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

13th Proficiency Test on the detection of *Anisakis spp.* L3 larvae in fish fillets

Federica Santolamazza, Azzurra Santoro, Marco Lalle

XIX Workshop of National Reference Laboratories for Parasites
6th and 7th november 2024
Istituto Superiore di Sanità

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

- ✓ 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
Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
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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 2024

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

February 21st

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

March 11th

March 18th

March 29th

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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MOIMAQ11.3 rev. 3

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Individual Report PT-04

May 31st

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

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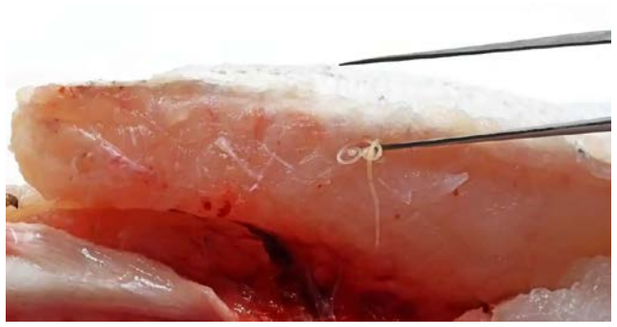
Final Report PT-04: An 1/2024

Test material

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

- ✓ A panel of 3 items (fish fillet sandwiches) has been prepared
- ✓ Each spiked with a single larva

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



European horse mackerel

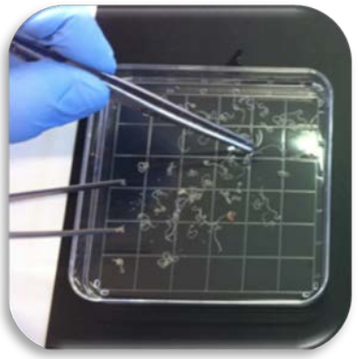
- ✓ Fillets of farmed rainbow trout were freshly prepared and used to guarantee an Anisakidae-free matrix

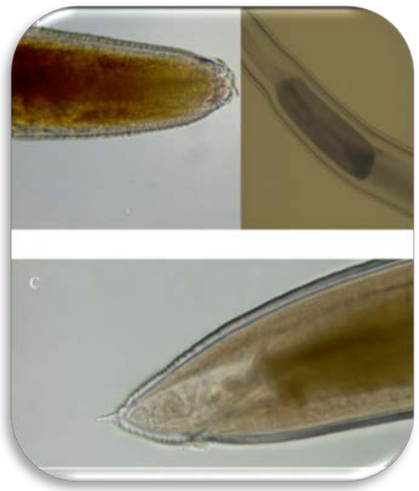
Rainbow trout

Test material

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

- ✓ 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-2024

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

- ✓ Artificial digestion
- ✓ UV on squeezed and frozen
- ✓ Candling by lighting
- ✓ Compression system

Artificial digestion

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

UV examination

INTERNATIONAL STANDARD **ISO 23036-1**

First edition
2021-04

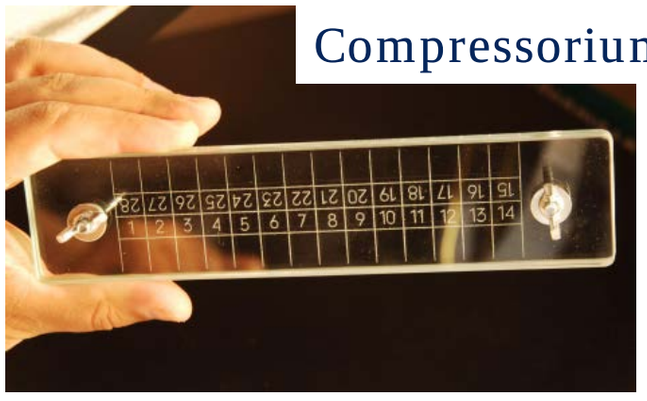
Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products —

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

Candling



Compressorium

PT Evaluation criteria

The PT evaluation is qualitative (presence or absence of larvae)

