

## Final report PT-04: An 1/2022

### 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"> <li>• 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.</li> <li>• 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.</li> <li>• EC Regulation No 2074/2005 (Candling)</li> </ul>	
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	93
Public laboratories	1			

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Private laboratories			PT panel composition	3 fish fillet sandwiches: each spiked with 1 larva.
NRL	29		Shipping	DHL
Shipping dates	14/03/2022			

## 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
<b>A3</b>	<b>2</b>	<b>1</b>	<b>Artificial digestion;</b>	<b>Negative</b>
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
A9	3	0	Artificial digestion;	Positive
A10	3	0	Candling;Artificial digestion;	Positive
A12	3	0	Artificial digestion;	Positive
A13	3	0	Artificial digestion;	Positive
A15	3	0	UV examination after freezing (UV-Press);	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
A21	3	0	Artificial digestion;	Positive
A25	3	0	Artificial digestion;	Positive

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A26	3	0	Artificial digestion;	Positive
<b>A28</b>	<b>2</b>	<b>1</b>	<b>UV examination after freezing (UV-Press);</b>	<b>Negative</b>
A29	3	0	UV examination after freezing (UV-Press);	Positive
A30	3	0	Artificial digestion;Candling;	Positive
A31	3	0	Artificial digestion;	Positive
<b>A32</b>	<b>2</b>	<b>1</b>	<b>Artificial digestion;</b>	<b>Negative</b>
<b>A35</b>	<b>0</b>	<b>3</b>	<b>Artificial digestion;</b>	<b>Negative</b>
A36	3	0	Artificial digestion;	Positive
<b>A38</b>	<b>2</b>	<b>1</b>	<b>Artificial digestion;Compressorium;</b>	<b>Negative</b>
A39	3	0	UV examination after freezing (UV-Press);	Positive
A43	3	0	Artificial digestion;	Positive
A44	3	0	Candling;	Positive

**Legend:**

- Laboratories that failed the PT are marked in bold.

**Summary of results:**

Total number of PT panels	31
Number of participant laboratories	30
Number of participants that passed the PT	25
Number of participants that failed the PT	5

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**Overtime comparison of results**

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

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

**Comments:**

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For the PT round 2022, 83% of the laboratories (25/30) passed the PT. Five laboratories failed the PT, and among them four reported one false negative whereas one laboratory reported all samples as false negative. Reasons for the failure were due to new unexperienced analysts and/or problems with the detection procedure. In particular, four used the Artificial Digestion (AD) method (with or without candling), and one used the UV-press (UV) method. Noteworthy, one laboratory overestimated the number of spiked larvae in one sample and the discrepancy could be explained by poor expertise of the analyst with did not confirm by microscopic observation the morphology of the recovered larvae. Under 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 over-/under-estimation and to take appropriate actions including further training of the analyst on morphological identification of the larvae or proper application of the used method. Laboratories that failed the PT were invited to consider appropriate corrective actions (e.g. shipping of additional sample panel for an EQA).

In PT 2022 round no increase in the overall performance of the laboratories was observed compared with the previous year (2021). However, a renewed and broader interest in the PT was observed with the participation of one new public laboratories (not NRL) and of two reference laboratories that did not participate for several years. 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 the negative result.

In comparison to the previous years (30 in 2021, 23 in 2020; 26 in 2019; 28 in 2018, 27 in 2017 and 30 in 2016), we observed positive trend in the number of laboratories that subscribe to the PT (Figure 1), as well as in the failure percentage (13% in 2021 vs 16% in 2022).

The relative percentage of detection methods adopted did not change substantially (Figure 1) with the Artificial digestion (AD) still being the prevalent method applied (24 laboratories, 80%), largely because it does not require special equipment. UV-press method (UV) alone was used in 5 laboratories (16.5%). Except for one laboratory, candling (C) was used in combination with artificial digestion. The use of candling (i.e. visual inspection) could be explained as it is the only method clearly reported in the EU regulation 2074/2005.

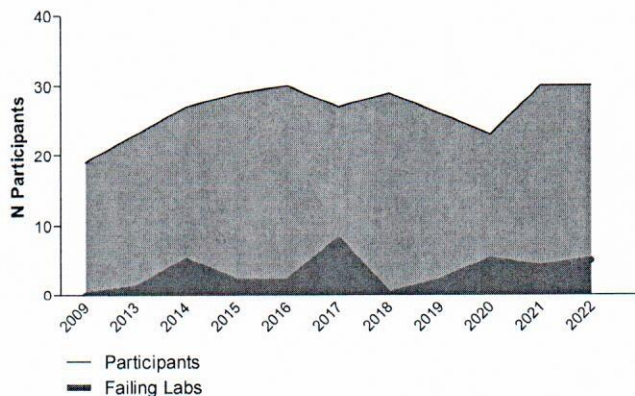


Figure 1. PT04 trend overtime

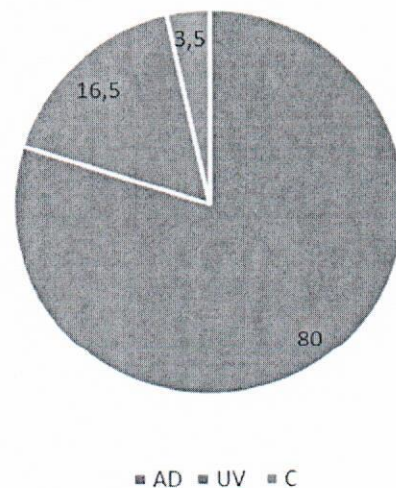


Figure 2. Percentage of the detection methods applied

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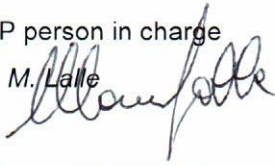
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PTP person in charge

Dr. M. Lalle



Date 17/05/2022

The Director

Dr. S.M. Cacciò



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

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