Istituto Superiore di Sanità
Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases
European Union Laboratory for Parasites

Final report PT-03: Tm 1/2026

PT-03: Identification of *Trichinella* larvae at the species level by a molecular method

Design

Purpose	Evaluation of laboratories competence in molecular identification of *Trichinella* larvae species	
Timetable	Invitation mail: 27/01/2026 Website updates: 31/01/2026 Registration deadline: 20/02/2026 PT items production: 09/03/2026 Shipping: 16/03/2026 Results submission deadline: 17/04/2026 Publication of the Final PT Report: within 29/05/2026	
Participants	National Reference Laboratories for parasites, Public and private, national and international institution	
Number of participants	Depending on requests	
PT items	Matrix	Ethanol 96%
	Item	*Trichinella* spp. Larvae preserved in ethanol
	Number of PT items	4 per each participant (10 larvae/each species)
	Panel composition	*T. nativa, T. pseudospiralis, Trichinella* T6 and *T. papuae*
	Number of surplus items	n.a.
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Tonanzi D., Marucci G.

Compliance with planned timelines: YES
Participant number and type: 24 public institution
0 private institution

Acquisition of matrix and analyte: Larvae, stored in 96% ethanol, were obtained by the International Trichinella Reference Center and maintained at EURL-P as reference material.

Production of PT items: The PT items were prepared on 09/03/2026 and consisted of four 1.5 mL tubes containing *Trichinella* larvae preserved in 96% ethanol, with one tube representing each species. Samples could be analyzed individually or pooled, depending on the sensitivity of the analytical method and the experience of the technical staff.

Activities provided by external providers: the organizer entrusts the shipment of PT items to a qualified transport company. The company providing the shipping service was DHL.

Homogeneity and stability of PT items: Larvae of each species used in the PT belongs to the same *Trichinella* reference strain as certified by the ITRC. Moreover, 10 larvae from each species included in the PT panel were tested to confirm their identity by accredited EURL-P internal molecular method (MI-02: Identification of *Trichinella* muscle stage larvae at the species level by Multiplex PCR) on 26/02/2026. Homogeneity is further ensured through an accurate verification of larvae in the tube performed by two operators. The stability of the PT items was evaluated by ad hoc experiments carried out by EURL-P. Larvae preserved in 96% ethanol and stored between -20°C and +20°C maintain their stability for up to five years.

Distribution of PT items: PT items were shipped on 16/03/2026, and the deadline for submission of results was set for 17/04/2026. Each set of PT items consisted of 50 ml vial marked with the participant's identification code. Each set contained four 1.5 ml vials identified with the code assigned by PTP to the PT items. The packaging consisted of a polystyrene and cardboard container containing an adequate number of cooling bricks to ensure that a temperature between 4 and 15°C was maintained inside the package.

Instructions for participants: Participants were informed on the shipment date via email on 16/03/2026. The email also contained a link for submitting results, which was active from 16/03/2026 (coinciding with shipment) to 17/04/2026. Participant instructions were made available on the PTP website from 31/01/2026. These instructions also contained information for sending feedback and submitting results. This information was sent to participants along with the PT announcement email and remarked in the email sent on 16/03/2026 (shipment date).

Data analysis: Feedback and participant results were collected via the online Forms application and transferred from the PTP to an Excel file used for data processing and further used to generate the tables with the participant results contained in this PT report.

Assigned value: The assigned value is determined by the expertise of the proficiency test manager and the technical staff involved in preparing the PT items.

Criteria for results evaluation: for each PT item, the result was evaluated by comparing the participant's reported result with the expected value. Result evaluation is qualitative. Result was considered "correct" if larvae of the PT item were properly identified at species level, and "incorrect" in case of wrong identification. Results have to be expressed reporting the species assigned to each PT item. The participant was asked to correctly identify the *Trichinella* larvae species of each PT item.

Confidentiality of results: the confidentiality of the data contained in this report is ensured using a unique code guaranteeing participant anonymity. The identity of participants in a PT scheme is kept confidential and subject to official secrecy. The PT provider reserves the right to provide participant PT results to competent authority and accreditation body, upon request. Participants will be notified in writing if a competent legislative authority requests access to their PT results.

