Final report PT-07: AnMol 1/2023

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	14	PT items	DNA	28
Public laboratories	/		Larvae fragments	28
Private laboratories	/		PT panel composition	2 samples with a single species DNA each(*A. simplex ss* and *Contracoecum rudoplphi C)*, 2 samples with a single larva fragment each (*P. decipiens sl and A. pegreffii)*

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NRL	14		Shipping	DHL
Shipping dates	13/03/2023			

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
A3	**3**	**1**	**EURLP method 1 (PCR_RFLP);**	**Negative**
A6	4	0	EURLP method 2 (multiplex PCR);	Positive
A7	4	0	EURLP method 1 (PCR_RFLP);EURLP method 2 (multiplex PCR);	Positive
A8	**1**	**3**	In house method;	Negative
A10	4	0	EURLP method 1 (PCR_RFLP);	Positive
A12	**3**	**1**	**EURLP method 1 (PCR_RFLP);**	**Negative**
A15	**3**	**1**	**EURLP method 1 (PCR_RFLP);In house method;**	**Negative**
A16	4	0	EURLP method 2 (multiplex PCR);	Positive
A26	**3**	**1**	**EURLP method 1 (PCR_RFLP);**	**Negative**
A28	4	0	In house method;	Positive
A31	**3**	**1**	**EURLP method 1 (PCR_RFLP);**	**Negative**
A38	**3**	**1**	**EURLP method 2 (multiplex PCR);**	**Negative**
A39	4	0	Published method	Positive
A40	4	0	EURLP method 2 (multiplex PCR);	Positive

Legend:

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

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Summary of results:

Total number of PT panels	56
Number of participant laboratories	14
Number of participants that passed the PT	7
Number of participants that failed the PT	7

Overtime comparison of results

Laboratory code	2017	2018	2019	2020	2021	2022	2023
A1	NA						
A3			**N**		P	**N**	**N**
A6	P	P	P	P	P	P	P
A7	P	P	P	P	P	P	P
A8		P		**N**	P	**N**	**N**
A10	P	P	P	P	P	P	P
A11		NA					
A12	P	P	P	**N**	P	P	**N**
A15				P	P	P	**N**
A16	P	P	P	P	P	P	P
A17	**N**	P	P	P	P		
A20	P	P	P	P	P	P	
A26					NA	P	**N**
A28	P	P	**N**	P	P	P	P
A31	P	NA			P	P	**N**
A38		P					**N**
A39			P		P	P	P
A40				NA	P	P	P

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

Comments:

In the PT round of 2023, all laboratories received the PT items within 72 hours. Only Seven out of 14 participant laboratories successfully accomplished the PT, whereas 7 laboratories (50%) failed. In particularly, four did not correctly identify the DNA sample of *C. rudolphi C*, two did not identify the fragment larva of *A. pegreffii* and, one failed to identify three items (*A. pegreffii* larva and *A. simplex ss* and *C.* rudoplphi C DNAs).
The reasons for the PT failure were multiple: i) miss-interpretation of the PCR-RFLP profile; ii) limitation of the applied PCR test in fully distinguish between *A. pegreffii* and *A. simplex*; iii) poor discriminatory power of the applied in-house molecular test. Only one laboratory that failed the PT provided investigated the reason of failure. Two laboratories have repeatedly failed the PT round in the previous years (table with overtime comparison of the results). The main reason of PT failure can be identified in the use of DNA of an uncommon species, *C. rudolphi C)* as well as to newly enrolled personnel with limited expertise. Compared to the previous

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years the number of participant laboratories was constant. However, the number of laboratories that failed the 2023 PT round was very high (50%) in comparison to previous years (Figure 1), indicating the need to further improve the correct application of the molecular identification tests in place. Concerning the applied molecular method(s) (Table 1): 5 laboratories applied only the PCR-RFLP method (EURLP 1; MI04); 4 used only the multiplex-PCR (EURLP 2; MI10); 1 used both methods; 1 used PCR of the EURLP 1 followed by Sanger sequencing instead of RFLP; and, finally, 3 applied in house or published methods based on PCR and Sanger sequencing.

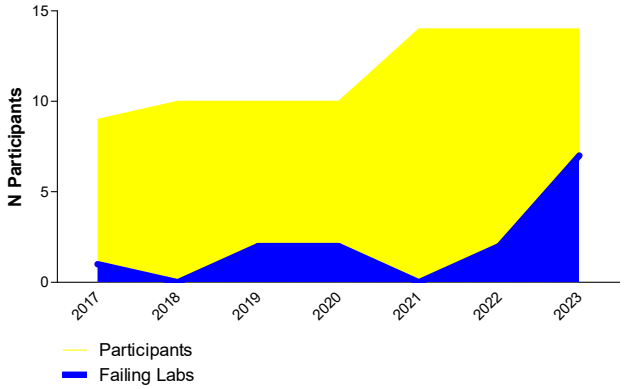

Figure 1. PT07 trend overtime

PTP person in charge
Dr. M. Lalle

Date 22-05-2023

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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