

Department of Infectious, Parasitic and Immunomediated Diseases Unit of Gastroenteric and Tissue Parasitic Diseases Istituto Superiore di Sanità



### Final PT report n. 1/2014

### PT "Identification of *Trichinella* larvae at the species level by a molecular method"

#### Design

Purpose	Evaluation of laboratories in charge for official control on food		
Scheme type	Single		
Participants	NRLs of EU		
N. of participants	Depending on request		
Method	not regulated		
Test method	chosen by the participant		
	Matrix	not applicable	
DT its and	Item	Trichinella spp. larvae in 96% ethanol	
PT items	N. of samples	4 (10 larvae/each) or 12 (1 larva/each) 1.5 ml vials for each participant	
	Distribution	Immediate shipment after preparation	
Subcontracted activities	PT item transport and delivery		
Results evaluation	Qualitative		

#### **Implementation**

N. of participants	20		PT panel 4 vials	12
Public laboratories	0	PT items		
Private laboratories	0	1 1 Items	PT panel 12 vials	8
NRLs	20		Subcontractor	TNT Express
Shipping dates	March 17,	2014		

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#### **Qualitative results**

The PT final evaluation was qualitative only. The PT was considered passed when all isolates or, in case of single larvae at least one of them for each isolate, are correctly identified.

Laboratory code	Method used	N. correct identification	N. incorrect identification	N. missed identification	Final evaluation
NRL16	Zarlenga et al. 1999 with modifications	4	0	0	positive
NRL7	EURLP protocol	11	0	1	positive
NRL11	EURLP protocol	4	0	0	positive
NRL44	EURLP protocol	3	1	0	negative
NRL23	EURLP protocol	4	0	0	positive
NRL1	EURLP protocol	12	0	0	positive
NRL10	EURLP protocol	12	0	0	positive
NRL8	Pozio and La Rosa 2003	4	0	0	positive
NRL22	EURLP protocol with modifications	9	3	0	negative
NRL42	EURLP protocol with modifications	3	1	0	negative
NRL4	EURLP protocol	12	0	0	positive
NRL3	EURLP protocol	12	0	0	positive
NRL6	Zarlenga et al. 1999 and 5S sequencing	11	0	1	positive
NRL24	EURLP protocol	12	0	0	positive
NRL17	EURLP protocol	4	0	0	positive
NRL34	EURLP protocol	4	0	0	positive
NRL25	EURLP protocol	4	0	0	positive
NRL2	EURLP protocol with modifications	3	1	0	negative
NRL21	Pozio and La Rosa 2003 with ESV and ITS1 primers only	4	0	0	positive
NRL35	Zarlenga et al. 1999	2	0	2	negative

**Legend**: Laboratories that failed the PT are marked in bold.

#### References:

- Zarlenga DS, Chute MB, Martin A, Kapel CM. A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of Trichinella. Int J Parasitol. 1999 Nov;29(11):1859-67.
- Pozio E, La Rosa G. PCR-derived methods for the identification of Trichinella parasites from animal and human samples. Methods Mol Biol. 2003;216:299-309.
- http://www.iss.it/crlp/index.php?lang=2&anno=2014&tipo=33

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#### Summary of qualitative results:

Total number of PT panels	20
Number of participant laboratories	20
Number of participants that passed the PT	15
Number of participants that failed the PT	5

#### Overtime comparison

Laboratory code	PT 2011	PT 2012	PT 2013	PT 2014
NRL1	positive	positive	positive	positive
NRL2	-	-	-	negative
NRL3	negative	-	positive	positive
NRL4	positive	positive	positive	positive
NRL6	positive	positive	negative	positive
NRL7	-	negative	negative	positive
NRL8	positive	positive	positive	positive
NRL9	negative	-	negative	-
NRL10	negative	positive	-	positive
NRL11	positive	negative	positive	positive
NRL12	-	-	positive	-
NRL16	positive	positive	positive	positive
NRL17	-	-	-	positive
NRL21	positive	negative	positive	positive
NRL22	-	positive	negative	negative
NRL23	-	positive	positive	positive
NRL24	negative	-	positive	positive
NRL25	-	-	positive	positive
NRL34	negative	negative	positive	positive
NRL35	negative	negative	-	negative
NRL38	positive	-	-	-
NRL40	-	positive	-	-
NRL42	-	-	-	negative
NRL44	-	-	-	negative

#### Legend:

• Laboratories that failed the PT are marked in bold.

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**Comments:** The main reasons leading to PT failure were:

- the use of not suitable diagnostic protocols (e.g. not all the five primer sets originally developed by Zarlenga et al. 1999 were used in the multiplex PCR) or tools;
- the lack of experience in multiplex PCR band pattern analysis.

The Director Dr. E. Pozio

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Date	10/04/2014

#### 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 subcontracts PT item transport and delivery to a qualified transportation company.
- 3. Each participating laboratory receives a PT panel according to the PT scheme. Each PT item consists of 4 or 12 1.5 ml vials containing four different *Trichinella spp*. The homogeneity of PT items is ensured by an accurate control of the number of larvae spiked into each vial (item), made by two operators using a stereo microscope. PT items are stable for 5 years 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 issued for each PT round shows the PT program implementation.

End of the report

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