

Final report PT-04: An 1/2026

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

Design

Purpose	Evaluation of laboratories competence in detection Anisakidae larvae in fish fillet	
Timetable	Invitation mail: 27/01/2026 Website updates: 31/01/2026 Registration deadline: 20/02/2026 PT items production: 16/03/2026 Shipping: 16/03/2026 Results submission deadline: 23/03/2026 Publication of the Final PT Report: 29/05/2026	
Participants	National Reference Laboratories for parasites, Public and private, national and international institution	
Number of participants	Depending on requests	
PT items	Matrix	Fresh water farmed fish fillet
	Item	Anisakidae live larvae
	Number of PT items	3 per participant
	Panel composition	2 positive PT items (1 larva and 2 larvae) and 1 negative PT item
	Number of surplus items	18
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Celani F., Di Grazia A., Santolamazza F., Tartarelli I

Compliance with planned timelines: YES NO

Participant number and type: **31** public institutions

Acquisition of matrix and analyte: Fillets of fresh water farmed trout (*Salmo trutta*) were purchased from qualified suppliers n 13/03/2026 and stored at 4°C until PT items preparation. Viscera of European hake (*Merluccius merluccius*) infested with L3 larvae of Anisakidae (*Anisakis pegreffii*) were provided by qualified suppliers on 13/03/2026 and stored a 4°C until PT items preparation. Alive and capsule-free larvae were individually collected by tweezers from the European hake viscera on the day of PT items preparation (16/03/2026)

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Production of PT items: PT items were prepared on 16/03/2026. Each PT panel consist of three sandwiches of fish fillet either contaminated or not with L3 larvae of Anisakidae, alive and capsule-free.

Activities provided by external providers: The PT provider (PTP) entrusted the shipment of PT items to a qualified transport company. The company that provided the shipping service was DHL.

Homogeneity and stability of PT items: Since PT items are individually spiked, homogeneity is ensured by accurate counting of the number of larvae spiked into each PT item, made by two operators. The stability of PT items in the package has been evaluated by ad hoc experiments made by PTP on vacuum sealed samples stored between +4°C and +15°C. Capsule-free larvae remain viable up to seven days from the date of preparation. Quality control requires that PTP's staff analyse PT items produced at the same time as those sent to participant and analysed on the deadline date for submitting results. The analysis is performed with an internal procedure based on 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.

Distribution of PT items: PT items were shipped on 16/03/2026, and the deadline for submission of results was set for 23/03/2026. Each set of PT items sent to participants consisted of a vacuum-sealed bag labeled with the participant's identification code. Inside were three smaller bags, each containing a fish fillet sandwich. Each small bag was vacuum-sealed and identified with the code assigned by the PTP to the PT item. Packaging consisted of a polystyrene and cardboard container with enough refrigerant packs to ensure a temperature between 4°C and 15°C inside the package.

Instructions for participants: Participants were informed of the shipment date via email on 16/03/2026. The email also contained a link for submitting results, which was active from 16/03/2026 (coinciding with shipment); during the PT, the deadline for the form was postponed to 27/03/2026 to allow one country, whose PT items had been held at customs until 23/03/2026, to complete the PT. Participant instructions were made available on the PTP website from 27/01/2026. These instructions also contained information for sending feedback and submitting results. This information was sent to participants along with the PT announcement email and remarked in the email sent on 16/03/2026 (shipment date).

Data analysis: Feedback and participant results were collected via the online Forms application and transferred from the PTP to an Excel file used for data processing and further used to generate the tables with the participant results contained in this PT report.

Assigned value: the assigned value is determined by the expertise of the proficiency test manager and the technical staff involved in preparing the PT items, as well as by parasite counting carried out by at least two operators.

