Final Report PT-08: EcMol 1/2026

PT-08: "Molecular identification of *Echinococcus granulosus, Echinococcus multilocularis* and *Taenia* spp."

Design

Purpose	Evaluation of laboratory competence in the molecular identification of *Echinococcus granulosus, Echinococcus multilocularis*, and *Taenia* spp.	
Timetable	Invitation mail: 27/01/2026 Website updates: 27/01/2026 Registration deadline: 20/02/2026 PT items production: 10/03/2026 Shipping: 16/03/2026 Results submission deadline: 17/04/2026 Publication of the Final PT Report: 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	Elution buffer
	Item	DNA
	Number of PT items	4 per each participant
	Panel composition	*Echinococcus granulosus s.s., Echinococcus multilocularis, Taenia* spp., negative
	Number of surplus items	n.a.
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Azzurra Santoro, Federica Santolamazza

Compliance with planned timelines: YES x NO ☐

Participant number and type: 22 public institutions

0 private institution

Acquisition of matrix and analyte: genomic DNA was extracted with a commercial kit from parasitic isolates stored at the EURL-P. An aliquot of each DNA preparation was tested to confirm species identity. Identification was performed through the amplification and sequencing of the mitochondrial genes *cox1* and *nad1*, as well as by a multiplex PCR capable of distinguishing DNA from *Echinococcus granulosus s.l.*, *Echinococcus multilocularis*, and *Taenia* spp. (Trachsel et al., Parasitology, 134 (2007), pp. 911–920). In all amplification assays, 2 µl of extracted DNA were used.

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Production of PT items: The PT items were prepared on 10/03/2026; they consisted of four 1.5 mL tubes containing, respectively, one aliquot of *Echinococcus granulosus s.s.* DNA, one aliquot of *Echinococcus multilocularis* DNA, one aliquot of *Taenia krabbei* DNA (as the 3 positive items) and one aliquot of *Canis lupus familiaris* DNA (as the negative item).

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: The preparation procedure of PT items, consisting of aliquots derived from the same stock preparation, ensures their homogeneity. Based on the experience gained over the years by the EURL-P, it has been established that DNA stored frozen at a temperature ≤ −15°C remains suitable for analysis for up to 10 years from the date of preparation. DNA may also be stored under refrigeration (between +4°C and +8°C) for up to six months.

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 vial contained four 1.5 ml vials identified with the codes 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 of the shipment 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. Instructions for participants were made available on the PTP website starting from 27/01/2026. These instructions also include procedures for submitting feedback information and results.

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. The evaluation for each item is "correct" or "incorrect" based on the accurate identification of the positive item at the species level (in case of *Echinococcus* spp.) or the genus level (in case of *Taenia* spp.), and the identification of the negative item. A positive final evaluation was assigned when all four items were correctly identified; otherwise, it was negative.

Confidentiality of results: the confidentiality of the data contained in this report is ensured by the use of 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
Emol1	Multiplex PCR[1]; cox1 PCR & sequencing	Emol01	*Taenia sp.*	negative	incorrect	NEGATIVE
		Emol02	*E. multilocularis*	*E. multilocularis*	correct	
		Emol03	*Taenia* sp. (*krabbei*)	*Taenia* spp.	correct	
		Emol04	*E. granulosus*	*E. granulosus*	correct	

