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

XVIII Workshop of National Reference Laboratories for Parasites
16th and 17th november 2023
Istituto Superiore di Sanità

12th Proficiency Testing
"Detection of *Anisakis spp.* L3 larvae in fish fillets"

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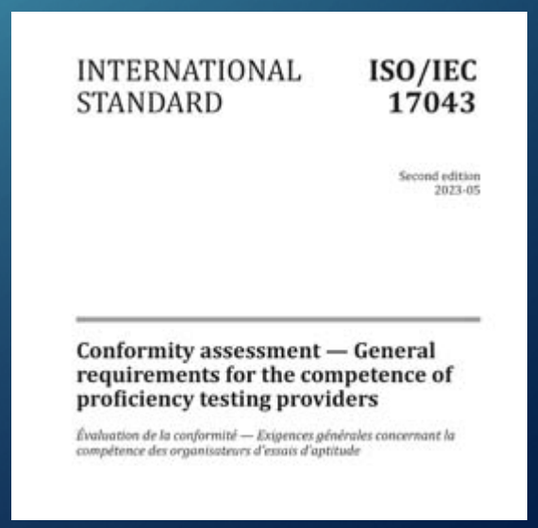
PT-04: Detection of Anisakidae L3 larvae in fish fillets

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

- ✓ PT is accredited according to the ISO 17043



INTERNATIONAL STANDARD **ISO/IEC 17043**

Second edition
2023-05

Conformity assessment — General requirements for the competence of proficiency testing providers

Évaluation de la conformité — Exigences générales concernant la compétence des organisateurs d'essais d'aptitude

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PT timing 2023

January 10th

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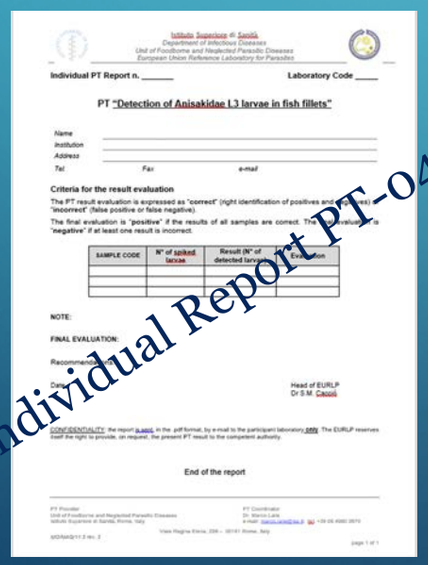
PTs REQUEST FORM 2023

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

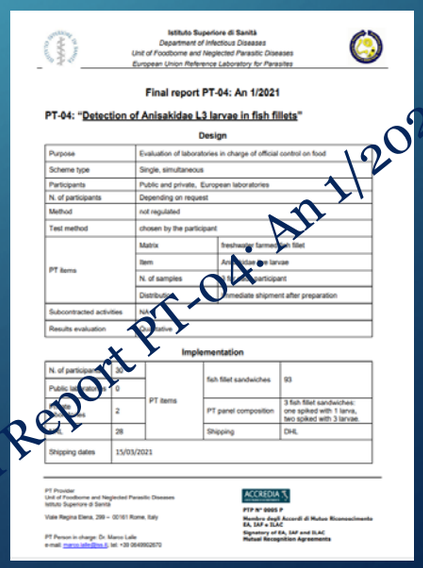
March 13th

April 4th



Individual Report PT-04

May 31st



Final Report PT-04: An 1/2023

March 20th

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Test material

- ✓ A panel of 3 items (fish fillet sandwiches) has been prepared
- ✓ one spiked with a single larva
- ✓ one spiked with three larvae
- ✓ one without larva

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

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

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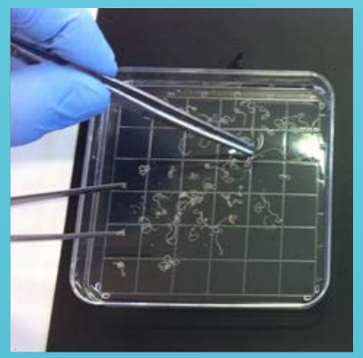
Test material

- ✓ 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

- ✓ Each PT panel was inserted in polystirene box with ice pack.

