



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”

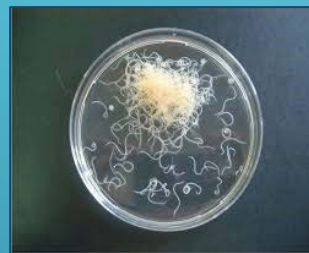
Federica Santolamazza, Azzurra Santoro, Marco Lalle



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*



European Union Reference Laboratory for Parasites
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Istituto Superiore di Sanità



PT timing 2023

January 10th



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



PTs REQUEST FORM 2023

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

March 13th



March 20th

DEADLINE
RESULT
REPORTING

April 4th

Individual PT Report n. _____ Laboratory Code _____

PT "Detection of Anisakidae L3 larvae in fish filets"

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) and "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 samples	Result (N° of detected larvae)	Conclusion

NOTE:
FINAL EVALUATION:
Recommendation:
Complimented by: _____ Head of EURLP Dr S.M. Cassioli

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

End of the report

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Page 1 of 1

May 31st

Final report PT-04: An 1/2021

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

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
Matrix	freezing fish fillet
Item	Anisakidae larvae
N. of samples	1 per participant
Distribution	immediate shipment after preparation
Subcontracted activities	not
Results evaluation	relative

Implementation

N. of participants	Public	Private	fish fillet sandwiches	03
2	PT items	PT panel composition	3 fish fillet sandwiches, one spiked with 1 larva, two spiked with 3 larvae	
28		Shipping	DHL	

Shipping dates: 15/03/2021

PT Provider
Unit of Foodborne and Neglected Parasitic Diseases
Istituto Superiore di Sanità
Via Regina Elena, 288 - 00158 Rome, Italy
PT Person in charge: Dr. Maria Lilla
e-mail: parassiti@iss.it Tel: +39 06 4980 2070

ACCREDITED
PTP N° 0001 P
Member Accredited of Italian Accreditation
RA, IAF e ILAC
Agreement of RA, IAF and ILAC
Mutual Recognition Agreements



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



Merluccius merluccius



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

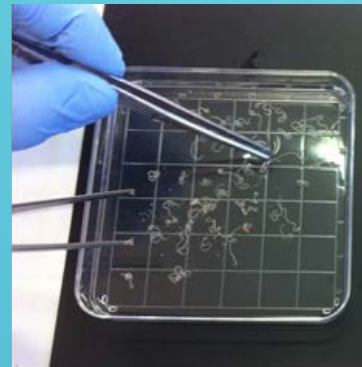
Oncorhynchus mykiss irideus





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 polystyrene box with ice pack.



- ✓ The parcels were sent to participants by international courier





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

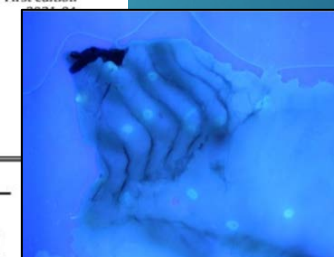
INTERNATIONAL STANDARD ISO
23036-1

First edition

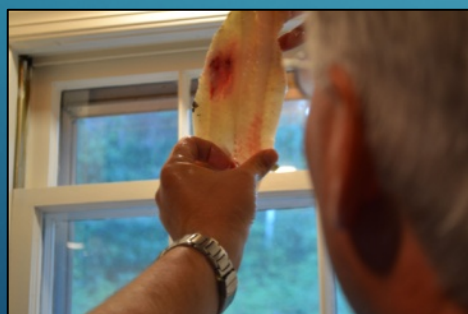
Microbiology of the food chain —
Methods for the detection of
Anisakidae L3 larvae in fish and
fishery products —

Part 1:
UV-press method

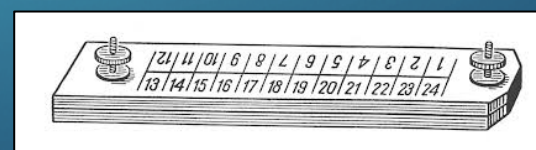
Microbiologie de la chaîne alimentaire — Méthodes de recherche des
larves L3 d'Anisakidae dans le poisson et les produits de la pêche —
Partie 1: Méthode presse/UV