Results provided by participants and performance EVALUATION

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
TM01	EURLP method	Trich1	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich2	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich3	*T6*	*T6*	correct	
		Trich4	*T. papuae*	*T. papuae*	correct	
TM02	EURLP method and sequencing	Trich5	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich6	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich7	*T6*	*T6*	correct	
		Trich8	*T. papuae*	*T. papuae*	correct	
TM03	EURLP method	Trich9	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich10	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich11	*T6*	*T6*	correct	
		Trich12	*T. papuae*	*T. papuae*	correct	
TM04	EURLP method	Trich13	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich14	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich15	*T6*	*T6*	correct	
		Trich16	*T. papuae*	*T. papuae*	correct	
TM05	In house method (magnetic beads and PCR and sequencing of 16S and COI)	Trich17	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich18	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich19	*T6*	*T6*	correct	
		Trich20	*T. papuae*	*T. papuae*	correct	
TM06	EURLP method	Trich21	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich22	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich23	*T6*	*T6*	correct	
		Trich24	*T. papuae*	*T. papuae*	correct	
TM07	EURLP method	Trich25	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich26	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich27	*T6*	*T6*	correct	
		Trich28	*T. papuae*	*T. papuae*	correct	
TM08	EURLP method (old version)	Trich29	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich30	*T. pseudospiralis*	*T. pseudospiralis*	incorrect	
		Trich31	*T6*	*T6*	correct	
		Trich32	*T. papuae*	*T. papuae*	incorrect	
TM09	Pozio and La Rosa 2003 (protease K incubation and multiplex PCR)	Trich33	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich34	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich35	*T6*	*T6*	correct	
		Trich36	*T. papuae*	*T. papuae*	correct	
TM10	EURLP method	Trich37	*T. nativa*	*T. nativa*	correct	NEGATIVE
		Trich38	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich39	*T. papuae*	*T6*	incorrect	
		Trich40	*T. britovi*	*T. papuae*	incorrect	

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
TM11	EURLP method	Trich41	*T. nativa*	*T. nativa*	correct	NEGATIVE
		Trich42	*impossible*	*T. pseudospiralis*	correct	
		Trich43	*T6*	*T6*	correct	
		Trich44	*T. papuae*	*T. papuae*	correct	
TM12	EURLP method	Trich45	*T. nativa*	*T. nativa*	correct	NEGATIVE
		Trich46	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich47	*T6*	*T6*	correct	
		Trich48	*T. zimbabwensis*	*T. papuae*	incorrect	
TM13	Pozio and La Rosa 2003 protease K incubation and multiplex PCR)	Trich49	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich50	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich51	*T6*	*T6*	correct	
		Trich52	*T. papuae*	*T. papuae*	correct	
TM14	EURLP method	Trich53	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich54	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich55	*T6*	*T6*	correct	
		Trich56	*T. papuae*	*T. papuae*	correct	
TM15	EURLP method and PCR and sequencing of 5S gene	Trich57	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich58	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich59	*T6*	*T6*	correct	
		Trich60	*T. papuae*	*T. papuae*	correct	
TM16	EURLP method	Trich61	*T. nativa*	*T. nativa*	correct	NEGATIVE
		Trich62	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich63	*T. britovi*	*T6*	incorrect	
		Trich64	*T. papuae*	*T. papuae*	correct	
TM17	EURLP method	Trich65	*T. nativa*	*T. nativa*	correct	NEGATIVE
		Trich66	*T. murrelli*	*T. pseudospiralis*	incorrect	
		Trich67	*T6*	*T6*	correct	
		Trich68	*T. britovi*	*T. papuae*	incorrect	
TM18	EURLP method	Trich69	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich70	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich71	*T6*	*T6*	correct	
		Trich72	*T. papuae*	*T. papuae*	correct	
TM19	EURLP method	Trich73	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich74	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich75	*T6*	*T6*	correct	
		Trich76	*T. papuae*	*T. papuae*	correct	
TM20	Pozio and La Rosa 2003 (protease K incubation and multiplex PCR)	Trich77	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich78	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich79	*T6*	*T6*	correct	
		Trich80	*T. papuae*	*T. papuae*	correct	

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
TM21	EURLP method and sequencing	Trich81	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich82	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich83	*T6*	*T6*	correct	
		Trich84	*T. papuae*	*T. papuae*	correct	
TM22	Pozio and La Rosa 2003 (silica column DNA purification kit and multiplex PCR)	Trich85	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich86	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich87	*T6*	*T6*	correct	
		Trich88	*T. papuae*	*T. papuae*	correct	
TM23	EURLP method	Trich89	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich90	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich91	*T6*	*T6*	correct	
		Trich92	*T. papuae*	*T. papuae*	correct	
TM24	EURLP method	Trich93	*T. nativa*	*T. nativa*	correct	POSITIVE
		Trich94	*T. pseudospiralis*	*T. pseudospiralis*	correct	
		Trich95	*T6*	*T6*	correct	
		Trich96	*T. papuae*	*T. papuae*	correct	

Legenda: Laboratories that failed the PT are marked in red.