- ✓ The parcels were sent to participants by international courier

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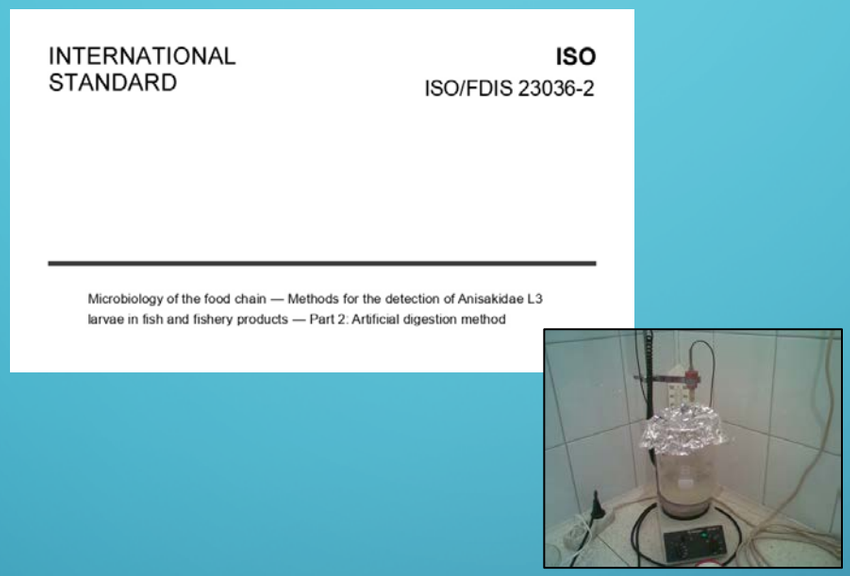
Instructions to participants and Detection Methods

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

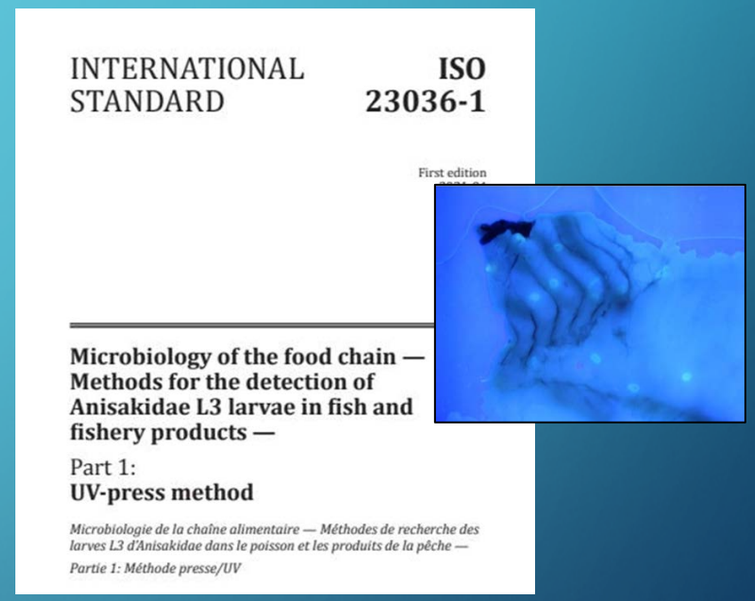
- ✓ Artificial digestion
- ✓ UV on squeezed and frozen
- ✓ Candling by lighting
- ✓ Compression system


INTERNATIONAL STANDARD

ISO
ISO/FDIS 23036-2

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

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

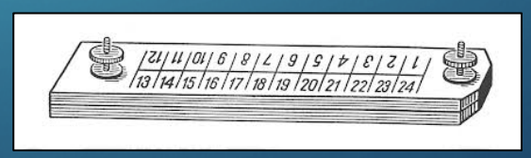
Part 1:
UV-press method

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

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PT Evaluation criteria

The PT evaluation is <u>qualitative</u> (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

Lab code	expected	observed	Result (correct/incorrect)	evaluation (positive/negative)
Ax	3	3	correct	Positive
	1	1	correct	
	3	3	correct	
Axx	3	0	incorrect	Negative
	1	1	correct	
	3	3	correct	

The PT is considered “POSITIVE” if “correct” results were obtained

The PT is considered “NEGATIVE” if at least one “incorrect” result was obtained

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PT Participants N=28

Country
Albania
Austria
Belgium
Bulgaria
Czech Republic
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

