# Final report PT-AnMol 1/2020

# PT report on "Molecular identification of Anisakid nematodes at the species level"

## Design

| | | |
|---|---|---|
| Purpose | Evaluation of laboratories in charge for official control on food | |
| Scheme type | Single | |
| Participants | Public and private, European laboratories | |
| N. of participants | Depending on request | |
| Method | not regulated | |
| Test method | chosen by the participant | |
| PT items | Matrix | fresh water farmed fish fillet |
| | 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 | 11 | PT items | DNA | 22 |
| Public laboratories | | | Larvae fragments | 22 |
| Private laboratories | | | PT panel composition | 2 samples with single species DNA, 2 samples with a single larva fragment each |
| NRL | 11 | | Shipping | DHL Express |
| Shipping dates | | 09/03/2020 | | |

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## 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 | Final evaluation |
|---|---|---|---|
| A6 | 4 | 0 | Positive |
| A7 | 4 | 0 | Positive |
| **A8** | **3** | **1** | **Negative** |
| A10 | 4 | 0 | Positive |
| **A12** | **0** | **4** | **Negative** |
| A15 | 4 | 0 | Positive |
| A16 | 4 | 0 | Positive |
| A17 | 4 | 0 | Positive |
| A20 | 4 | 0 | Positive |
| A28 | 4 | 0 | Positive |
| A40[a] | NA | NA | NA |
| | | | |

**Legend**:

- Laboratories that failed the PT are marked in bold.
- [a] due to Covid-19 pandemic the laboratory could not test the samples.
- NA: not applicable

**Summary of results:**

| | |
|---|---|
| Total number of PT panels | 11 |
| Number of participant laboratories | 10 (1 laboratory withdrawed) |
| Number of participants that passed the PT | 8 |
| Number of participants that failed the PT | 2 |

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**Overtime comparison of results**

| Laboratory code | 2017 | 2018 | 2019 | 2020 |
|---|---|---|---|---|
| A1 | *NA* | | | |
| A3 | | | **N** | |
| A6 | P | P | P | P |
| A7 | P | P | P | P |
| A8 | | P | | **N** |
| A10 | P | P | P | P |
| A11 | | NA | | |
| A12 | P | P | P | **N** |
| A15 | | | | P |
| A16 | P | P | P | P |
| A17 | **N** | P | P | P |
| A20 | P | P | P | P |
| A28 | P | P | **N** | P |
| A31 | P | NA | | |
| A38 | | P | | |
| A39 | | | P | |
| A40 | | | | NA |

**Note:** P, positive; N, negative; NA, not applicable, results not received; grey boxed, no participation

**Comments:**

In the 2020 PT round, only 8 out of 10 participant laboratories successfully accomplish the PT. One laboratory that agree to participate at the PT round could not perform the test and send the result due to limitation in work activities due to Covid-19 pandemic. Compared to the previous year one new laboratory participated. All laboratories correctly received the PT items within 72 hours.
Concerning the two laboratories that failed the PT: i) one did not correctly identifies one DNA sample by using an in house PCR and sequencing method targeting the 5S gene; ii) the other laboratory failed to identify all the samples (1 was PCR negative and 3 were misidentified). For the latest one, the reason of the failure was largely due to an incorrect labelling of samples by the analyst while performing the tests. This laboratory request to EURLP a further sample panel for an EQA scheme.
Concerning the applied molecular method(s) (Figure 1): 3 laboratories applied only the PCR-RFLP method (EURLP MI04); 2 used only the multiplex-PCR (EURLP MI10); 1 used both methods; 1 used PCR-RLFP in combination with a sequencing publish method; and, finally, 3 applied in house or published methods based on PCR and Sanger sequencing. No relation between the applied methods and successful identification was evident.

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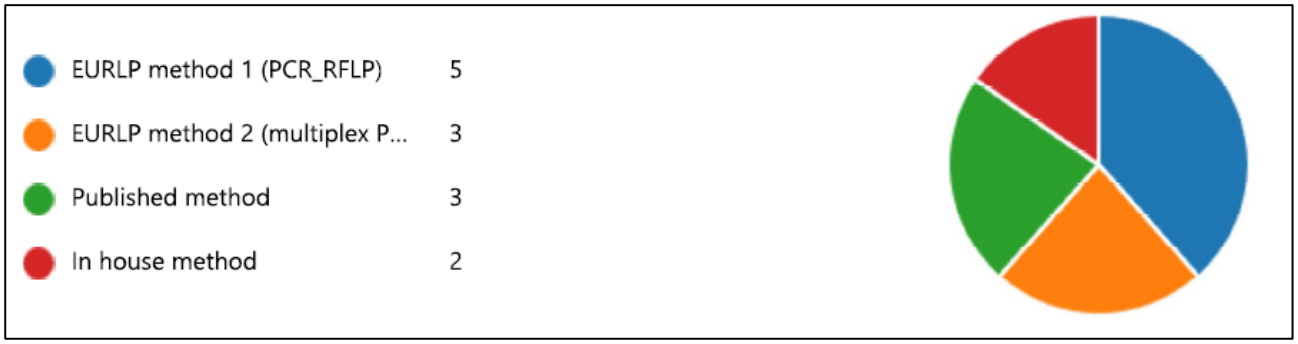


**Figure 1.** Detection method(s) used in the PT participant lab. Note that some laboratories use more than one method.

Compared to the previous years, no differences in the number of laboratories providing the results occurred. No relevant variation in the overall performance could be highlighted and, at least in one case, the low performance could be sue to inexperience of laboratory personnel.

The Director
*Dr. S.M. Cacciò*

**Date** 13/10/2020

Notes:

1. To guarantee confidentiality, participant laboratories are identified by alphanumeric codes. PT participant identity is kept confidential and bound by professional secrecy. If PT results have to be provided directly to a competent authority, the organizer shall send a written notice to inform the involved participants.
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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