Final report PT-AnMol 1/2018

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	Ethanol (larvae) and saline buffer (DNA)
	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	12	PT items	DNA	24
Public laboratories	2		Larvae fragments	24
Private laboratories			PT panel composition	2 samples with single species DNA, 2 samples with a single larva fragment each
NRL	10		Shipping	TNT Express
Shipping dates	12/03/2018			

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; tel: +39 0649902670

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	4	0	Positive
A10	4	0	Positive
A11	NA	NA	NA
A12	4	0	Positive
A16	4	0	Positive
A17	4	0	Positive
A20	4	0	Positive
A28	4	0	Positive
A31	NA	NA	NA
A38	4	0	Positive

Legend:

- Laboratories that failed the PT are marked in bold.
- NA, Not applicable. The laboratory didn't send the results

Summary of results:

Total number of PT panels	12
Number of participant laboratories	12
Number of participants that passed the PT	10
Number of participants that failed the PT	0

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Overtime comparison of results

Laboratory code	2017	2018
A1	NA	-
A6	P	P
A7	P	P
A8	-	P
A10	P	P
A11	-	NA
A12	P	P
A16	P	P
A17	N	P
A20	P	P
A28	P	P
A31	P	NA
A38	-	P

Comments:
In the 2018 PT round, only 10 out of 12 participant laboratories sent the results. No reason was communicated by the other two lab for not providing the results. One lab was allowed to provide the results one week after the deadline since it could not perform the test in time due to troubles with reagents' delivery All the laboratories that provided results successful accomplish the PT. Six laboratories applied the PCR-RFLP method (EURLP MI04), two laboratories the multiplex PCR (EURLP MI10) and two applied in house methods based on PCR and Sanger sequencing. Compared to the previous year two new laboratories participated. The only laboratory that failed in 2017 was positive in 2018. Participant laboratories proved to be highly competent in the identification of Anisakidae at the species level, irrespective of the molecular method used.

The Director
Dr. E. Pozio

Date 03/05/2018

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 each sample (item) consists 4 tubes: 2 tubes containing a single fragment of Anisakidae L3 larva each in 96% ethanol; and 2

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tubes containing DNA extracted from a single Anisakidae L3 larva in saline buffer solution. All larvae have been individually identified at species level by analyzing one of their fragments by the EURLP method "Identification at species level of parasites of the family Anisakidae by PCR/RFLP" (http://old.iss.it/crlp/index.php?lang=2&anno=2018&tipo=33). The DNAs have been extracted from single larvae and also identified at species level by the above method. Homogeneity of PT items is ensured by providing to each participant aliquots of the same DNA preparations. PT items are stable as follow: larvae preserved in 96% ethanol, and stored between -20 and +30° C maintain their stability up to 5 years after the date of preparation. DNA stored below 15°C are stable for years.

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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