Final report PT-11/2026

PT-11: "Molecular identification of *Echinococcus granulosus sensu lato* (*s.l.*) and *Echinococcus multilocularis*"

Design

Purpose	Assessment of the competence of participants in the molecular identification of *E. granulosus s.l.* and *E. multilocularis*.	
Timetable	Invitation mail: 15/06/2026 Website updates: within 15/06/2026 Registration deadline: 31/08/2026 PT items production: 11/09/2026 Shipping: 16/09/2026 Results submission deadline: 02/10/2026 Publication of the Final PT Report: 13/11/2026	
Participants	Operational Contact Point (OCP), National Focal Point (NFP) of the FWD-Net (Food and Waterborne Diseases and Zoonoses Network) coordinated by European Centre for Disease Prevention and Control (ECDC)	
Number of participants	Depending on requests	
PT items	Matrix	Elution buffer (buffered solution used to elute DNA and preserve its stability and integrity)
	Item	Genomic DNA
	Number of PT items	4 per each participant
	Panel composition	*Echinococcus granulosus s.s.* (2 items), *Echinococcus multilocularis* (1 item), *Taenia* spp. (negative) (1 item)
	Number of surplus items	NA
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Azzurra Santoro, Federica Santolamazza, Marianna Ascierto, Eleonora Bello, Ilaria Bellini

Compliance with planned timelines: YES X NO ☐

Participant number and type: 16 public institutions

0 private institution

Acquisition of matrix and analyte: genomic DNA was provided by the EURL-P (European Union Reference Laboratory for Parasites). The DNA had been extracted from parasitic isolates held in the EURL-P reference collection using a commercial DNA extraction kit, according to the manufacturer's instructions. 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*

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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.

Production of PT items: The Proficiency Testing (PT) items were prepared on 11/09/2026; they consisted of four 1.5 mL tubes containing, respectively, two aliquots of *Echinococcus granulosus s.s.* DNA, one aliquot of *Echinococcus multilocularis* DNA (as the 3 positive items) and one aliquot of *Taenia saginata* DNA (as the negative item). The two *Echinococcus granulosus s.s.* aliquots were prepared from the same stock DNA preparation, one aliquot was used undiluted, whereas the second aliquot was diluted 1:2.

Activities provided by external providers: the PT Provider (PTP) entrusted the shipment of PT items to a qualified transport company. The company providing the shipping service was DHL.

Homogeneity and stability of PT items: Homogeneity was ensured by preparing each PT items, referred to the same species, from the same stock DNA preparations. Stability was guaranteed based on documented evidence of long-term DNA preservation under refrigerated conditions. Based on established literature, DNA remains stable for up to 10 years at a temperature ≤−15°C and up to six months if refrigerated (temperature between +4°C and +8°C). PT items were stored at ≤8°C until shipment and distributed under refrigerated conditions.

Distribution of PT items: PT items were shipped on 16/09/2026, and the deadline for submission of results was set for 02/10/2026. Each set of PT items consisted of a 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 holding an adequate number of cooling bricks to ensure that a temperature between 4°C and 15°C was maintained inside the package.

Instructions for participants: Participants were informed of the shipment via email on 16/09/2026. The email also enclosed a link for submitting results, which was active from 16/09/2026 (coinciding with shipment) to 02/10/2026. Instructions for participants were made available on the PTP website starting from 15/06/2026. 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 was established based on the results obtained by the EURL-P during the characterization of the PT items and confirmed in the corresponding test report.

Criteria for results evaluation: For each PT item, the participant's result was evaluated against the assigned value and classified as "correct" or "incorrect". Correct results consisted of the identification of *E. granulosus s.l.* (two items), *E. multilocularis* (one item), and the correct recognition of the negative item as *Taenia saginata* or negative for *Echinococcus* spp. Participants received a positive final evaluation only if all four PT items were correctly identified; otherwise, the final evaluation was negative.

During the post-distribution quality assessment performed by the PTP on residual PT items, low levels of non-target DNA were detected in some samples by qPCR, specifically *E. multilocularis* DNA in the negative (*Taenia saginata*) item and *E. granulosus* DNA in the *E. multilocularis* positive item. The original DNA stock solutions used for PT item preparation showed no unexpected signals by qPCR. However, further investigations demonstrated the presence of environmental DNA in the areas used for PT item preparation. It was therefore concluded that the additional non-target DNA resulted from environmental contamination occurring during the preparation of the PT items. Consequently, reports of additional non-target DNA by participants detections matching the patterns verified by the PTP were not considered analytical errors. Such findings did not affect either the evaluation of the individual item or the participant's overall performance assessment.

