

Final report PT-04: An 1/2023

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

Design

Purpose	Evaluation of laboratories competence in detection anisakidae larvae in fish fillet		
Scheme type	Single, simultaneous		
Participants	National reference laboratories for parasites. Public and private laboratories		
N. of participants	Depending on request		
Method	<ul style="list-style-type: none"> • ISO 23036-1:2021 Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 1: UV-press method. • ISO 23036-2:2021 - Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method. • EC Regulation No 2074/2005 (Candling) 		
Test method	chosen by the participant		
PT items	Matrix	fresh water farmed fish fillet	
	Item	Anisakidae live larvae	
	N. of samples	3 for each participant	
	Distribution	Immediate shipment after preparation	
Subcontracted activities	NA		
Results evaluation	Qualitative		

Implementation

N. of participants	28	PT items	fish fillet sandwiches	93
Public laboratories	/			

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
 e-mail: marco.lalle@iss.it; tel: +39 0649902670



PTP N° 0005 P
 Membro degli Accordi di Mutuo Riconoscimento
 EA, IAF e ILAC
 Signatory of EA, IAF and ILAC
 Mutual Recognition Agreements

Private laboratories	/		PT panel composition	3 fish fillet sandwiches: one spiked with 1 larva; one spiked with 3 larvae; one without larva
NRL	28		Shipping	DHL
Shipping dates	13/03/2023			

Results

The PT final evaluation was qualitative only. The PT was considered passed if all positive and all negative samples were correctly identified by the participant.

Laboratory code	N° of samples correctly identified	N° of samples NOT correctly identified	Method applied	Final evaluation
A1	3	0	Artificial digestion;	Positive
A2	3	0	Artificial digestion	Positive
A3	3	0	Artificial digestion	Positive
A4	3	0	Artificial digestion	Positive
A5	3	0	Artificial digestion	Positive
A6	3	0	Candling; Artificial digestion	Positive
A7	3	0	Candling; Artificial digestion	Positive
A8	3	0	Artificial digestion	Positive
A9	3	0	Artificial digestion	Positive
A10	3	0	Candling; Artificial digestion	Positive
A12	2	1	Artificial digestion	Negative
A13	3	0	Artificial digestion	Positive
A15	3	0	Artificial digestion	Positive
A16	3	0	UV examination after freezing (UV-Press)	Positive
A18	3	0	Artificial digestion	Positive
A19	3	0	Artificial digestion	Positive
A20	3	0	Artificial digestion	Positive

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
 e-mail: marco.lalle@iss.it; tel: +39 0649902670



PTP N° 0005 P

Membro degli Accordi di Mutuo Riconoscimento
 EA, IAF e ILAC

Signatory of EA, IAF and ILAC
 Mutual Recognition Agreements

A21	3	0	Artificial digestion	Positive
A26	3	0	Artificial digestion	Positive
A28	3	0	UV examination after freezing (UV-Press)	Positive
A29	3	0	UV examination after freezing (UV-Press)	Positive
A30	3	0	Candling; Compressorium; Artificial digestion;;	Positive
A31	3	0	Artificial digestion	Positive
A35	3	0	Artificial digestion	Positive
A36	3	0	Artificial digestion	Positive
A38	3	0	UV examination after freezing (UV-Press); Artificial digestion	Positive
A39	3	0	UV examination after freezing (UV-Press)	Positive
A43	3	0	Artificial digestion	Positive

Legend:

- Laboratories that failed the PT are marked in bold.

Summary of results:

Total number of PT panels	31
Number of participant laboratories	28
Number of participants that passed the PT	27
Number of participants that failed the PT	1

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
 e-mail: marco.lalle@iss.it; tel: +39 0649902670



PTP N° 0005 P
 Membro degli Accordi di Mutuo Riconoscimento
 EA, IAF e ILAC
 Signatory of EA, IAF and ILAC
 Mutual Recognition Agreements

Overtime comparison of results

Laboratory code	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
A1	P	P	P	P	P	P	N	P	P	P
A2	P	P	P	N	P	P	P	P	P	P
A3	N	P	P	P	P	P		P	N	P
A4	P	P	N	N					P	P
A5	P	P	P	P	P	P		P	P	P
A6	P	P	P	P	P	P	P	P	P	P
A7	P	P	P	P	P	P	P	P	P	P
A8	P	P	P		P					P
A9	P		P	N	P		P	P	P	P
A10	P	P	N	P	P	P	P	P	P	P
A11	P	N	P	N	P	P	N	P		
A12	P	N	P	P	P	P	P	P	P	N
A13	P	P	P	N	P	P	P	P	P	P
A15	P	P	P	P	P	P	P	P	P	P
A16	N	P	P	N	P	P	P	P	P	P
A17	NR		P	P	P	P	P	N		
A18	P	P	P	P	P	P	N	P	P	P
A19	P	P	P	P	P	N	P	P	P	P
A20	P	P	P	P	P	P	N	P	P	P
A21	P	P	P	P	P	P	P	P	P	P
A23/A33/A25*	N	P	P	N	P	P	P	P	P	
A26	P	P	P	P	P	P	P	P	P	P
A28	P		P	P	P	P	P	P	N	P
A29			P		P	P	P	P	P	P
A30		P	P	P	P	P	N	P	P	P
A31		P	P	P	P	P		N	P	P
A32		P							N	
A35					P	N	P	P	N	P
A36				P	P	P	P	P	P	P
A37					N					
A38					P			N	N	P
A39						P		P	P	P
A41								N		
A42								P		
A43									P	P
A44									P	

Legend: *Different code were assigned to the lab in different years

Comments:

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
 e-mail: marco.lalle@iss.it; tel: +39 0649902670



PTP N° 0005 P

Membro degli Accordi di Mutuo Riconoscimento
 EA, IAF e ILAC

Signatory of EA, IAF and ILAC
 Mutual Recognition Agreements

For the PT round 2023, 96% of the laboratories (27/28) passed the PT. Only one laboratory failed the PT reporting one false negative and, in addition, reported a lower number of larvae in the sample spiked with 3 larvae. No explanation was provided by the participant for the PT failure and low performance, likely due to analysts with limited experience and/or problems with the detection procedure.