Summary of results:

Total number of PT panels	24
Number of participant laboratories	24
Number of participants that passed the PT	19
Number of participants that failed the PT	5

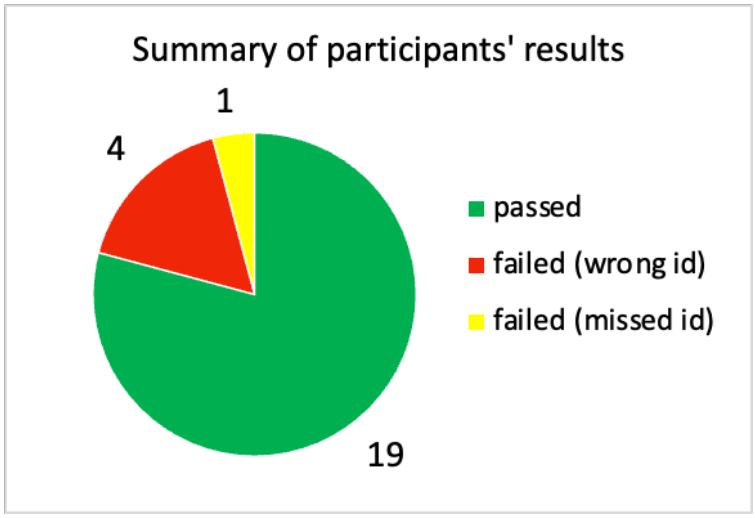
Overtime comparison of results

Laboratory code	2021	2022	2023	2024	2025	2026
TM01	NEG	POS	POS	POS	POS	POS
TM02	POS	POS	POS	POS	POS	POS
TM03	POS	POS	POS	POS	POS	POS
TM04	POS	POS	POS	POS	POS	POS
TM05	POS	POS	POS	POS	POS	POS
TM06	POS	POS	POS	POS	POS	POS
TM07	NEG	POS	POS	POS	POS	POS
TM08	POS	POS	POS	POS	POS	POS
TM09	POS	POS	POS	POS	POS	POS
TM10	NEG	NP	NP	NP	NP	NEG
TM11	NP	NP	POS	NP	NP	NEG
TM12	NEG	NEG	NEG	NEG	POS	NEG
TM13	POS	POS	POS	POS	POS	POS
TM14	POS	POS	POS	POS	NEG	POS
TM15	POS	POS	POS	POS	POS	POS

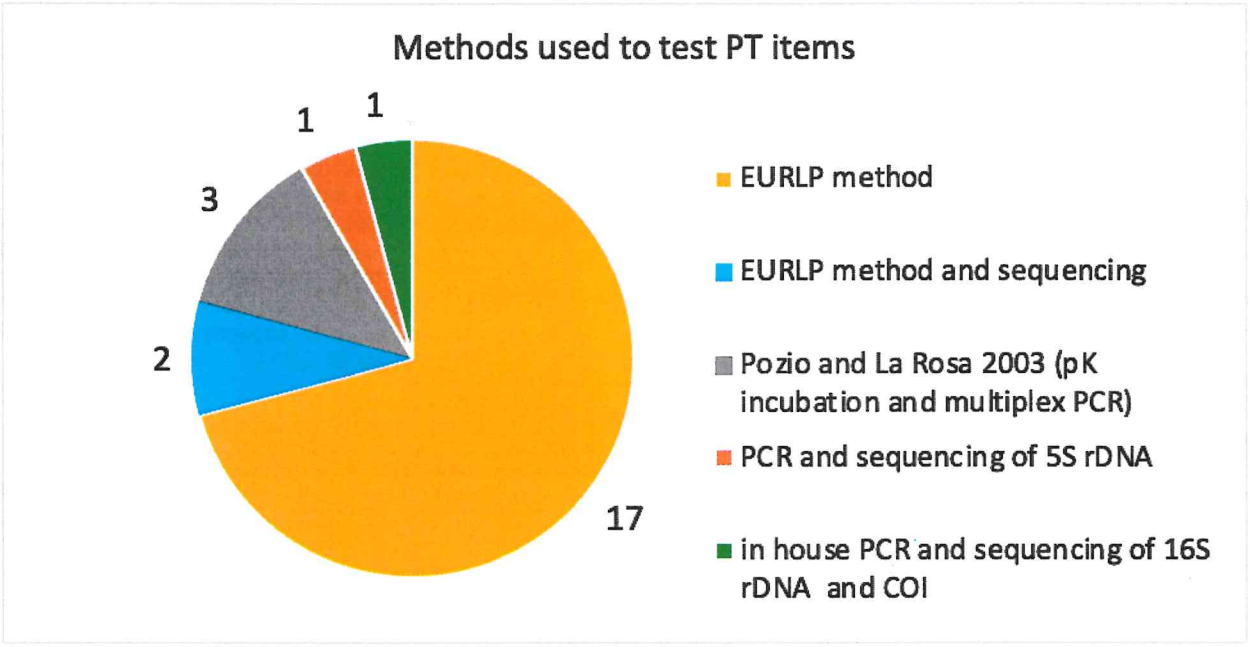
Laboratory code	2021	2022	2023	2024	2025	2026
TM16	POS	POS	POS	POS	POS	NEG
TM17	NEG	POS	POS	POS	POS	NEG
TM18	NEG	POS	POS	POS	POS	POS
TM19	POS	POS	POS	POS	POS	POS
TM20	NEG	POS	POS	NP	POS	POS
TM21	POS	POS	POS	POS	POS	POS
TM22	POS	POS	POS	NEG	POS	POS
TM23	POS	POS	POS	POS	POS	POS
TM24	POS	POS	NEG	POS	POS	POS