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PT Results

Lab code	N° of spiked/detected larvae[1]			Method(s)	Final Evaluation
	1	0	3		
A1	1	0	2	Artificial digestion	Positive
A2	1	0	3	Artificial digestion	Positive
A3	1	0	3	Artificial digestion	Positive
A4	1	0	3	Artificial digestion	Positive
A5	1	0	3	Artificial digestion	Positive
A6	1	0	3	Candling; Artificial digestion	Positive
A7	1	0	3	Candling; Artificial digestion	Positive
A8	1	0	3	Artificial digestion	Positive
A9	1	0	3	Artificial digestion	Positive
A10	1	0	3	Candling; Artificial digestion	Positive
A12	0	0	2	Artificial digestion	Negative
A13	1	0	3	Artificial digestion	Positive
A15	1	0	3	Artificial digestion	Positive
A16	1	0	3	UV examination after freezing (UV-Press)	Positive
A18	1	0	3	Artificial digestion	Positive
A19	1	0	3	Artificial digestion	Positive
A20	1	0	3	Artificial digestion	Positive
A21	1	0	3	Artificial digestion	Positive
A26	1	0	4	Artificial digestion	Positive
A28	1	0	1	UV examination after freezing (UV-Press)	Positive
A29	1	0	3	UV examination after freezing (UV-Press)	Positive
A30	1	0	3	Candling;Compressorium;Artificial digestion	Positive
A31	1	0	3	Artificial digestion	Positive
A35	1	0	3	Artificial digestion	Positive
A36	1	0	2	Artificial digestion	Positive
A38	1	0	3	UV examination after freezing (UV-Press) Artificial digestion	Positive
A39	1	0	3	UV examination after freezing (UV-Press)	Positive
A43	1	0	3	Artificial digestion	Positive

larvae are reported

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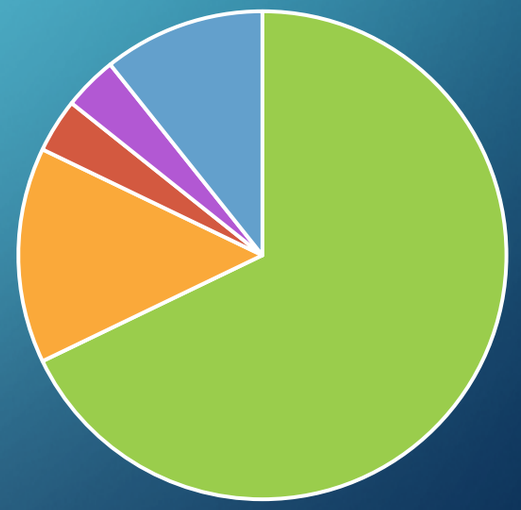
PT **Results**

Participation
28/28 labs sent the results

Methods

19 Artificial digestion alone (68%) (6 labs followed ISO 23036-2)
4 UV-Press (16%) (1 labs followed ISO 23036-1)
3 Candling + Artificial digestion
1 UV-Press + Artificial digestion
1 Candling + Artificial digestion + compressorium



Detection

- 27 labs of 28 passed the PT
- 1 labs reported one false negative
- 1 lab overestimated the number of spiked larvae (n=4)