In addition, three laboratories underestimated the number of larvae in sample spiked with 3 larvae with two using Artificial Digestion (AD) method and one using the UV-press (UV) method. The last one for instance failed the PT2022 thus indicating problems in the correct application of the UV-press method. Since the PT is only qualitative (presence/absence of the larvae), the PT results was considered positive but the laboratories were invited to consider the underestimation and to take appropriate actions including further training of the analyst on morphological identification of the larvae or proper application of the used method. The laboratory that failed the PT was invited to consider appropriate corrective actions (e.g. shipping of additional sample panel for an EQA).

In PT 2023 round a slight increase in the overall performance of the laboratories was observed in comparison to the previous year (2022)., In comparison to the previous years, we observed a constant trend in the number of laboratories that subscribe to the PT (Figure 1), although now limited only to NRLs. A decrease in the failure percentage was observed (4% in 2023 vs 16% in 2022), although this reflect a periodic trend (maybe reflecting turnover of technical personnel in each laboratory). Poor performance (failure) was not related to irregular or first time participation to the PT. Lack of experience of technical personnel (either new or not familiar with the test methods) could mainly explain negative results.

The relative percentage of detection methods adopted did not change substantially (Figure 2) with the Artificial digestion (AD) still being the prevalent method applied (22 laboratories, 78%), largely because it does not require special equipment. UV-press method (UV) alone, or followed by AD, was used in five laboratories (22%). Candling (C) (i.e. visual inspection) was always used in combination with artificial digestion. Candling is the only method clearly reported in the EU regulation 2074/2005.

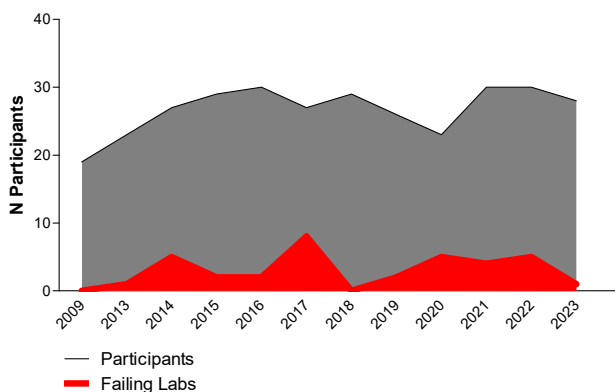


Figure 1. PT04 trend overtime

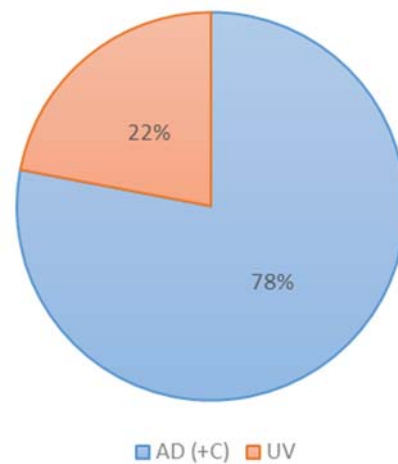


Figure 2. Percentage of the detection methods applied

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
 e-mail: marco.lalle@iss.it; tel: +39 0649902670



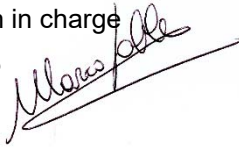
PTP N° 0005 P

Membro degli Accordi di Mutuo Riconoscimento
 EA, IAF e ILAC

Signatory of EA, IAF and ILAC
 Mutual Recognition Agreements

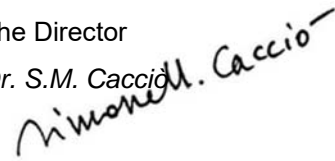
PTP person in charge

Dr. M. Lalle



The Director

Dr. S.M. Caccio



Date 22/05/2022

Notes:

1. To guarantee confidentiality, participant laboratories are identified by alphanumeric codes. PT participant identity is kept confidential and bound by professional secrecy. The PTP reserves itself the right to provide the laboratory PT result to the competent authority on request.
2. The organizer designates a qualified company for the transport and delivery of PT items.
3. Each participating laboratory receives a PT panel according to the PT scheme. Each PT item consists of a fish fillet sandwich spiked or not with live Anisakidae larvae. The homogeneity of PT items is ensured by an accurate control of the number of larvae spiked into each sample (item) made by two operators. PT items are stable for 7 days from the date of preparation (corresponding to the shipping date), provided that they are maintained in suitable conditions.
4. At the beginning of each year, the organizer draws up a PT program and makes it known by sending an email to the NRLs
5. The final report issue of each PT round shows the PT program implementation.

End of the report

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
e-mail: marco.lalle@iss.it; tel: +39 0649902670



PTP N° 0005 P

Membro degli Accordi di Mutuo Riconoscimento
EA, IAF e ILAC

Signatory of EA, IAF and ILAC
Mutual Recognition Agreements