# Final report PT-07: AnMol 1/2025

## PT-07: "Molecular identification of Anisakid nematodes at the species level"

### Design

| | | |
|---|---|---|
| Purpose | Evaluation of laboratories competence in molecular identification of anisakidae nematodes species | |
| Scheme type | Single, simultaneous | |
| Participants | National reference laboratories for parasites.<br>Public and private laboratories | |
| N. of participants | Depending on request | |
| Method | not regulated | |
| Test method | chosen by the participant | |
| PT items | Matrix | Ethanol (larvae) and saline buffer (DNA) |
| | Item | anisakid nematodes (DNAs or larvae fragments) |
| | N. of samples | 4 vials for each participant |
| | Distribution | Preparation and packaging can be performed before shipment |
| Subcontracted activities | NA | |
| Results evaluation | Qualitative | |

### Implementation

| | | | | |
|---|---|---|---|---|
| N. of participants | 17 | PT items | DNA | 34 |
| Public laboratories | / | | Larvae fragments | 34 |
| Private laboratories | / | | PT panel composition | 2 items with a single species DNA each (*Contracoecum osculatum or Anisakis pegreffii*), 2 items with a single larva fragment each (*P. decipiens sl or A. simplex ss)* |
| NRL | 17 | | Shipping | DHL |
| Shipping dates | 17/03/2025 | | | |

PT Provider
Unit of Foodborne and Neglected Parasitic Diseases
Istituto Superiore di Sanità

viale Regina Elena, 299 – 00161 Rome, Italy

Person in charge of PT: Dr. Marco Lalle
e-mail: marco.lalle@iss.it;
tel: +39 0649902670

00629

## Results

The PT final evaluation was qualitative only. The PT was considered passed if all species were correctly identified by the participant.

| Laboratory code | N° of samples correctly identified | N° of samples NOT correctly identified | Method applied | Final evaluation |
|---|---|---|---|---|
| **AMM01** | **3** | **1** | **In house method (PCR and sequencing)** | **NEGATIVE** |
| AMM02 | 4 | 0 | EURLP method 2 (multiplex PCR); | POSITIVE |
| AMM03 | 4 | 0 | Published method (PCR and Sequencing) | POSITIVE |
| AMM04 | 4 | 0 | EURLP method 2 (multiplex PCR); | POSITIVE |
| **AMM05** | **3** | **1** | **EURLP method 1 (PCR_RFLP);** | **NEGATIVE** |
| **AMM06** | **3** | **1** | **EURLP method 1 (PCR_RFLP);** | **NEGATIVE** |
| AMM7 | 4 | 0 | EURLP method 2 (multiplex PCR); | POSITIVE |
| AMM8 | 4 | 0 | EURLP method 1 (PCR_RFLP); | POSITIVE |
| **AMM9** | **2** | **2** | **EURLP method 2 (multiplex PCR);** | **NEGATIVE** |
| AMM10 | 4 | 0 | EURLP method 2 (multiplex PCR); | POSITIVE |
| AMM11 | 4 | 0 | EURLP method 2 (multiplex PCR);In house method (PCR-RFLP and multiplex PCR) | POSITIVE |
| AMM12 | 4 | 0 | Published method (PCR and sequencing) | POSITIVE |
| AMM13 | 4 | 0 | EURLP method 1 (PCR_RFLP);EURLP method 2 (multiplex PCR); | POSITIVE |
| AMM14 | 4 | 0 | EURLP method 1 (PCR_RFLP);EURLP method 2 (multiplex PCR);In house method (PCR and sequencing) | POSITIVE |
| AMM15 | 4 | 0 | EURLP method 2 (multiplex PCR); | POSITIVE |
| AMM16 | 4 | 0 | EURLP method 1 (PCR_RFLP); | POSITIVE |
| AMM17 | 4 | 0 | EURLP method 1 (PCR_RFLP); | POSITIVE |

**Legend**:

- Laboratories that failed the PT are gey highlited and marked in bold.