The result is “correct” if the laboratory detected Anisakidae larvae in the three spiked samples
The result is “incorrect” if the laboratory did not detect any larva in the spiked samples

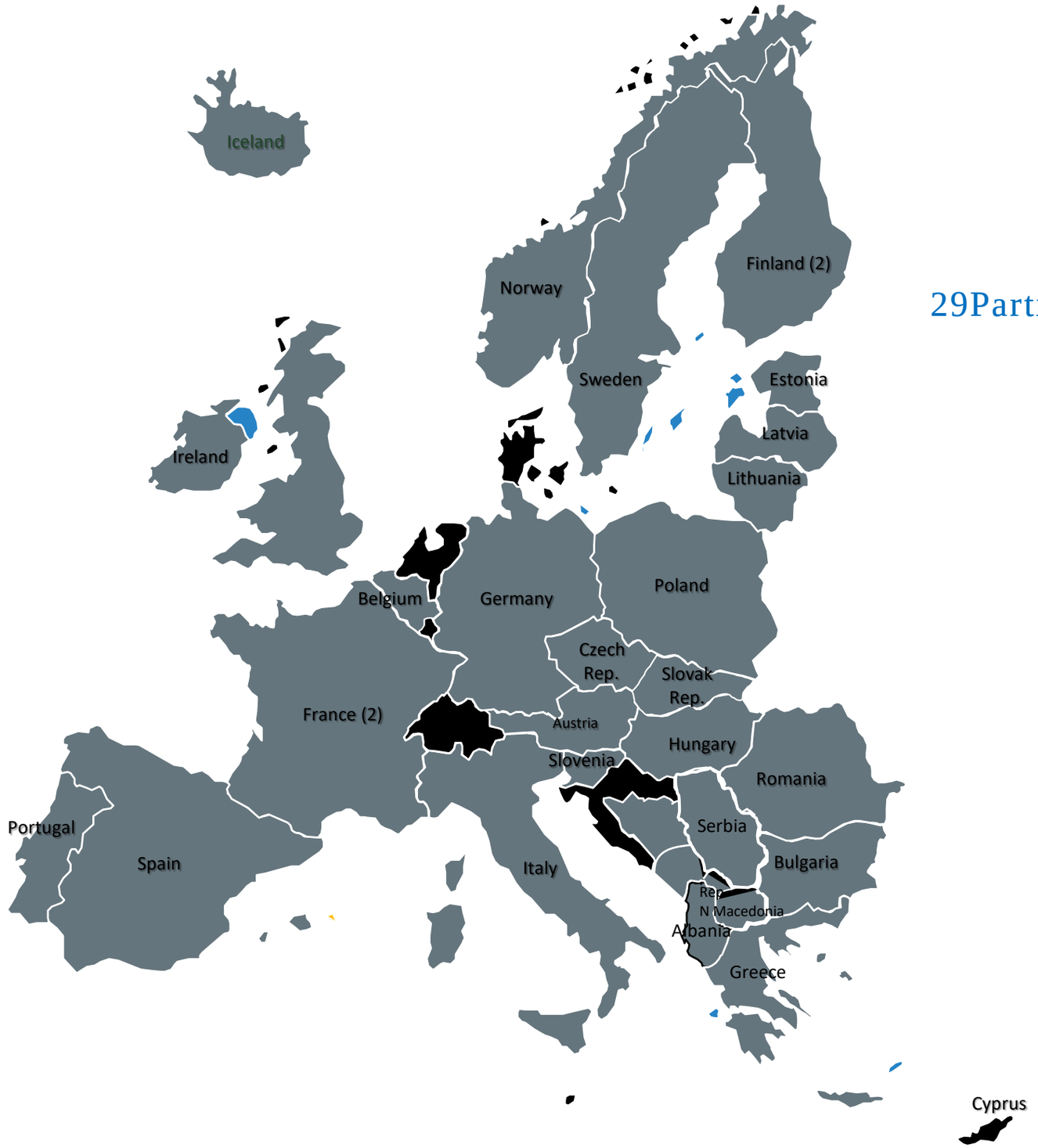
The PT is considered “POSITIVE” if “correct” results were obtained
The PT is considered “NEGATIVE” if at least one “incorrect” result was obtained

