Final Report PT-05: Ec 1/2026

PT-05: "Detection of *Echinococcus* sp. worms in the intestinal mucosa of the definitive host"

Design

Purpose	Evaluation of laboratory competence in the accurate identification of *Echinococcus* spp. worms within a matrix of intestinal mucosa.	
Timetable	Invitation mail: 27/01/2026 Website updates: 27/01/2026 Registration deadline: 20/02/2026 PT items production: 16/03/2026 Shipping: 16/03/2026 Results submission deadline: 23/03/2026 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	Intestinal mucosa of canids
	Item	worms of *Echinococcus* spp.
	Number of PT items	3 per each participant
	Panel composition	3 items: 1 positive (20 worms) and 2 negatives
	Number of surplus items	15 (5 panels)
Activities provided by external providers	Shipping	
Provider name	DHL	
Results evaluation	Qualitative evaluation	

Implementation

PT staff: Azzurra Santoro, Federica Santolamazza, Francesco Celani, Antonio Di Grazia, Irene Tartarelli

Compliance with planned timelines: YES x NO ☐

Participant number and type: 32 public institutions

0 private institution

Acquisition of matrix and analyte: Intestinal mucosa from the definitive host was provided by the NRL Finland upon request of the PTP. The animals from which the mucosa was collected had tested negative for *Echinococcus* by molecular methods by the provider. The mucosa was inactivated at −80 °C. Worms, preserved in ethanol, were collected and provided upon request of the PTP by the NRL Croatia.

Production of PT items: the PT scheme provides for the distribution of three PT items to each participating laboratory. Each PT item consists of homogenized intestinal mucosa of *Echinococcus* definitive host, either contaminated or not with *Echinococcus* worms.

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: homogeneity is ensured by counting the number of parasites under a stereomicroscope by two operators. PT items are considered stable for a period of 21 days from the date of preparation (which coincides with the shipping date). Stability was assessed by the PTP through ad hoc experiments.

Distribution of PT items: PT items were shipped on 16/03/2026, and the deadline for submission of results was set for 23/03/2026. Each set of PT items sent to participants consisted of a vacuum-sealed bag labeled with the participant's identification code. Inside, there were three 15 mL tubes filled with homogenized mucosa, each labeled with the item code. Packaging consisted of a polystyrene and cardboard container with a sufficient number of refrigerant packs to ensure a temperature between 4°C and 15°C 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); during the PT, the deadline for the form submission was postponed to 27/03/2026 to allow one country whose PT items had been held at customs until 23/03/2026 to complete the PT. 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 information and participants' results were collected via the web app. The tables in this document were prepared, based on the data submitted by participants, using Excel software.

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, as well as by parasite counting carried out by at least two operators.

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 item as positive or negative, regardless of the number of worms identified. A positive final evaluation is assigned when all three items were correctly identified; otherwise, it is 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
Emu1	SCT	Emu01	8	20	correct	Positive
		Emu02	0	0	correct	
		Emu03	0	0	correct	
Emu2	In house method	Emu04	10	20	correct	Positive
		Emu05	0	0	correct	
		Emu06	0	0	correct	

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
Emu3	SCT	Emu07	8	20	correct	Negative
		Emu08	5	0	incorrect	
		Emu09	0	0	correct	
Emu4	In house method	Emu10	2	20	correct	Negative
		Emu11	4	0	incorrect	
		Emu12	2	0	incorrect	
Emu5	SCT	Emu13	0	20	incorrect	Negative
		Emu14	0	0	correct	
		Emu15	0	0	correct	
Emu6	SCT	Emu16	10	20	correct	Negative
		Emu17	6	0	incorrect	
		Emu18	6	0	incorrect	
Emu7	SCT	Emu19	0	20	incorrect	Negative
		Emu20	5	0	incorrect	
		Emu21	3	0	incorrect	
Emu8	SCT	Emu22	6	20	correct	Negative
		Emu23	3	0	incorrect	
		Emu24	4	0	incorrect	
Emu9	SCT	Emu25	7	20	correct	Negative
		Emu26	9	0	incorrect	
		Emu27	0	0	correct	
Emu10	SCT	Emu28	12	20	correct	Positive
		Emu29	0	0	correct	
		Emu30	0	0	correct	
Emu11	SCT	Emu31	12	20	correct	Negative
		Emu32	3	0	incorrect	
		Emu33	7	0	incorrect	
Emu12	SCT	Emu34	15	20	correct	Positive
		Emu35	0	0	correct	
		Emu36	0	0	correct	
Emu13	SCT	Emu37	6	20	correct	Negative
		Emu38	5	0	incorrect	
		Emu39	7	0	incorrect	
Emu14	SCT	Emu40	3	20	correct	Positive
		Emu41	0	0	correct	
		Emu42	0	0	correct	
Emu15	SCT	Emu43	1	20	correct	Positive
		Emu44	0	0	correct	
		Emu45	0	0	correct	
Emu16	SCT	Emu46	9	20	correct	Negative
		Emu47	4	0	incorrect	
		Emu48	4	0	incorrect	
Emu17	SCT	Emu49	3	20	correct	Negative
		Emu50	2	0	incorrect	
		Emu51	0	0	correct	