Criteria for results evaluation: Result evaluation is qualitative. Result is considered "correct" if the participant properly identifies PT items spiked or not with larvae and "incorrect" in case of false positive or false negative identification. Results must be expressed indicating the number of larvae detected in each PT item, allowing PTP for a more accurate evaluation of participant performance. Due to the nature of PT items, no statistical parameters are applied for result evaluation. Results are evaluated against the value assigned by the PTP. The final evaluation is "positive" if all PT items are correctly identified, otherwise it is "negative".

Confidentiality of results: The confidentiality of this report is guaranteed using a unique code that allows the anonymity of participants. The identity of participants is kept confidential and subject to professional secrecy. The EURL-P reserves the right to provide the results of participation in the PT scheme to the competent authorities upon request. The participant will be notified in writing if a competent legislative authority requests the provision of the PT results.

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Results provided by participants and performance EVALUATION

Participant code	Applied method	Item code	Result	Assigned value	Outcome	Final evaluation
AF01	AD	A1400	0	0 (neg)	CORRECT	POSITIVE
		A1401	1	1 (pos)	CORRECT	
		A1402	2	2 (pos)	CORRECT	
AF02	UV-Press	A1403	0	0 (neg)	CORRECT	POSITIVE
		A1404	1	1 (pos)	CORRECT	
		A1405	2	2 (pos)	CORRECT	
AF03	AD	A1406	0	0 (neg)	CORRECT	POSITIVE
		A1407	1	1 (pos)	CORRECT	
		A1408	2	2 (pos)	CORRECT	
AF04	UV-Press	A1409	0	0 (neg)	CORRECT	NEGATIVE
		A1410	0	1 (pos)	INCORRECT	
		A1411	2	2 (pos)	CORRECT	
AF05	AD	A1412	0	0 (neg)	CORRECT	POSITIVE
		A1413	1	1 (pos)	CORRECT	
		A1414	2	2 (pos)	CORRECT	
AF06	AD	A1415	0	0 (neg)	CORRECT	POSITIVE
		A1416	1	1 (pos)	CORRECT	
		A1417	2	2 (pos)	CORRECT	
AF07	AD	A1418	0	0 (neg)	CORRECT	NEGATIVE
		A1419	0	1 (pos)	INCORRECT	
		A1420	2	2 (pos)	CORRECT	
AF08	UV-press	A1421	0	0 (neg)	CORRECT	POSITIVE
		A1422	1	1 (pos)	CORRECT	
		A1423	2	2 (pos)	CORRECT	
AF09	Ca+Co+AD	A1424	0	0 (neg)	CORRECT	POSITIVE
		A1425	1	1 (pos)	CORRECT	
		A1426	2	2 (pos)	CORRECT	
AF10	AD	A1427	0	0 (neg)	CORRECT	POSITIVE
		A1428	1	1 (pos)	CORRECT	
		A1429	2	2 (pos)	CORRECT	
AF11	AD	A1430	0	0 (neg)	CORRECT	POSITIVE
		A1431	1	1 (pos)	CORRECT	
		A1432	2	2 (pos)	CORRECT	
AF12	Ca+UV-Press+AD	A1433	0	0 (neg)	CORRECT	POSITIVE
		A1434	1	1 (pos)	CORRECT	
		A1435	2	2 (pos)	CORRECT	
AF13	AD	A1436	0	0 (neg)	CORRECT	POSITIVE
		A1437	1	1 (pos)	CORRECT	
		A1438	2	2 (pos)	CORRECT	
AF14	AD	A1439	0	0 (neg)	CORRECT	POSITIVE
		A1440	1	1 (pos)	CORRECT	
		A1441	2	2 (pos)	CORRECT	
AF15	AD	A1442	0	0 (neg)	CORRECT	POSITIVE
		A1443	1	1 (pos)	CORRECT	
		A1444	2	2 (pos)	CORRECT	
AF16	AD	A1445	0	0 (neg)	CORRECT	POSITIVE
		A1446	1	1 (pos)	CORRECT	
		A1447	2	2 (pos)	CORRECT	
AF17	Ca+UV-press+AD	A1448	0	0 (neg)	CORRECT	POSITIVE
		A1449	1	1 (pos)	CORRECT	
		A1450	2	2 (pos)	CORRECT	
AF18	AD	A1451	0	0 (neg)	CORRECT	POSITIVE
		A1452	1	1 (pos)	CORRECT	
		A1453	2	2 (pos)	CORRECT	