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

PT Participants

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

Country
Albania
Austria
Belgium
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



29Participants: NRLs

PT Results

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

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

PT Results

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

Participation

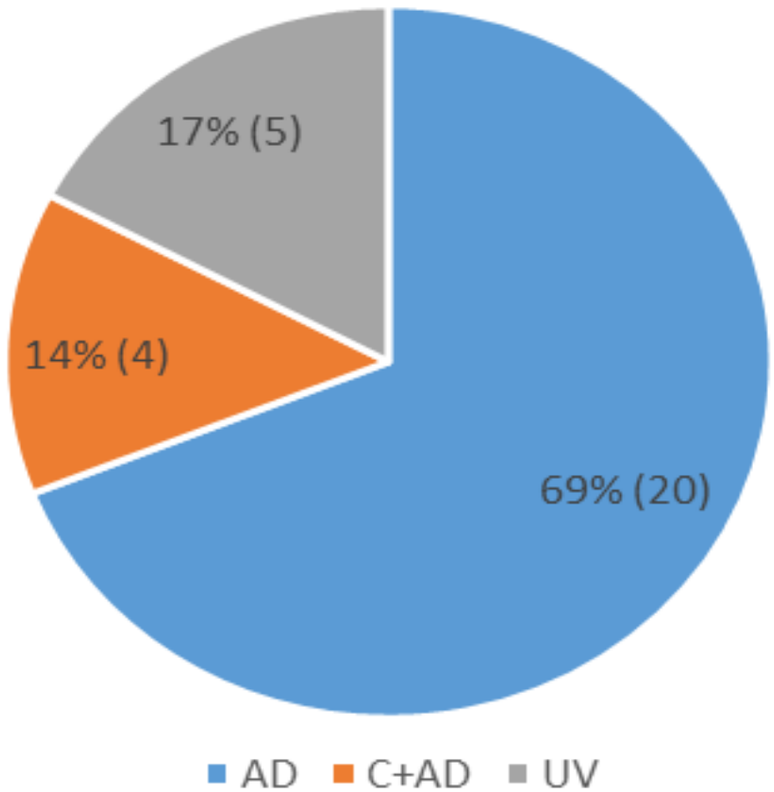
29/29 labs sent the results

Methods

20 Artificial digestion (69%)

4 Candling + Artificial digestion (14%)

5 UV-Press (17%)

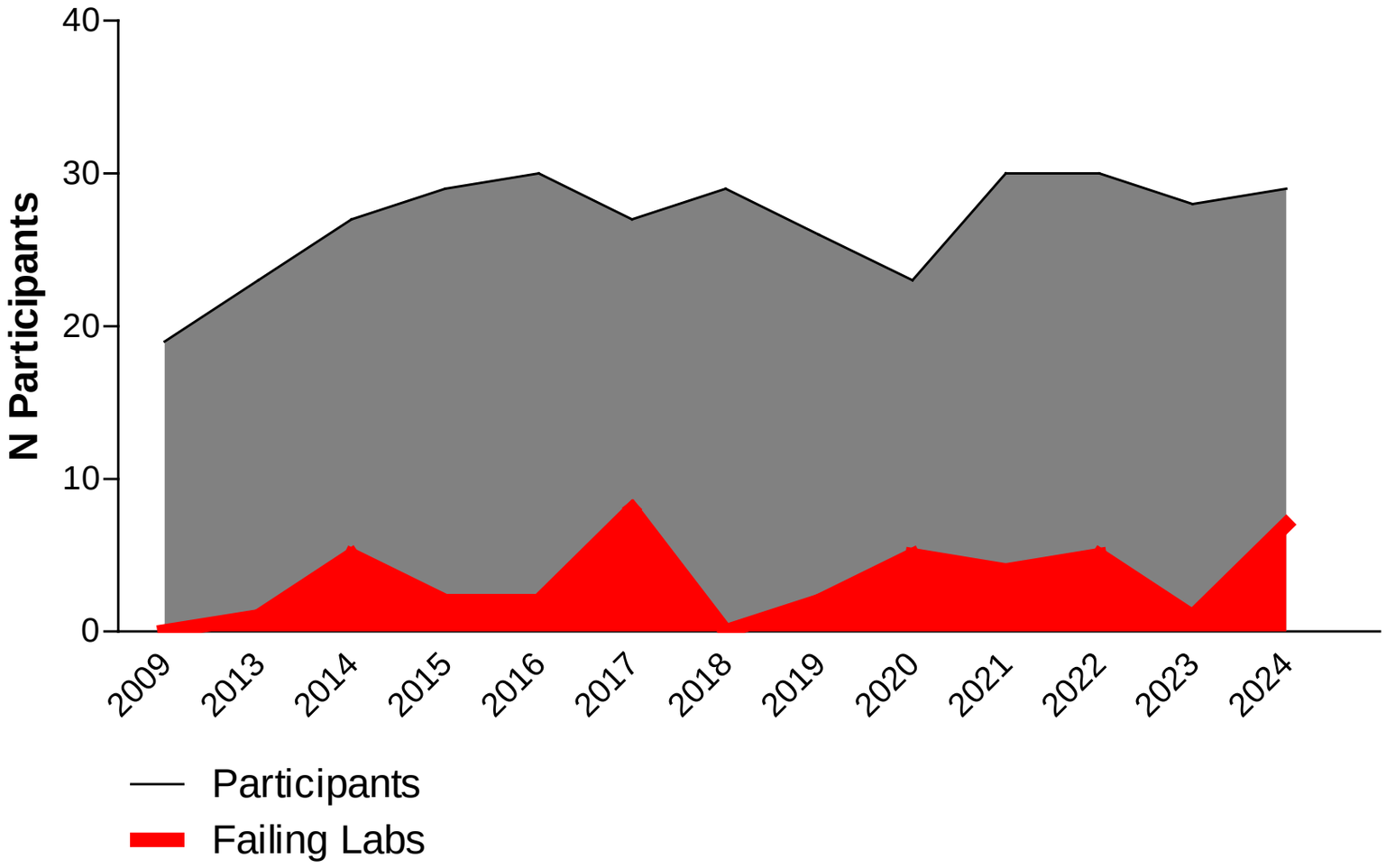


Detection

- 22 labs of 29 passed the PT
- 7 labs reported one false negative

PT04 Trend

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024



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

Percentage of participants failing the PT overtime