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Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
Emol2	Multiplex PCR[1]	Emol05	negative	negative	correct	POSITIVE
		Emol06	*E. multilocularis*	*E. multilocularis*	correct	
		Emol07	*Taenia* spp.	*Taenia* spp.	correct	
		Emol08	*E. granulosus*	*E. granulosus*	correct	
Emol3	Multiplex PCR[1] & sequencing	Emol09	negative	negative	correct	POSITIVE
		Emol10	*E. multilocularis*	*E. multilocularis*	correct	
		Emol11	*Taenia* spp.	*Taenia* spp.	correct	
		Emol12	*E. granulosus*	*E. granulosus*	correct	
Emol4	Multiplex PCR[1]	Emol13	negative	negative	correct	POSITIVE
		Emol14	*E. multilocularis*	*E. multilocularis*	correct	
		Emol15	*Taenia* spp.	*Taenia* spp.	correct	
		Emol16	*E. granulosus*	*E. granulosus*	correct	
Emol5	Multiplex PCR[1]	Emol17	negative	negative	correct	POSITIVE
		Emol18	*E. multilocularis*	*E. multilocularis*	correct	
		Emol19	*Taenia* spp.	*Taenia* spp.	correct	
		Emol20	*E. granulosus*	*E. granulosus*	correct	
Emol6	RLFP-PCR[2]	Emol21	negative	negative	correct	POSITIVE
		Emol22	*E. multilocularis*	*E. multilocularis*	correct	
		Emol23	*Taenia* spp.	*Taenia* spp.	correct	
		Emol24	*E. granulosus s.s.*	*E. granulosus*	correct	
Emol7	Multiplex PCR[1]	Emol25	negative	negative	correct	POSITIVE
		Emol26	*E. multilocularis*	*E. multilocularis*	correct	
		Emol27	*Taenia* spp.	*Taenia* spp.	correct	
		Emol28	*E. granulosus*	*E. granulosus*	correct	
Emol8	Multiplex PCR[1]; Nested PCR[3]; end-point PCR[4]	Emol29	negative	negative	correct	POSITIVE
		Emol30	*E. multilocularis*	*E. multilocularis*	correct	
		Emol31	*Taenia* spp.	*Taenia* spp.	correct	
		Emol32	*E. granulosus s.l.*	*E. granulosus*	correct	
Emol9	Multiplex PCR[1]	Emol33	negative	negative	correct	POSITIVE
		Emol34	*E. multilocularis*	*E. multilocularis*	correct	
		Emol35	*Taenia* spp.	*Taenia* spp.	correct	
		Emol36	*E. granulosus*	*E. granulosus*	correct	
Emol10	cox1 PCR & sequencing[5]	Emol37	negative	negative	correct	POSITIVE
		Emol38	*E. multilocularis*	*E. multilocularis*	correct	
		Emol39	*Taenia krabbei*	*Taenia* spp.	correct	
		Emol40	*E. granulosus*	*E. granulosus*	correct	
Emol11	EURL-P MI-15[6] & sequencing	Emol41	negative	negative	correct	POSITIVE
		Emol42	*E. multilocularis*	*E. multilocularis*	correct	
		Emol43	*Taenia krabbei*	*Taenia* spp.	correct	
		Emol44	*E. granulosus s.l.* (G1/G3)	*E. granulosus*	correct	
Emol12	Multiplex PCR[1]	Emol45	negative	negative	correct	POSITIVE
		Emol46	*E. multilocularis*	*E. multilocularis*	correct	
		Emol47	*Taenia* spp.	*Taenia* spp.	correct	
		Emol48	*E. granulosus*	*E. granulosus*	correct	
Emol13	Nested PCR[7] & sequencing	Emol49	negative	negative	correct	POSITIVE
		Emol50	*E. multilocularis*	*E. multilocularis*	correct	
		Emol51	*Taenia multiceps*	*Taenia* spp.	correct	
		Emol52	*E. granulosus*	*E. granulosus*	correct	
Emol14	Multiplex PCR[1]	Emol53	negative	negative	correct	POSITIVE
		Emol54	*E. multilocularis*	*E. multilocularis*	correct	
		Emol55	*Taenia* spp.	*Taenia* spp.	correct	
		Emol56	*E. granulosus*	*E. granulosus*	correct	
Emol15	Multiplex PCR[1]; real-time PCR[8,9,10]	Emol57	negative	negative	correct	POSITIVE
		Emol58	*E. multilocularis*	*E. multilocularis*	correct	
		Emol59	*Taenia* spp.	*Taenia* spp.	correct	
		Emol60	*E. granulosus*	*E. granulosus*	correct	

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Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
Emol16	Multiplex PCR[1]; real-time PCR[8]	Emol61	negative	negative	correct	POSITIVE
		Emol62	*E. multilocularis*	*E. multilocularis*	correct	
		Emol63	*Taenia* spp.	*Taenia* spp.	correct	
		Emol64	*E. granulosus*	*E. granulosus*	correct	
Emol17	Published method, not specified	Emol65	negative	negative	correct	POSITIVE
		Emol66	*E. multilocularis*	*E. multilocularis*	correct	
		Emol67	*Taenia* sp.	*Taenia* spp.	correct	
		Emol68	*E. granulosus*	*E. granulosus*	correct	
Emol18	Multiplex PCR[1]	Emol69	negative	negative	correct	POSITIVE
		Emol70	*E. multilocularis*	*E. multilocularis*	correct	
		Emol71	*Taenia* spp.	*Taenia* spp.	correct	
		Emol72	*E. granulosus*	*E. granulosus*	correct	
Emol19	EURL-P SOP[11]	Emol73	negative	negative	correct	POSITIVE
		Emol74	*E. multilocularis*	*E. multilocularis*	correct	
		Emol75	*Taenia krabbei*	*Taenia* spp.	correct	
		Emol76	*E. granulosus* G3	*E. granulosus*	correct	
Emol20	EURL-P MI-15[6]; real time PCR[12]	Emol77	negative	negative	correct	POSITIVE
		Emol78	*E. multilocularis*	*E. multilocularis*	correct	
		Emol79	*Taenia* sp.	*Taenia* spp.	correct	
		Emol80	*E. granulosus s.s.* G1/G3	*E. granulosus*	correct	
Emol21	Multiplex PCR[1]	Emol81	negative	negative	correct	POSITIVE
		Emol82	*E. multilocularis*	*E. multilocularis*	correct	
		Emol83	*Taenia serialis*	*Taenia* spp.	correct	
		Emol84	*E. granulosus*	*E. granulosus*	correct	
Emol22	Multiplex PCR[1]; Real-time multiplex PCR[13] and sequencing	Emol85	negative	negative	correct	POSITIVE
		Emol86	*E. multilocularis*	*E. multilocularis*	correct	
		Emol87	*Taenia* spp.	*Taenia* spp.	correct	
		Emol88	*E. granulosus*	*E. granulosus*	correct	

