one false negative and one false positive by applying UV-press method
- ✓ 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

The method applied for the artificial digestion (AD) was not correctly reported (EURLP method) by 6 out of 30 participants

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets- 2025

Remarks

Some laboratory still report EURLP method as the method applied for the Artificial Digestion. **This is NOT CORRECT**.

For Artificial Digestion the only allowed procedure is the ISO 23036-2:2021 or, eventually, an internal procedure of the laboratory but in compliance with the ISO 23036-2:2021.

We invite all the NRL to either use the ISO 23036-2:2021 or to adopt an internal method in compliance with the ISO 23036-2:2021

Thanks for your attention

ACKNOWLEDGMENTS

Marco Lalle
Francesco Celani
Simona Cherchi
Irene Tartarelli

EURLP