

Istituto Superiore di Sanità

Department of Infectious Diseases Unit of Foodborne and Neglected Parasitic Diseases European Union Reference Laboratory for Parasites



Final report PT-AnMol 1/2019

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	10		DNA	20
Public laboratories	1		Larvae fragments	20
Private laboratories		PT items	PT panel composition	2 samples with single species DNA, 2 samples with a single larva fragment each
NRL	9		Shipping	TNT Express
Shipping dates	11/03	/2019		

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PTP N° 0005

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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	Final evaluation
А3	2	2	Negative
A6	4	0	Positive
A7	4	0	Positive
A10	4	0	Positive
A12	4	0	Positive
A16	4	0	Positive
A17	4	0	Positive
A20	4	0	Positive
A28	3	1	Negative
A39	4	0	Positive

Legend:

• Laboratories that failed the PT are marked in bold.

Summary of results:

Total number of PT panels	10
Number of participant laboratories	10
Number of participants that passed the PT	8
Number of participants that failed the PT	2

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Overtime comparison of results

Laboratory code	2017	2018	8019
A1	NA		
A3			N
A6	Р	Р	Р
A7	Р	Р	Р
A8		Р	
A10	Р	Р	Р
A11		NA	
A12	Р	Р	Р
A16	Р	Р	Р
A17	N	Р	Р
A20	Р	Р	Р
A28	Р	Р	N
A31	Р	NA	
A38		Р	
A39			Р

Note: P, positive; N, negative; NR, no result received; gray boxed, no participation

Comments:

In the 2019 PT round, only 8 out of 10 participant laboratories successfully accomplish the PT. One did not correctly identify one DNA sample and one larval sample. The reason was identified on technical troubles in the application of the PCR-RFLP method (EURLP MI04), due to the unexperienced analyst. The other laboratory reported a problem in the DNA extraction protocol that prevented the analysis of one larval sample to be performed. Both laboratories informed the EURLP that further training of the analysts will be implemented as corrective action.

Four laboratories applied the PCR-RFLP method (EURLP MI04) or its modification alone, three laboratories the multiplex PCR (EURLP MI10), one laboratory applied both methods and, finally, two applied in house methods based on PCR and Sanger sequencing. Compared to the previous year two new laboratories participated. No relation between the applied methods and successful identification was evident.

Compared to the previous years, no differences in the number of laboratories providing the results occurred. However, a decrease in the performance was observed and mainly due to the inexperience of new personnel joining the participant laboratories.

The Director

Dr. S.M. Cacciò

Linemal Cem

Date 02-05-2019

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Notes:

- 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 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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