

## Final report PT-07: AnMol 1/2024

### 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. Public and private 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	15	PT items	DNA	30
Public laboratories	/		Larvae fragments	30
Private laboratories	/		PT panel composition	2 items with a single species DNA each ( <i>Contracoecum osculatum</i> ), 2 items with a single larva fragment each ( <i>P. decipiens</i> sl and <i>A. pegreffii</i> )
NRL	15		Shipping	DHL

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

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle

e-mail: [marco.lalle@iss.it](mailto:marco.lalle@iss.it); tel: +39 0649902670



**PTP N° 0005 P**

**Membro degli Accordi di Mutuo Riconoscimento  
 EA, IAF e ILAC**

**Signatory of EA, IAF and ILAC  
 Mutual Recognition Agreements**

Shipping dates	11/03/2024
----------------	------------

## 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	4	0	In house method;EURLP method 1 (PCR_RFLP);	Positive
AMM02	4	0	EURLP method 2 (multiplex PCR);	Positive
<b>AMM03</b>	<b>2</b>	<b>2</b>	<b>EURLP method 2 (multiplex PCR);In house method; (PCR-RFLP)</b>	<b>Negative</b>
AMM04	4	0	Published method;	Positive
AMM05	4	0	EURLP method 2 (multiplex PCR);	Positive
AMM06	4	0	EURLP method 1 (PCR_RFLP);	Positive
AMM07	4	0	EURLP method 2 (multiplex PCR);EURLP method 1 (PCR_RFLP);In house method;	Positive
AMM08	4	0	EURLP method 1 (PCR_RFLP);	Positive
<b>AMM09</b>	<b>3</b>	<b>1</b>	<b>Published method;</b>	<b>Negative</b>
AMM10	4	0	EURLP method 1 (PCR_RFLP);	Positive
AMM11	4	0	EURLP method 2 (multiplex PCR);	Positive
AMM12	4	0	EURLP method 1 (PCR_RFLP);EURLP method 2 (multiplex PCR);	Positive
AMM13	4	0	EURLP method 1 (PCR_RFLP);	Positive
AMM14	4	0	EURLP method 2 (multiplex PCR);	Positive
AMM15	4	0	EURLP method 1 (PCR_RFLP);	Positive

### Legend:

- Laboratories that failed the PT are marked in bold and highlighted in grey.

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

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle

e-mail: [marco.lalle@iss.it](mailto:marco.lalle@iss.it); tel: +39 0649902670



**PTP N° 0005 P**

**Membro degli Accordi di Mutuo Riconoscimento  
 EA, IAF e ILAC**

**Signatory of EA, IAF and ILAC  
 Mutual Recognition Agreements**

### Summary of results:

Total number of PT panels	60
Number of participant laboratories	15
Number of participants that passed the PT	13
Number of participants that failed the PT	2

### Overtime comparison of results

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

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

### Comments:

In the PT round of 2024, all but one laboratories, received the PT items within 72 hours. Thirteen participants successfully accomplished the PT, whereas two laboratories (13%) failed. In particular, one laboratory (AMM03) did not correctly identify both DNA samples of *Contracaecum osculatum* (they were reported as *A. typical*), whereas the other (AMM09) did not correctly identify the fragment larva of *A. pegreffii* (it was reported as *A. simplex*).

The reasons for the PT failure were analyzed and reported to the participants. AMM03 reported difficulties in sample amplification and related this to the low amount of DNA in samples. However, the same batch of DNAs were forwarded to all PT participants and no problem about amplification was reported by other participants, independently on the method applied. No problem with items storage during the shipping could be also envisaged, according to temperature check reported by the participant. Based on the information provided in the Result form and additional information provided from the participant by email, it was recommended to optimize both in-house method as well as the EURLP-multiplex PCR (e.g. test alternative Taq polymerase).

AMM09 used a published method that appears to have limitations in detecting several Anisakidae species being specific for *C. osculatum* but not for other species. It is unclear if additional method(s) were used for typing. The laboratory was invited to further improve the in-house method as well as to perform it in parallel

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

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle

e-mail: [marco.lalle@iss.it](mailto:marco.lalle@iss.it); tel: +39 0649902670



**PTP N° 0005 P**

**Membro degli Accordi di Mutuo Riconoscimento  
 EA, IAF e ILAC**

**Signatory of EA, IAF and ILAC  
 Mutual Recognition Agreements**



with recommended EURLP methods. Over the years, this laboratory have failed four out of five times the PT (table "overtime comparison of the results").

Concerning the applied molecular method(s) (Figure 1): 5 laboratories applied only the PCR-RFLP method (EURLP 1; MI04); 5 used only the multiplex-PCR (EURLP 2; MI10); 2 used both methods; 3 used 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. The number of laboratories that failed the 2024 PT round low (13%) in comparison to previous years (Figure 2), indicating that efforts were done to improve the correct application of the molecular identification tests in place.

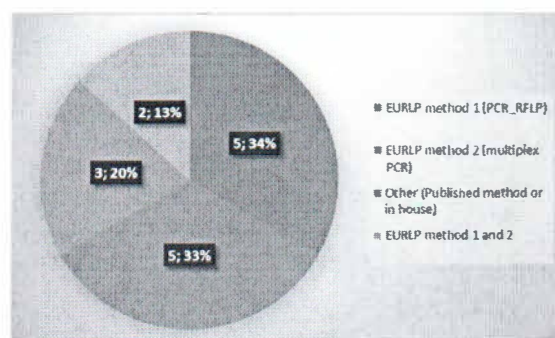


Figure 1. Percentage of Method applied for the PT2024

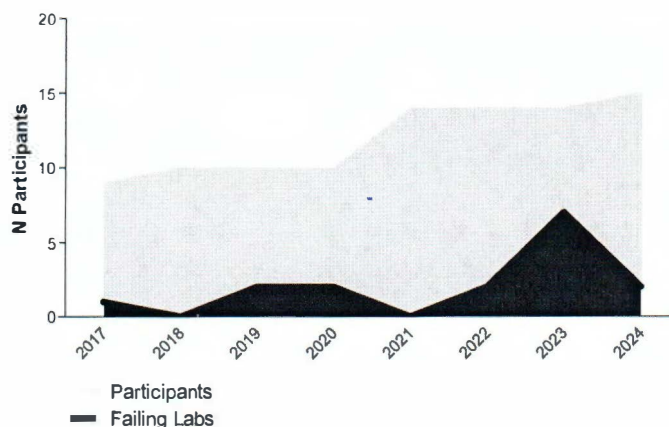


Figure 2. PT07 trend overtime

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

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle

e-mail: [marco.lalle@iss.it](mailto:marco.lalle@iss.it); tel: +39 0649902670



PTP N° 0005 P

Membro degli Accordi di Mutuo Riconoscimento  
 EA, IAF e ILAC

Signatory of EA, IAF and ILAC  
 Mutual Recognition Agreements



Written and elaborated by

PTP person in charge

Dr. M. Lalle

Verified and issued by

The Director

Dr. A. Casulli

Date 22-05-2024

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

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

Viale Regina Elena, 299 – 00161 Rome, Italy

PT Person in charge: Dr. Marco Lalle

e-mail: [marco.lalle@iss.it](mailto:marco.lalle@iss.it); tel: +39 0649902670



**PTP N° 0005 P**

**Membro degli Accordi di Mutuo Riconoscimento  
EA, IAF e ILAC**

**Signatory of EA, IAF and ILAC  
Mutual Recognition Agreements**