Final report PT-07: AnMol 1/2022

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 single species DNA (both with *A. simplex ss)*, 2 samples with a single larva fragment each (*P. decipiens sl and A. pegreffii)*
NRL	14		Shipping	DHL
Shipping dates	14/03/2022			

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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	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) and EURLP method 2 (multiplex PCR)"	Positive
A8	**3**	**1**	**In house method (CO1 PCR and Sanger sequencing)**	**Negative**
A10	4	0	EURLP method 1 (PCR_RFLP)	Positive
A12	4	0	EURLP method 1 (PCR_RFLP)	Positive
A15	4	0	"EURLP method 1 (for PCR) and In house method (sanger sequencing)"	Positive
A16	4	0	EURLP method 2 (multiplex PCR)	Positive
A20	4	0	EURLP method 1 (PCR_RFLP)	Positive
A26	4	0	EURLP method 1 (PCR_RFLP)	Positive
A28	4	0	*In house method (sanger sequencing)*	Positive
A31	4	0	EURLP method 1 (PCR_RFLP)	Positive
A39	4	0	*EURLP method 1 (PCR_RFLP)*	Positive
A40	4	0	EURLP method 2 (multiplex PCR)	Positive

Legend:

- Laboratories that failed the PT are marked in bold.

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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	12
Number of participants that failed the PT	2

Overtime comparison of results

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

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

Comments:

In the PT round of 2022, 12 out of 14 participant laboratories successfully accomplished the PT. Two laboratory failed, in particularly both did not correctly identify the DNA sample of *A. pegreffii*. The reason for the incorrect identification could be due to miss-interpretation of the PCR-RFLP profile, in one laboratory, and to cross contamination or limitation of the PCR test applied that could not distinguish between the sibling species *A. pegreffii* and *A. simplex*, in the other laboratory. None of the laboratory that failed the PT provided any explanation. However this two laboratories failed the PT round once in the previous years (table with overtime comparison of the results). The PT failure could be due to newly enrolled personnel with limited expertise. Compared to the previous years the number of participant laboratories was constant. All laboratories received the PT items within 72 hours. Concerning the applied molecular method(s) (Table 1): 7 laboratories applied only the PCR-RFLP method (EURLP 1; MI04); 3 used only the multiplex-PCR (EURLP 2; MI10); 1 used both

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methods; 1 used PCR of the EURLP 1 followed by Sanger sequencing instead of RFLP; and, finally, 2 applied in house or published methods based on PCR and Sanger sequencing.

PTP person in charge
Dr. M. Lalle

The Director
Dr. S.M. Cacciò

Date 23-05-2022

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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