NP= no participation. **POS**= positive. **NEG**= negative.

Comments on participants' performance: Five participants failed the PT because of incorrect or missed identifications. In particular: Lab 17 misidentified *T. pseudospiralis* as *T. murrelli* and *T. papuae* as *T. britovi*; TM10 and TM16 misidentified genotype T6 as *T. britovi;* Lab TM10 also confused two results by assigning them to the wrong item code; Lab TM12 misidentified *T. papuae* as *T. zimbabwensis*; Lab TM11 failed to obtain an identification for the PT item containing *T. pseudospiralis* larvae.



Performance evaluation if different methods are applied: all methods used were considered equivalent and were evaluated in the same way. Nineteen participants tested PT items using the EURL-P internal molecular method (MI-02: Identification of *Trichinella* muscle-stage larvae at the species level by multiplex PCR) and two of them also confirmed results by sequencing. Three participants applied the method published in La Rosa and Pozio 2003, based on larvae incubation with proteinase K and DNA amplification by multiplex PCR. One participant used the method published in Rombout et al. 2001, based on PCR and sequencing of 5S gene. One participant used an in-house method based on PCR and sequencing of the 16S and COI genes. No direct correlation between PT failure and the method used for species identification was observed.



Comments and recommendations based on the outcomes of PT: Lab 17 observed the presence of non-specific bands in the PCR, probably due to incorrect primer synthesis, which led to the misidentification of two PT items. Labs 10, 11, and 16 failed to identify T6 genotype and *T. papuae*, confusing them with *T. britovi* and *T. zimbabwensis*, respectively, which show similar banding patterns. This suggests that the error was most likely due to limited experience of laboratory staff with the specific banding patterns produced by the multiplex PCR.

General recommendations:
The PT failure (wrong or missed species identification) may depend on several factors, such as:

- Exchange of PT objects during the analysis phase.
- DNA contamination during the analysis phase.
- Incomplete removal of ethanol used to preserve the larvae.
- Errors or inappropriate changes made by the operator during the DNA extraction or PCR amplification steps.
- Use of unsuitable reagents during the DNA extraction or PCR amplification steps.
- Incorrect interpretation of DNA fragments observed during the electrophoresis run.

Written and elaborated by
PTP person in charge

Dr. G. Marucci

Verified and issued by
The Director

Dr. A. Casulli

Date 13/05/2026

Notes:

1. Personal data are processed in compliance with the regulatory provisions referred to in EU Regulation 2016/679 and Privacy Code, as reported in Legislative Decree no. 101/2018. The data controller of personal data is the Istituto Superiore di Sanità with registered office in Viale Regina Elena n. 299 - 00161 Rome, in the person of its President. In addition, the ISS has appointed its own Data Protection Officer (D.P.O.), e-mail address: responsabile.protezionedati@iss.it. Data are processed exclusively for carrying out the PT activities, for this purpose adequate physical, technical and organizational security measures have been set up to prevent and avoid their destruction and/or loss of integrity, as well as their illicit or incorrect use. Data is accessible only to authorized personnel who has their own credentials and their own operating station. The participant has the rights referred to in art. 15 GDPR et seq., more precisely right of access, right of rectification, right of treatment limitation, right to data portability, right of opposition, as well as the right to lodge a complaint with the Guarantor Authority (art. 77 GDPR and 141 Privacy Code, as reported by Legislative Decree 101/2018). The ISS, in its capacity as Data Controller, undertakes to keep the records of processing activities correctly pursuant to art. 30 GDPR.
2. The original raw data and a copy of Final PT Report are kept for 10 years at the PTP site.
3. Participants may use this report to support their skills to the accreditation body and other interested parties.
4. The accreditation, according to the ISO/IEC 17043 international standard, is regulated by a specific agreement and recognizes the technical competence of the PTP to organize PT schemes. The accreditation body, ACCREDIA (www.accredia.it), does not take any responsibility for the activities related to production of PT items and participants results evaluation.

End of the report