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 PTP reserves the right to provide participant PT results to competent authority and ECDC, upon request. Participants will be notified in writing if a competent legislative authority requests access to their PT results.

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Results provided by participants and performance EVALUATION

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
ECHI26_1	PCR & sequencing	ECHI01	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI02	Echinococcus granulosus G3	Echinococcus granulosus	correct	
		ECHI03	Negative (Taenia saginata)	Negative (T. saginata)	correct	
		ECHI04	Echinococcus granulosus G3	Echinococcus granulosus	correct	
ECHI26_2	qPCR	ECHI05	Echinococcus multilocularis	Echinococcus multilocularis	correct	**Negative**
		ECHI06	negative	Echinococcus granulosus	incorrect	
		ECHI07	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI08	negative	Echinococcus granulosus	correct	
ECHI26_3	qPCR	ECHI09	Echinococcus multilocularis, Echinococcus granulosus sl	Echinococcus multilocularis	correct	Positive
		ECHI10	Echinococcus granulosus sl	Echinococcus granulosus	correct	
		ECHI11	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI12	Echinococcus granulosus sl	Echinococcus granulosus	correct	
ECHI26_4	PCR & sequencing	ECHI13	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI14	Echinococcus granulosus sensu stricto G3	Echinococcus granulosus	correct	
		ECHI15	Negative (Taenia saginata)	Negative (T. saginata)	correct	
		ECHI16	Echinococcus granulosus sensu stricto G3	Echinococcus granulosus	correct	
ECHI26_5	NA	ECHI17	NA	Echinococcus multilocularis	NA	NA
		ECHI18	NA	Echinococcus granulosus	NA	
		ECHI19	NA	Negative (T. saginata)	NA	
		ECHI20	NA	Echinococcus granulosus	NA	
ECHI26_6	PCR & sequencing	ECHI21	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI22	Echinococcus granulosus sensu stricto G3	Echinococcus granulosus	correct	
		ECHI23	Taenia saginata	Negative (T. saginata)	correct	
		ECHI24	Echinococcus granulosus sensu stricto G3	Echinococcus granulosus	correct	

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Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
ECHI26_7	PCR & sequencing	ECHI25	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI26	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI27	Taenia saginata	Negative (T. saginata)	correct	
		ECHI28	Echinococcus granulosus	Echinococcus granulosus	correct	
ECHI26_8	qPCR	ECHI29	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI30	Echinococcus granulosus s.s.	Echinococcus granulosus	correct	
		ECHI31	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI32	Echinococcus granulosus s.s.	Echinococcus granulosus	correct	
ECHI26_9	PCR (endpoint)	ECHI33	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI34	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI35	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI36	Echinococcus granulosus	Echinococcus granulosus	correct	
ECHI26_10	PCR & sequencing; multiplex PCR (endpoint)	ECHI37	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI38	Echinococcus granulosus s.s.	Echinococcus granulosus	correct	
		ECHI39	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI40	Echinococcus granulosus s.s.	Echinococcus granulosus	correct	
ECHI26_11	qPCR	ECHI41	Echinococcus multilocularis, Echinococcus granulosus	Echinococcus multilocularis	correct	Positive
		ECHI42	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI43	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI44	Echinococcus granulosus	Echinococcus granulosus	correct	
ECHI26_12	PCR & sequencing	ECHI45	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI46	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI47	Echinococcus multilocularis	Negative (T. saginata)	correct	
		ECHI48	Echinococcus granulosus	Echinococcus granulosus	correct	