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Continue

Participant code	Applied method	Item code	Result	Assigned value	Outcome	Final evaluation
AF19	UV-Press	A1454	0	0 (neg)	CORRECT	POSITIVE
		A1455	1	1 (pos)	CORRECT	
		A1456	2	2 (pos)	CORRECT	
AF20	Ca+ AD	A1457	0	0 (neg)	CORRECT	POSITIVE
		A1458	1	1 (pos)	CORRECT	
		A1459	2	2 (pos)	CORRECT	
AF21	AD	A1460	0	0 (neg)	CORRECT	POSITIVE
		A1461	1	1 (pos)	CORRECT	
		A1462	2	2 (pos)	CORRECT	
AF22	AD	A1463	0	0 (neg)	CORRECT	POSITIVE
		A1464	1	1 (pos)	CORRECT	
		A1465	2	2 (pos)	CORRECT	
AF23	AD	A1466	0	0 (neg)	CORRECT	POSITIVE
		A1467	1	1 (pos)	CORRECT	
		A1468	2	2 (pos)	CORRECT	
AF24	AD	A1469	0	0 (neg)	CORRECT	POSITIVE
		A1470	1	1 (pos)	CORRECT	
		A1471	2	2 (pos)	CORRECT	
AF25	AD	A1472	0	0 (neg)	CORRECT	POSITIVE
		A1473	1	1 (pos)	CORRECT	
		A1474	2	2 (pos)	CORRECT	
AF26	UV-Press	A1475	0	0 (neg)	CORRECT	POSITIVE
		A1476	1	1 (pos)	CORRECT	
		A1477	2	2 (pos)	CORRECT	
AF27	AD	A1478	0	0 (neg)	CORRECT	NEGATIVE
		A1479	0	1 (pos)	INCORRECT	
		A1480	2	2 (pos)	CORRECT	
AF28	AD	A1481	0	0 (neg)	CORRECT	POSITIVE
		A1482	1	1 (pos)	CORRECT	
		A1483	2	2 (pos)	CORRECT	
AF29	AD	A1484	0	0 (neg)	CORRECT	NEGATIVE
		A1485	0	1 (pos)	INCORRECT	
		A1486	2	2 (pos)	CORRECT	
AF30	AD	A1487	0	0 (neg)	CORRECT	POSITIVE
		A1488	1	1 (pos)	CORRECT	
		A1489	2	2 (pos)	CORRECT	
AF31	AD	A1490	0	0 (neg)	CORRECT	POSITIVE
		A1491	1	1 (pos)	CORRECT	
		A1492	2	2 (pos)	CORRECT	

Legenda:

- Laboratories that failed the PT are dark grey boxed and marked in bold.
- AD=Artificial digestion; UV-Press=UV examination after pressing and freezing; Ca=Candling; Co=Compressorium

Summary of results:

Total number of PT panels	31
Number of participants	31
Number of participants that passed the PT	27
Number of participants that failed the PT	4

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Overtime comparison of results

