Final report PT-04: An 1/2025

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	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.	
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	30	PT items	fish fillet sandwiches	108
Public laboratories	/			
Private laboratories	/		PT panel composition	3 fish fillet sandwiches (2 sandwiches spiked with 2 larvae each and 1 sandwich with no larva)

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NRL	30		Shipping	DHL
Shipping dates	17/03/2025			

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
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

Legend: Laboratory that failed the PT is grey boxed and marked in bold

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Summary of results:

Total number of PT panels	36
Number of participant laboratories	30
Number of participants that passed the PT	29
Number of participants that failed the PT	1

Overtime comparison of results

Laboratory code 2025	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
AF01		P	P	P	P	P		P	P	P	P	P
AF02		P	P	P	P	P	P	P	P	P	P	P
AF03			P		P	P	P	P	P	P	P	P
AF04						P		P	P	P	P	P
AF05		P	P	P	P	P	P	P	P	P	P	P
AF06		P	P	P	P	P	N	P	P	P	P	P
AF07			P	P	P	P	P	P	N	P	P	P
AF08					P	N	P	P	N	P	P	P
AF09		N	P	P	P	P	P	P	P	N	N	P
AF10		P	P	N	P	P	P	P	P	P	P	P
AF11				P	P	P	P	P	P	P	P	P
AF12		P	P	P	P	P		N	P	P	P	P
AF13		P	P	P	P	P	N	P	P	P	P	P
AF14		P	P	N	P	P	P	P	P	P	P	N
AF15		P	P	N	P	P	P	P	P		N	P
AF16		P	P	N	P	P	P	P	P	P	N	P
AF17		P	N	N					P	P	P	P
AF18		P	P	P	P	P	P	P	P	P	P	P
AF19												P
AF20		P	P	P	P	P	N	P	P	P	P	P
AF21											P	P
AF22		P	P	P	P	N	P	P	P	P	P	P
AF23		P	P	P	P	P	P	P	P	P	N	P
AF24		P	N	P	P	P	P	P	P	P	N	P
AF25					P			N	N	P	P	P
AF26		P	P	P	P	P		P	N	P	P	P
AF27									P	P	N	P
AF28		P	P	P	P	P	P	P	P	P	P	P
AF29			P	N	P		P	P	P	P	P	P
AF30		P	P	P	P	P	N	P	P	P	N	P

Legend: P, positive result; black boxed N, negative result; empty gray boxed, not attending/not sending result

Comments:

For the PT round 2025, 97% of the laboratories (29/30) passed the PT. Only one laboratory failed the PT reporting one false negative (0 instead of 2) and one false positive (2 instead of 0) by applying UV-press method. Following the request to provide explanation for the negative results the laboratory answered that it the reason would have been investigated. It can be hypothesized that shuffling of samples during analysis occurred.

In PT 2025 round, the overall number of participant laboratories appears to have reached a plateau when compared with the last four years (Figure 1) and it is currently limited to NRLs. A newly appointed National Reference Laboratory joined the first time the PT round. A remarkable increase in the overall performance of the laboratories was observed, particularly in comparison to the previous year (2024). Indeed, the percentage of failing laboratories was 3% in 2025 vs 24% in 2024. Nevertheless, a trend with a periodic fluctuation in failure percentage can be observed overtime and it might reflecting turnover of technical personnel in each laboratory. Indeed, poor performance (failure) could not be related to irregular or first time participation to the PT. Compared to the previous year laboratories provided correct information regarding the method applied. In particular, in the Result Form submitted, the reference to the method applied for the artificial digestion (AD) was correctly reported as ISO 23036-2:2021 or "in compliance with ISO 23036-2:2021. However, 6 laboratory still reported EURLP method.

The relative percentage of detection methods adopted did not change substantially in comparison with previous years (Figure 2) with AD, with or without the preliminary analysis by candling (C), still being the prevalent method applied (24 laboratories, 79%), largely because it does not require special equipment. UV-press method (UV) was used alone in four laboratories (14%) or followed by AD in two laboratories (7%)

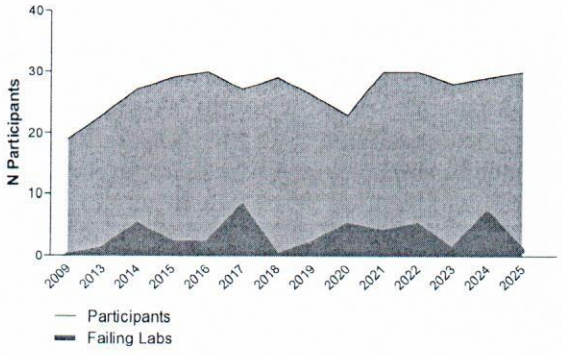

Figure 1. PT04 trend overtime

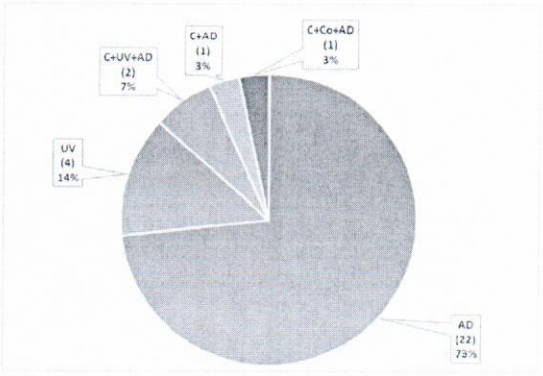

Figure 2. Percentage of the detection methods applied

Written and elaborated by
PTP person in charge
Dr. M. Lalle

Date 19/05/2025

Verified and issued by
The Director
Dr. A. Casulli

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