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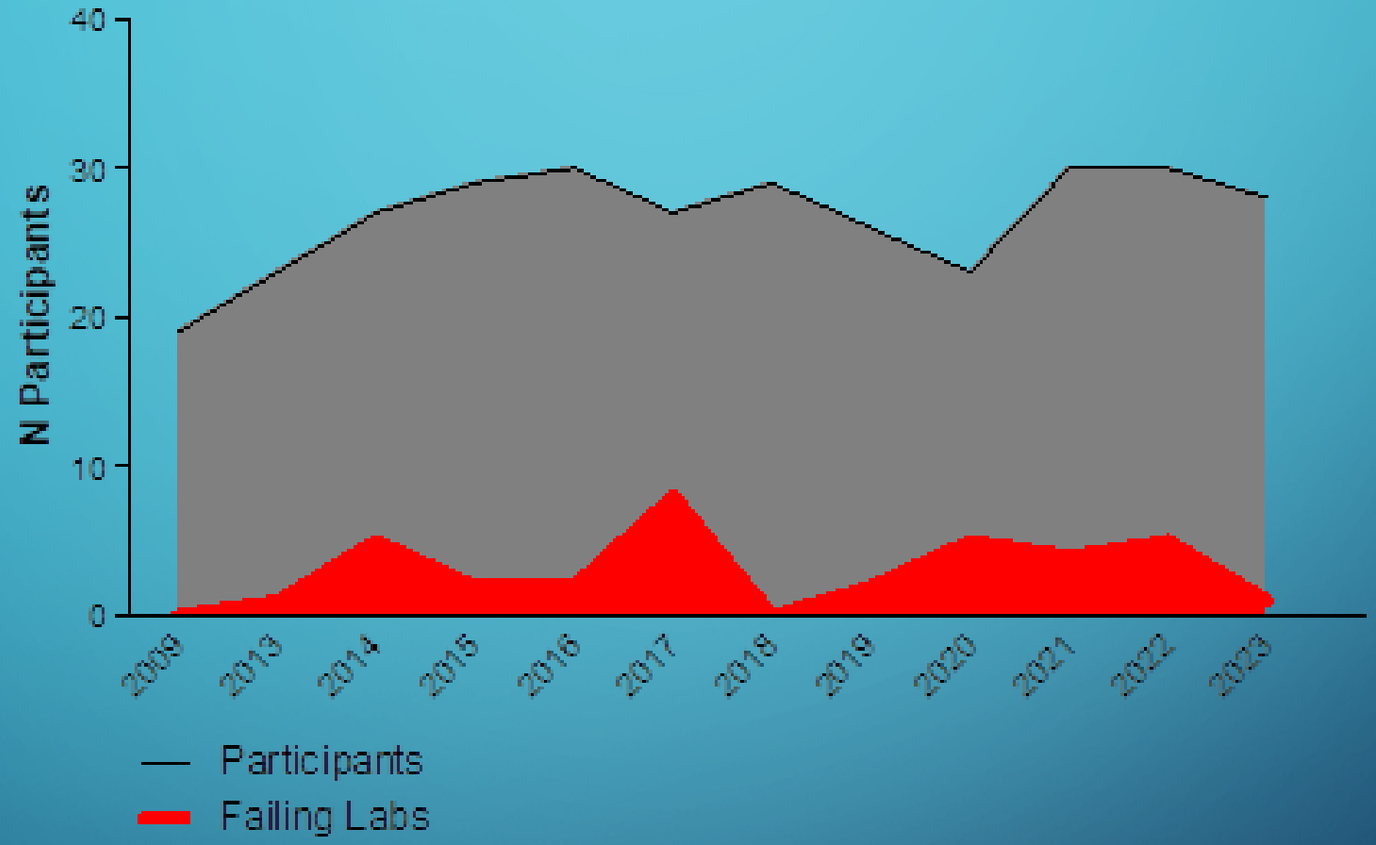
PT04 Trend



2009	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
0%	4%	18.5%	7%	7%	30%	0%	7.5%	22%	13%	16%	3.5%

Percentage of participants failing the PT overtime

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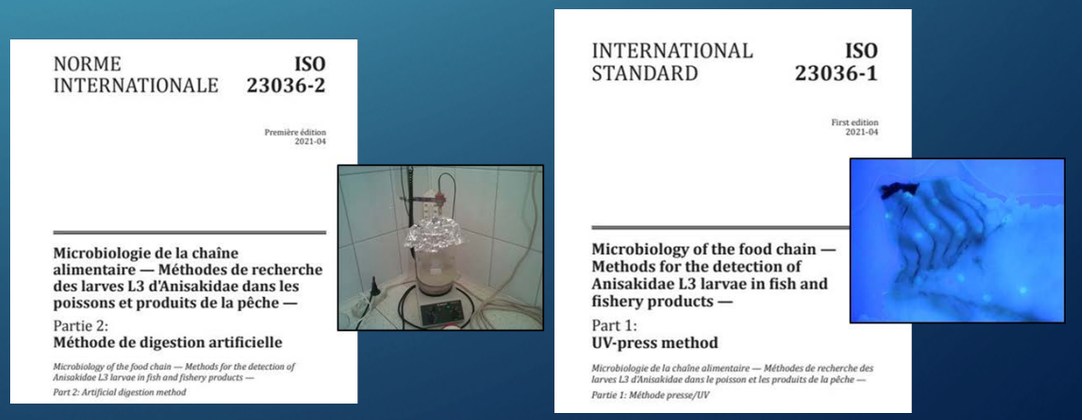
Conclusions
12th PT on the detection of *Anisakis spp.* L3 larvae in fish fillets

- ✓ A stable number of PT participants was recorded in 2023 compared to previous years

- ✓ Only one laboratory failed the PT
- ✓ Only one laboratory overestimated (n=4) the number of spiked larvae and 3 labs underestimated
- ✓ 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

More info: marco.lalle@iss.it