Laboratory code	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026
AF01	NP	NP	NP	NP	POS	NEG	POS	POS	NEG	POS	POS	POS	POS
AF02	NP	NP	NP	NP	NP	POS	NP	POS	POS	POS	POS	POS	POS
AF03	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	NP	NEG	POS	POS
AF04	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	NEG
AF05	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF06	NP	NP	NP	NP	POS	NP	NP	NEG	NEG	POS	POS	POS	POS
AF07	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	POS	NEG
AF08	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF09	NP	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF10	POS	POS	NEG	NEG	NP	NP	NP	NP	POS	POS	POS	POS	POS
AF11	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS	POS
AF12	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
AF13	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF14	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS	POS
AF15	NP	POS	POS	POS	POS	POS	NP	NEG	POS	POS	POS	POS	POS
AF16	NEG	POS	POS	POS	POS	POS	NP	POS	NEG	POS	POS	POS	POS
AF17	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF18	NP	NP	NP	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF19	NP	NP	POS	NP	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF20	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
AF21	POS	POS	POS	POS	POS	POS	NP	POS	POS	POS	POS	POS	POS
AF22	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
AF23	POS	NP	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS
AF24	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS	NEG	POS	POS
AF25	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
AF26	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS
AF27	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG
AF28	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	POS	POS
AF29	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG
AF30	POS	NEG	POS	NEG	POS	POS	NEG	POS	NP	NP	NP	NP	POS
AF31	POS	NP	POS	NEG	POS	NP	POS	POS	POS	POS	POS	POS	POS

NP= no participation (grey). POS= positive (white). NEG= negative (black).

Comments on participants' performance: In the PT round of 2026 30 out of 31 participants received the PT items within 72 hours. The late delivery of the package for one participant was due to customs' delay. For the PT round 2026, 87% of the participants (27/31) passed the PT. Four participants failed the PT, all reporting a false negative in the item spiked with 1 larva. Three of the four used the artificial digestion method (AD) and one the UV examination after freezing (UV-Press). This last participant (AF04) also failed in the previous year, but at that time probably the cause was the shuffling of PT items. Another participant (AF07) also failed in the 2024 round. The other two participants instead showed good performance in the past five years. In PT 2026 round, the overall number of participant laboratories appears to have reached a plateau when compared with the last four years (Figure 1) and, as for the 2025 round, it is currently limited to NRLs. A National Reference Laboratory that did not participate for the previous four years, joined again the PT round. A slight decrease in the overall performance of the laboratories was observed, particularly in comparison to the previous year (2025). Indeed, the percentage of failing laboratories was 12% in 2026, thus much higher than 3% in 2025 but lower than 24% in 2024. Nevertheless, a trend with a periodic fluctuation in failure percentage can be observed overtime and it might reflect turnover of technical personnel in each laboratory. Indeed, poor performance (failure) could not be related to irregular or first-time participation in the PT.

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Performance evaluation if different methods are applied: 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 (Ca) or compressorium (Co), still being the prevalent method applied (24 laboratories, 78%), largely because it does not require special equipment. UV-press method (UV) was used alone in five laboratories (16%) or followed by AD in two laboratories (7%). Compared to the previous year almost all participants provided correct information regarding the method applied. It is relevant to mention that, also in 2026 three participants still reported EURLP method as reference to AD, and two were among those failing the PT. In particular, one of the failing participants (AF27) reported that fish fillets were pulled apart by fingers instead of being chopped by scissors or a knife and that digestion temperature: 45° C, not in compliance with either the ISO 23036-2:2021 or the EURL-P internal standard operating procedure (POP04) based on ISO 23036-2:2021). Regarding the UV-press method, the participant that failed reported a variation compared to the ISO 23036-1:2021, with samples being put in a bag and then frozen at -20°C, the defrost and pressed under a hydraulic press with a 1mm frame and finally examined under UV light. Independently from the applied method (AD or UV-press) the percentage of failure was comparable, being 12,5% using AD vs 20% using UV-press.