UV examination



Candling



Compressorium



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

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



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



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

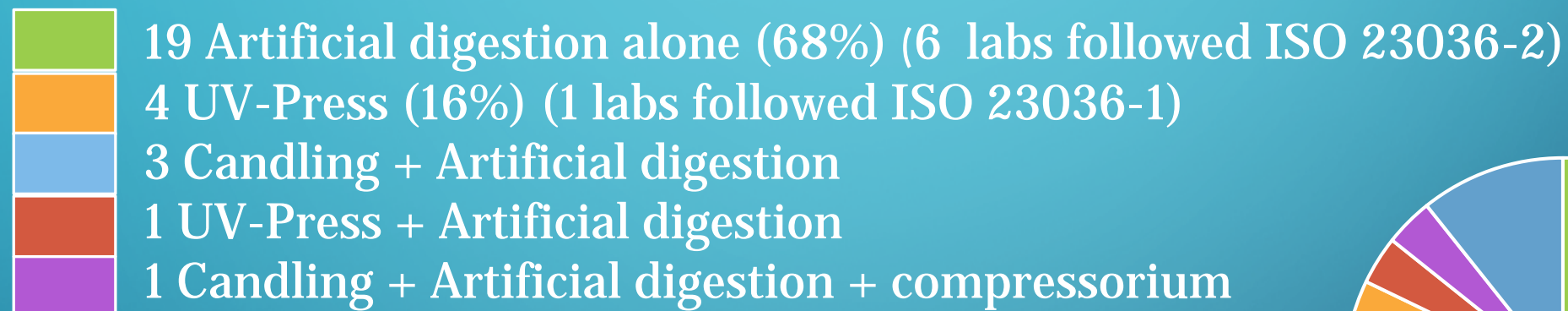


PT Results

Participation

28/28 labs sent the results

Methods

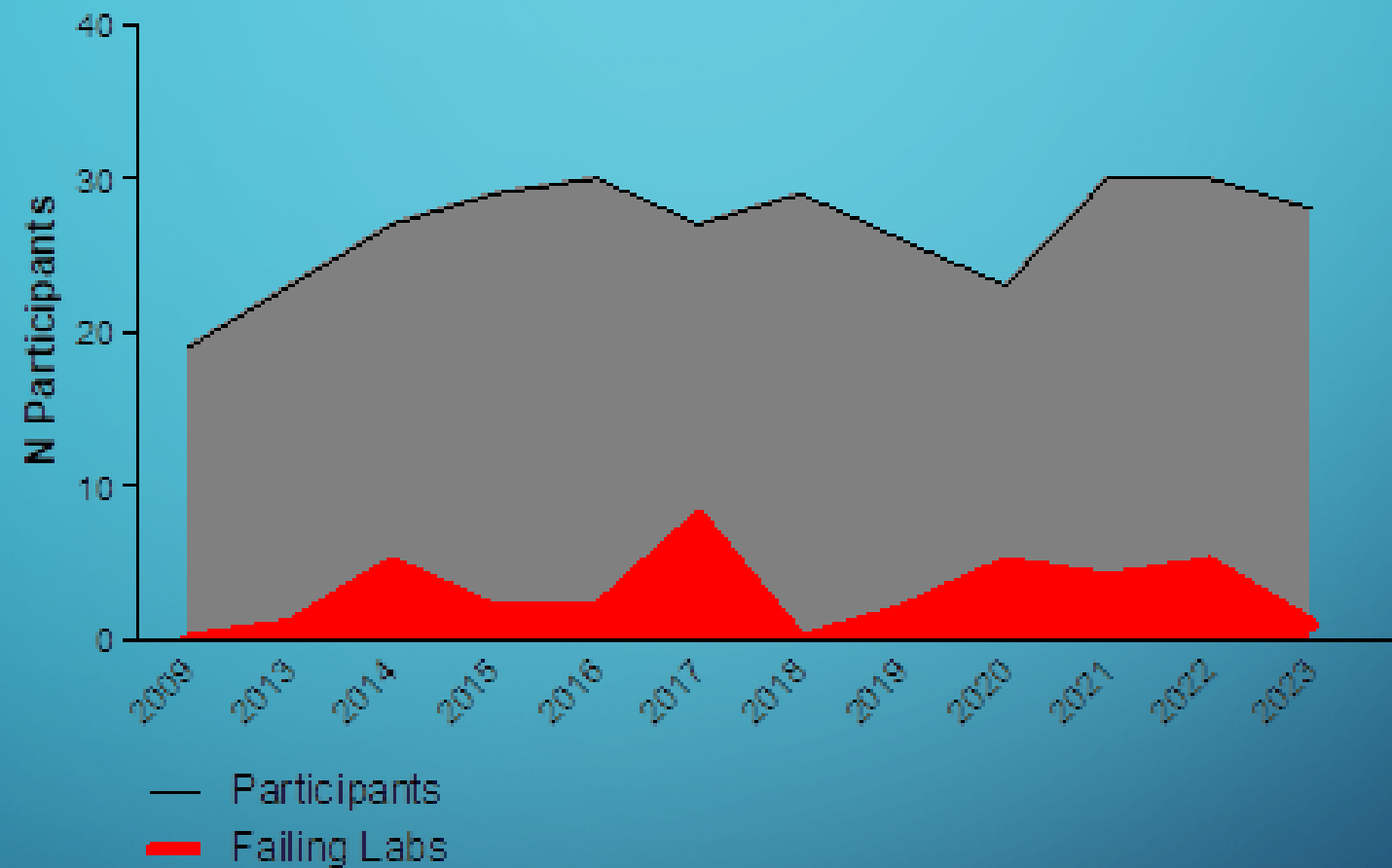


Detection

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



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



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



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