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## Summary of results:

| | |
|---|---|
| Total number of PT panels | 68 |
| Number of participant laboratories | 17 |
| Number of participants that passed the PT | 13 |
| Number of participants that failed the PT | 4 |

## Overtime comparison of results

| Laboratory code 2025 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 |
|---|---|---|---|---|---|---|---|---|---|
| AMM01 | | | | P | P | P | | P | |
| AMM02 | P | P | | P | P | P | P | P | P |
| AMM03 | | | P | | P | P | P | P | P |
| AMM04 | | | | NA | P | P | P | P | P |
| AMM05 | P | P | P | P | P | P | | P | |
| AMM06 | P | P | P | | P | P | | P | |
| AMM07 | P | P | P | P | P | P | P | P | P |
| AMM08 | P | NA | | | P | P | | P | P |
| AMM09 | | | | | | | | | |
| AMM10 | | | | | | | | | P |
| AMM11 | P | P | P | P | P | P | P | | P |
| AMM12 | | | | | | | | | P |
| AMM13 | P | P | P | P | P | P | P | P | P |
| AMM14 | | P | | | P | | | P | P |
| AMM15 | | P | | | | | | P | P |
| AMM16 | | | | | P | | | | P |
| AMM17 | | | | | NA | P | | P | P |

**Note:** P, positive; N, negative; NA, no result received; grey box, no participation

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**Comments:**

In the PT round of 2025, all but one laboratories, received the PT items within 72 hours. Thirteen participants (77%) successfully accomplished the PT, whereas four laboratories (23%) failed. In particularly, two laboratory (AMM01 and AMM06) had problem in either DNA isolation and/or DNA amplification from *Pseudoterranova decipiens* larva fragment or PCR amplification from *Contracaecum* osculatum DNA (AMM05). One did not correctly identify both DNA samples of *C. osculatum (*they were reported as *A. typica)*. The fourth laboratory (AMM09) did not correctly identify the larva fragment of *A. simplex* (it was reported as *A. pegreffii*) and the DNA from *C. osculatum* (it was reported as *Hysterotilacium aduncum*). This laboratory used the EURLP method 2 (multiplex PCR).

The reasons for the PT failure were analyzed and reported by two participants. AMM09 reported misreading of the method instructions and incorrect interpretation of the PCR fragment obtained. This laboratory participated for the first time and did not have reference material for comparison. AMM06 reported a mistake when loading the DNA in the PCR reaction. Following the Individual Report the PCR was run again with the same DNA and the result was *Pseudoterranova spp.* No feedback was received from the other two laboratories.

Concerning the applied molecular method(s) (Figure 1): 5 laboratories applied only the PCR-RFLP method (EURLP 1; MI04); 7 used only the multiplex-PCR (EURLP 2; MI10) alone or in addition to in-house method; 2 used both methods; 2 used only in house/published methods (one of which was PCR of the EURLP 1 followed by Sanger sequencing instead of RFLP).
Compared to the previous years the number of participant laboratories slightly increased and three new laboratories joint the PT. The number of laboratories that failed the 2025 PT round increased (23%) in comparison to previous years (Figure 2) except for 2023. The overtime comparison indicates that laboratories that have failed in previous year have the tendency to fail again time by time. However for some laboratories efforts were done to improve the correct application of the molecular identification tests in place.

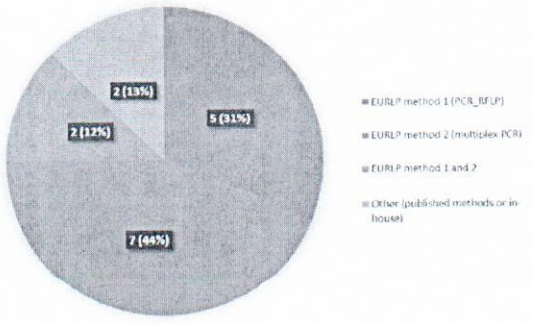


Figure 1. Number of laboratories applying the different methods (percentage are in brackets) for the PT2025

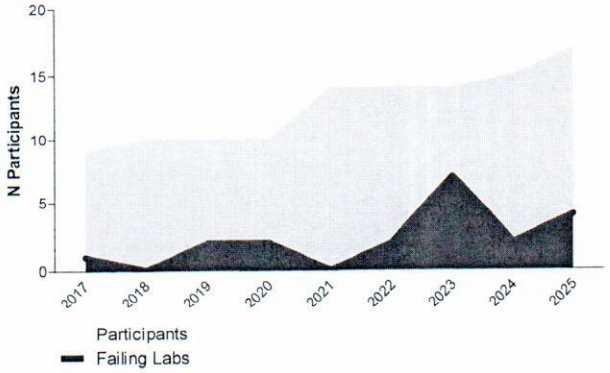


Figure 2. PT07 trend overtime

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tel: +39 0649902670

00629

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

Verified and issued by
The Director
*Dr. A. Casulli*

**Date** 19/05/2025

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