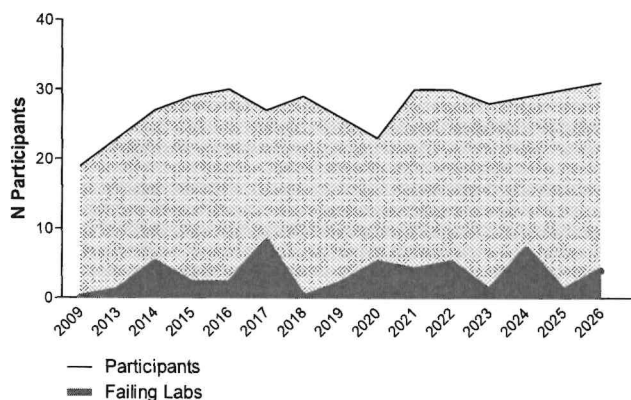
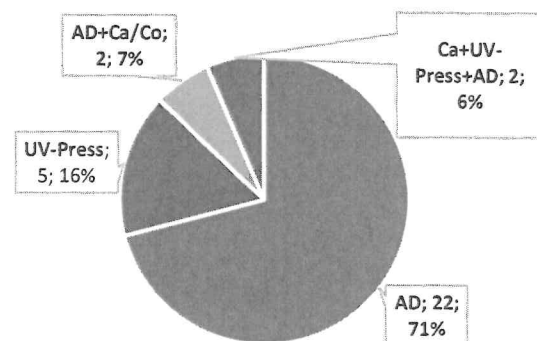


Figure 1. PT04 trend overtime



2. Percentage of the detection methods applied

Comments and recommendations based on the outcomes of PT: At the time of the preparation of this report, specific reasons for the PT failure could not be analyzed. A request to fill in a follow-up form will be forwarded to participants that failed the PT after this Report publication and eventually discussed during the NRL Annual Workshop to be held at Istituto Superiore di Sanità in October 2026. Reporting a false negative sample could depend on several factors such as:

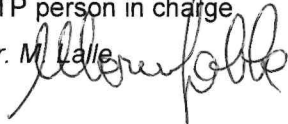
- Exchange of PT items during analysis.
- Destruction of larvae during the mechanical chopping phase of the sample.
- Incomplete digestion of the sample, indicated by a high amount of undigested residue present on the sieve. This may be due to unsuitable reagents (e.g., expired or low-activity pepsin) or inadequate digestion time and temperature.
- Insufficient rinsing of the apparatus used for artificial digestion (beaker, sieve), which may lead to the loss of larvae.

- Failure to recognize the larvae by the operator.
- Loss of the larvae during unpacking and sample manipulation.

We recommend to participant that failed the PT to carefully review their procedure, particularly since all have reported a false negative when the item was spiked with a single Anisakidae larva, suggesting problem with method sensitivity.

Written and elaborated by
PTP person in charge

Dr. M. Lalle



Verified and issued by
The Director

Dr. A. Casulli



Date 27/05/2026

Notes:

1. Personal data are processed in compliance with the regulatory provisions referred to in EU Regulation 2016/679 and Privacy Code, as reported in Legislative Decree no. 101/2018. The data controller of personal data is the Istituto Superiore di Sanità with registered office in Viale Regina Elena n. 299 - 00161 Rome, in the person of its President. In addition, the ISS has appointed its own Data Protection Officer (D.P.O.), e-mail address: responsabile.protezionedati@iss.it. Data are processed exclusively for carrying out the PT activities, for this purpose adequate physical, technical and organizational security measures have been set up to prevent and avoid their destruction and/or loss of integrity, as well as their illicit or incorrect use. Data is accessible only to authorized personnel who has their own credentials and their own operating station. The participant has the rights referred to in art. 15 GDPR et seq., more precisely right of access, right of rectification, right of treatment limitation, right to data portability, right of opposition, as well as the right to lodge a complaint with the Guarantor Authority (art. 77 GDPR and 141 Privacy Code, as reported by Legislative Decree 101/2018). The ISS, in its capacity as Data Controller, undertakes to keep the records of processing activities correctly pursuant to art. 30 GDPR.
2. The original raw data and a copy of Final PT Report are kept for 10 years at the PTP site.
3. Participants may use this report to support their skills to the accreditation body and other interested parties.
4. The accreditation, according to the ISO/IEC 17043 international standard, is regulated by a specific agreement and recognizes the technical competence of the PTP to organize PT schemes. The accreditation body, ACCREDIA (www.accredia.it), does not take any responsibility for the activities related to production of PT items and participants results evaluation.

End of the report

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