[1]Trachsel et al., Parasitology, 134 (2007), pp. 911–920.
[2]Umhang et al., Parasite, 33 (2026), 17.
[3]Dinkel et al., J. Clin. Microbiol., 36 (1998), pp. 1871–1876.
[4]Abbasi et al., Am. J. Trop. Med. Hyg., 69 (2003), pp. 324–330.
[5]Bowles et al., Mol. Biochem. Parasitol., 54 (1992), pp. 165–173.
[6]Santolamazza et al., Infect. Genet. Evol., 85 (2020), 104575.
[7]Geysen et al., J. Food Prot., 70 (2007), pp. 236–240.
[8]Knapp et al., Vet. Parasitol., 201 (2014), pp. 40–47.
[9]Isaksson et al., Parasit. Vectors, 7 (2014), 583.
[10]Maksimov et al., Pathogens, 9 (2020), 791.
[11]https://www.iss.it/en/-/standard-operating-procedures-sops-
[12]Øines et al., Parasit. Vectors, 7 (2014), 246.
[13]In house method.

Legend:

- Negative evaluations are marked in red.

Summary of results:

Total number of PT panels	22
Number of participants	22
Number of participants that passed the PT	21
Number of participants that failed the PT	1

Overtime comparison of results

Laboratory code (2026)	2022	2023	2024	2025	2026
EmoI1	POS	POS	NEG	POS	NEG
EmoI2	POS	POS	POS	POS	POS
EmoI3	NEG	POS	NP	POS	POS
EmoI4	NP	NP	POS	POS	POS
EmoI5	POS	POS	POS	POS	POS
EmoI6	POS	POS	POS	POS	POS
EmoI7	POS	POS	POS	POS	POS
EmoI8	POS	POS	POS	POS	POS
EmoI9	NP	NP	NP	NP	POS
EmoI10	NP	NP	NP	POS	POS
EmoI11	POS	POS	POS	POS	POS
EmoI12	POS	POS	POS	POS	POS
EmoI13	POS	POS	POS	POS	POS
EmoI14	POS	POS	POS	NEG	POS
EmoI15	POS	POS	POS	POS	POS
EmoI16	NP	POS	POS	NEG	POS
EmoI17	POS	POS	POS	POS	POS
EmoI18	POS	POS	POS	POS	POS
EmoI19	POS	POS	NEG	POS	POS
EmoI20	POS	POS	POS	POS	POS
EmoI21	POS	POS	POS	POS	POS
EmoI22	NP	NP	NP	POS	POS

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

Comments on participants' performance: In 2026, 21 out of 22 participating laboratories successfully passed the PT. In one case, the negative item was misclassified as *Taenia sp*., resulting in a final negative evaluation. Although not mandatory, four laboratories correctly identified the *Taenia* item as *Taenia krabbei*, and four laboratories further correctly identified the *E. granulosus* item as belonging to *E. granulosus sensu stricto*.

Performance evaluation if different methods are applied: the multiplex PCR by Trachsel et al., was the most commonly used method for PT execution. Some laboratories employed alternative methods, either in alternative to or in combination with the Trachsel method, including real-time PCRs and methods based on RFLP or sequencing. No correlation was observed between laboratory performance and the method applied.

Comments and recommendations based on the outcomes of PT:
General
Participating laboratories are generally able to fulfil the objectives of the PT, both by applying the recommended method and by using alternative methods of their own choice, whether in-house or published. Several laboratories also demonstrated the capability to apply multiple methods, including sequencing.

A failure to identify PT objects may be due to:

- Inappropriate temperature of storage of the DNA items.
- Errors or inappropriate modifications of the DNA extraction or PCR amplification protocols.
- Use of unsuitable reagents during the DNA extraction or PCR amplification steps.

An incorrect species identification could be due to:

- Errors or inappropriate changes made by the operator during the DNA extraction or PCR amplification protocol steps.
- Exchange of PT objects during the analysis phase.
- DNA contamination during the analysis phase.
- Incorrect interpretation of DNA fragments observed during the electrophoresis run.

Written and elaborated by
PTP person in charge

Dr. A. Santoro

Verified and issued by
The Director

Dr. A. Casulli

Date 29/05/2026

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.

End of the report