Final report PT-07: AnMol 1/2021

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	None		Larvae fragments	30
Private laboratories	None		PT panel composition	2 samples with single species DNA (*A. simplex ss)*, 2 samples with a single larva fragment each (*P. decipiens sl)*
NRL	15		Shipping	DHL Express
Shipping dates	15/03/2021			

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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.

Table1. Results

Laboratory code	N° of samples correctly identified	N° of samples NOT correctly identified	Method applied	Final evaluation
A3	4	0	PCR RFLP (EURLP 1)	Positive
A6	4	0	Multiplex PCR (EURLP 2)	Positive
A7	4	0	EURLP method 1 (PCR_RFLP) and EURLP method 2 (multiplex PCR)	Positive
A8	4	0	In house method (CO1 PCR and sequencing)	Positive
A10	4	0	PCR RFLP (EURLP 1)	Positive
A12	4	0	PCR RFLP (EURLP 1)	Positive
A15	4	0	In house method (EURLP method 1 but with sequencing)	Positive
A16	4	0	Multiplex PCR (EURLP 2)	Positive
A17	4	0	In house method (EURLP method 1 but with sequencing)	Positive
A20	4	0	PCR RFLP (EURLP 1)	Positive
A26[a]	NA	NA	NA	NA
A28	4	0	In house method (Cox2 gene PCR and sequencing)	Positive
A31	4	0	PCR RFLP (EURLP 1)	Positive
A39	4	0	In house method (EURLP method 1 but with sequencing)	Positive
A40	4	0	Multiplex PCR (EURLP 2)	Positive

Legend:

- Laboratories that failed the PT are marked in bold.
- [a]The laboratory could not provide results due to laboratory renovation in progress.
- NA: not applicable

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Summary of results:

Total number of PT panels	15
Number of participant laboratories	14 (one laboratory withdrawn)
Number of participants that passed the PT	14
Number of participants that failed the PT	0

Overtime comparison of results

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

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

Comments:

In the 2021 PT round, 14 out of 14 participant laboratories successfully accomplished the PT. One laboratory that agreed to participate at the PT round could not perform the test and did not provided result due to limitation in work activities (laboratory under renovation). Compared to the previous years the number of participant laboratories is constantly increasing. All laboratories received the PT items within 72 hours. Concerning the applied molecular method(s) (Table 1): 5 laboratories applied only the PCR-RFLP method (EURLP 1; MI04); 3 used only the multiplex-PCR (EURLP 2; MI10); 1 used both methods; 3 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. No relation between the applied methods and successful identification was evident.

PTP person in charge
Dr. M. Lalle

The Director
Dr. S.M. Cacciò

Date 21-09-2021

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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