PT-04 Detection of Anisakidae L3 larvae in Fish Fillets-2024

Conclusions

- ✓ A stable number of PT participants was recorded in 2024 compared to previous years
- ✓ Seven laboratories failed the PT reporting each one false negative (0 instead of 1) and all applied artificial digestion 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


NORME INTERNATIONALE **ISO 23036-2**

Première édition 2021-04

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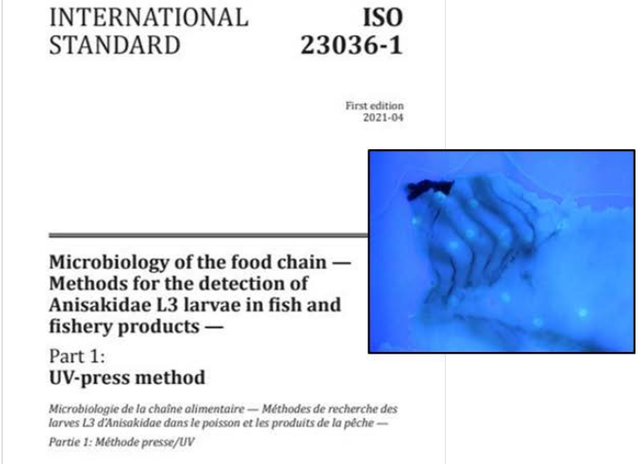
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Part 1:
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Partie 1: Méthode presse/UV

The method applied for the artificial digestion (AD) was not correctly reported (EURLP method) by 20 out of 29 participants

Thanks for your attention

ACKNOWLEDGMENTS

Marco Lalle
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EURLP