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Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
ECHI26_13	multiplex PCR (endpoint)	ECHI49	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI50	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI51	Negative	Negative (T. saginata)	correct	
		ECHI52	Echinococcus granulosus	Echinococcus granulosus	correct	
ECHI26_14	NGS	ECHI53	Echinococcus granulosus G3	Echinococcus multilocularis	incorrect	**Negative**
		ECHI54	Echinococcus granulosus G3	Echinococcus granulosus	correct	
		ECHI55	non-Echinococcus	Negative (T. saginata)	correct	
		ECHI56	Echinococcus granulosus G3	Echinococcus granulosus	correct	
ECHI26_15	multiplex PCR (endpoint)	ECHI57	Echinococcus multilocularis	Echinococcus multilocularis	correct	Positive
		ECHI58	Echinococcus granulosus	Echinococcus granulosus	correct	
		ECHI59	Taenia spp.	Negative (T. saginata)	correct	
		ECHI60	Echinococcus granulosus	Echinococcus granulosus	correct	
ECHI26_16	NA	ECHI61	NA	Echinococcus multilocularis	NA	NA
		ECHI62	NA	Echinococcus granulosus	NA	
		ECHI63	NA	Negative (T. saginata)	NA	
		ECHI64	NA	Echinococcus granulosus	NA	

Legenda:

- Laboratories that failed the PT are marked in **bold**.
- NA= not applicable

Summary of results:

Total number of PT panels	16
Number of participants	14§
Number of participants that passed the PT	12
Number of participants that failed the PT	2

§2 participants did not submit the results

Overtime comparison of results

Not applicable (N/A).

As this was the inaugural round of the proficiency test, no previous results were available for trend analysis or comparison over time.

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Comments on participants' performance: Many laboratories that submitted results (12/14, 85.71%) successfully detected and correctly identified the target DNA in all PT items. In one of the two cases that received a negative evaluation, the laboratory indicated in the results submission form that a specific method for the detection of *Echinococcus granulosus* had not yet been implemented in its routine workflow, which readily explains the failure to identify this target. In the second case, the laboratory reported during the follow-up investigation that the analysis had been repeated and that the correct identification was subsequently obtained. The initial misidentification was therefore considered likely attributable to a sporadic analytical error rather than to a systematic methodological issue. Overall, the results indicate that most participating laboratories can reliably detect and identify the target DNA even under challenging conditions. The overall performance of the participants can therefore be regarded as highly satisfactory.

Performance evaluation if different methods are applied: Participants were allowed to use one or more molecular methods of their choice and were encouraged to employ those routinely used in their laboratories. Although at least four laboratories relied exclusively on real-time PCR (qPCR), most participants used conventional PCR-based approaches, either coupled with sequencing for species identification (seven participants) or implemented in multiplex assays that allow species-specific identification (two participants). qPCR assays and PCR-and-sequencing approaches based on species-specific primers appeared to be more sensitive than conventional PCR assays employing generic primers, as they were able to detect low levels of non-target DNA contamination in some PT items. In contrast, conventional PCR assays employing generic primers generally identified only the predominant target DNA present in the sample, which is consistent with the expected behaviour of broad-range amplification approaches. Nevertheless, all methodological approaches proved suitable for achieving the objectives of the PT when correctly implemented.

Comments and recommendations based on the outcomes of PT: Given the overall high level of performance achieved by the participants, no major recommendations are considered necessary. The results indicate that the laboratories generally possess robust analytical capabilities for the detection and identification of the target DNA. Several participants reported the use of multiple, alternative methodological approaches in their routine activities, (e.g. different amplification targets or single-plex and multiplex assays). The availability of complementary methods may be beneficial for the investigation and resolution of doubtful or discordant findings. The PTP remains available to provide technical support to laboratories wishing to implement methods for targets that are not yet included in their diagnostic routine. Finally, the PTP encourages laboratories that are unable to submit results within the established deadline to forward their results at a later stage whenever possible. Although these laboratories are not eligible to receive a participation certificate, they may still receive an individual performance evaluation upon request.

A failure to identify PT items may be due to:

- Inappropriate storage conditions or temperatures of the DNA items.
- Inappropriate method used for the scope of the PT scheme.
- Errors or inappropriate modifications introduced during PCR amplification procedures.
- Use of unsuitable or expired reagents during PCR amplification steps.

Incorrect species identification may be due to:

- Errors or inappropriate modifications introduced during PCR amplification procedures.
- Accidental exchange or mislabeling of PT items during the analytical process.
- DNA contamination occurred during sample handling or analysis.
- Incorrect interpretation of the results, including electrophoretic banding patterns, real-time PCR amplification data, and sequencing data.

Written and elaborated by
PTP person in charge

Dr. A. Santoro Azzurra Santoro

Verified and issued by
The Director

Dr. A. Casulli

Date 12/11/26

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 have 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

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