

Department of Infectious Diseases Unit of Foodborne and Neglected Parasitic Diseases European Union Reference Laboratory for Parasites



# Final report PT-04: An 1/2024

# 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	the detection of A — Part 1: UV-pre ISO 23036-2:202 the detection of A — Part 2: Artific	the detection of Anisakidae L3 larvae in fish and fishery products — Part 1: UV-press method.					
Test method	chosen by the participant	chosen by the participant					
	Matrix	fresh water farmed fish fillet					
PT items	Item	Anisakidae live larvae					
Priteris	N. of samples	3 for each participant					
	Distribution	Immediate shipment after preparation					
Subcontracted activities	NA						
Results evaluation	Qualitative						

### Implementation

N. of participants	29	PT items	fish fillet sandwiches	405
Public laboratories	/			105

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Private laboratories	1	PT panel composition	3 fish fillet sandwiches each spiked with 1 larva;		
NRL	29	Shipping	DHL		
Shipping dates	11/03/202				

#### 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	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	artises 1 s laure	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	

Legend: Laboratories that failed the PT are grey boxed and marked in bold.

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### Summary of results:

Total number of PT panels	35	
Number of participant laboratories	29	
Number of participants that passed the PT	22	
Number of participants that failed the PT	7	

### Overtime comparison of results

Laboratory code	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
AF01	Р	Р	Р	Р	Р	Р		Р	Р	Р	Р
AF02	Р	Р	Р	N	Р	Р	Р	Р	Р	Р	N
AF03	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р
AF04	Р	Р	Р	Р	Р	Р	N	Р	Р	Р	Р
AF05	Р		Р	Р	Р	Р	Р	Р	N	Р	Р
AF06			Р		Р	Р	Р	Р	Р	Р	Р
AF07										1.5	Р
AF08	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р
AF09						Р		Р	Р	Р	Р
AF10	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р
AF11	Р	Р	Р	Р	Р	Р	N	Р	Р	Р	Р
AF12	N	Р	Р	N	Р	Р	Р	Р	Р		N
AF13	Р	Р	Р	N	Р	Р	Р	Р	Р	Р	Р
AF14					Р	N	Р	Р	N	Р	Р
AF15	Р	N	Р	Р	Р	Р	Р	Р	Р	N	N
AF16	N	Р	P	Р	Р	Р		Р	N	Р	Р
AF17									Р	Р	N
AF18	Р	Р	N	Р	Ρ.	Р	Р	Р	Р	Р	N
AF19	N	Р	Р	N	Р	Р	Р	Р	Р	Р	Р
AF20	Р	Р	N	N					Р	Р	Р
AF21	Р	Р	Р	Р	Р	N	Р	Р	Р	Р	Р
AF22			0 (4.5 %)	Р	Р	Р	Р	Р	Р	Р	Р
AF23	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р	N
AF24	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р	Р
AF25	Р		Р	N	P		Р	Р	Р	Р	Р
AF26	Р	Р	Р	Р	Р	Р	N	Р	Р	Р	N
AF27		Р	Р	Р	Р	Р		N	Р	Р	Р
AF28		oley (			Р	"""		N	N	Р	Р
AF29		Р	Р	Р	Р	Р	N	Р	Р	Р	Р

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

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#### Comments:

For the PT round 2024, 76% of the laboratories (22/29) passed the PT. Seven laboratories failed the PT reporting each one false negative (0 instead of 1) and all applied artificial digestion method. No explanation was provided by any of the participant for the PT failure and low performance. It can be hypothesized that limited experience of new analyst(s) could be the reason of poor performance since failure was observed in laboratories that usually performed well in previous years. However, we cannot exclude that problems occurred during analysis.

In PT 2024 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. However, a remarkable decrease in the overall performance of the laboratories was observed, particularly in comparison to the previous year (2023). Indeed, the percentage of failing laboratories was 4% in 2023 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. Lack of experience of technical personnel (either new or not familiar with the test methods) could mainly explain negative results. It is anyway to be remarked that several laboratories did not provide a 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 not correctly reported (EURLP method) by 20 out of 29 participants. Indeed, for AD the only allowed procedure is the ISO 23036-2:2021 or, eventually, an internal procedure of the laboratory but in compliance with the ISO 23036-2:2021. Similarly, 2 out to 29 laboratories reported incomplete reference to the UV-press-method (UV). This prevented the PT provider to express any further comment on the correct or incorrect application of the procedure

Also 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, 83%), largely because it does not require special equipment. UV-press method (UV) was used in five laboratories (17%).

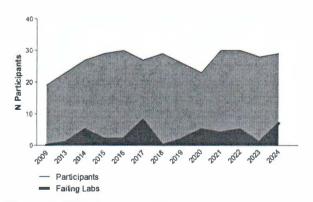


Figure 1. PT04 trend overtime

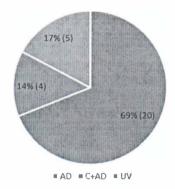


Figure 2. Percentage of the detection methods applied

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Written and elaborated by PTP person in charge

Date 22/05/2024

Verified and issued by The Director

Dr. A. Casulli

#### Notes:

- 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.
- 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.
- At the beginning of each year, the organizer draws up a PT program and makes it known by sending an email to the NRLs
- The final report issue of each PT round shows the PT program implementation.

End of the report

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