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
Emu18	SCT	Emu52	6	20	correct	Positive
		Emu53	0	0	correct	
		Emu54	0	0	correct	
Emu19	SCT	Emu55	17	20	correct	Negative
		Emu56	15	0	incorrect	
		Emu57	20	0	incorrect	
Emu20	SCT	Emu58	6	20	correct	Positive
		Emu59	0	0	correct	
		Emu60	0	0	correct	
Emu21	SCT	Emu61	4	20	correct	Positive
		Emu62	0	0	correct	
		Emu63	0	0	correct	
Emu22	--	Emu64	--	20	--	NA*
		Emu65	--	0	--	
		Emu66	--	0	--	
Emu23	SCT	Emu67	9	20	correct	Positive
		Emu68	0	0	correct	
		Emu69	0	0	correct	
Emu24	SCT	Emu70	4	20	correct	Negative
		Emu71	12	0	incorrect	
		Emu72	0	0	correct	
Emu25	SCT	Emu73	4	20	correct	Negative
		Emu74	0	0	correct	
		Emu75	1	0	incorrect	
Emu26	SCT	Emu76	0	20	incorrect	Negative
		Emu77	0	0	correct	
		Emu78	0	0	correct	
Emu27	SCT	Emu79	11	20	correct	Negative
		Emu80	6	0	incorrect	
		Emu81	7	0	incorrect	
Emu28	In house method	Emu82	11	20	correct	Positive
		Emu83	0	0	correct	
		Emu84	0	0	correct	
Emu29	SCT	Emu85	3	20	correct	Positive
		Emu86	0	0	correct	
		Emu87	0	0	correct	
Emu30	SCT	Emu88	9	20	correct	Positive
		Emu89	0	0	correct	
		Emu90	0	0	correct	
Emu31	SCT	Emu91	4	20	correct	Positive
		Emu92	0	0	correct	
		Emu93	0	0	correct	
Emu32	SCT	Emu94	8	20	correct	Negative
		Emu95	0	0	correct	
		Emu96	6	0	incorrect	

Legend:

- SCT: Sedimentation and Counting Technique
- Negative evaluations are marked in red.
- *this laboratory did not submit the results

Summary of results:

Total number of PT panels	32
Number of participants	32
Number of participants that passed the PT	14
Number of participants that failed the PT	17

Overtime comparison of results:

Laboratory code (2026)	2022	2023	2024	2025	2026
Emu1	POS	POS	POS	POS	POS
Emu2	POS	POS	POS	POS	POS
Emu3	POS	POS	POS	NEG	NEG
Emu4	NEG	POS	POS	POS	NEG
Emu5	POS	POS	POS	POS	NEG
Emu6	POS	POS	POS	POS	NEG
Emu7	NP	NP	NP	NP	NEG
Emu8	NEG	POS	POS	POS	NEG
Emu9	POS	POS	POS	POS	NEG
Emu10	POS	POS	POS	POS	POS
Emu11	NEG	POS	POS	POS	NEG
Emu12	POS	POS	POS	POS	POS
Emu13	NP	POS	POS	POS	NEG
Emu14	NP	POS	NP	NEG	POS
Emu15	NEG	POS	POS	POS	POS
Emu16	NEG	POS	POS	POS	NEG
Emu17	POS	POS	POS	POS	NEG
Emu18	POS	POS	POS	POS	POS
Emu19	POS	POS	POS	POS	NEG
Emu20	NEG	POS	POS	POS	POS
Emu21	NEG	POS	POS	POS	POS
Emu22	NEG	POS	POS	POS	NA
Emu23	NEG	POS	POS	POS	POS
Emu24	NEG	POS	POS	NEG	NEG
Emu25	POS	NEG	POS	POS	NEG
Emu26	NP	NP	POS	NEG	NEG
Emu27	NP	NP	NP	POS	NEG
Emu28	POS	POS	POS	POS	POS
Emu29	POS	POS	POS	POS	POS
Emu30	NEG	POS	POS	NEG	POS
Emu31	NEG	POS	NP	POS	POS
Emu32	NEG	POS	POS	POS	NEG

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

Comments on participants' performance: In 2026, 14 out of 32 participating laboratories successfully passed the proficiency test, whereas 17 out of 32 did not, and one laboratory didn't submit results. Although the majority of laboratories that submitted results correctly identified the positive item (28/31), the principal challenges were associated with the negative items, which were correctly identified by only 18 out of 31 laboratories (Item II) and 20 out of 31 (Item III) laboratories, respectively.

The considerable number of false positive results may be attributed to the natural occurrence, within the mucosa of the definitive host, of cestodes that can be misinterpreted as *Echinococcus* spp. These parasites are generally reported as *Amoebotaenia* spp. Nevertheless, a progressive improvement in the expertise of participating laboratories can be observed in the identification and correct differentiation of *Amoebotaenia* worms, supported by the fact that 11 of the laboratories that successfully passed the PT explicitly reported *Amoebotaenia* findings in their notes. Only one laboratory, despite recognizing the presence of *Amoebotaenia*, did not achieve a satisfactory outcome, as it identified all three samples as negative.

Performance evaluation if different methods are applied: The majority of laboratories (27/31) employed the SCT (sedimentation and counting technique) method, while three laboratories applied in-house methods, which generally involved sample filtration. No association between a method different from SCT and the performance was observed, as two of these three laboratories passed the PT, whereas one did not. Additionally, two laboratories complemented the SCT approach by applying molecular confirmation through PCR analysis of the isolated parasites. Both the laboratories passed the PT.

Comments and recommendations based on the outcomes of PT: although most laboratories demonstrate an adequate ability to process and analyze samples to ensure parasite detectability, improvements are still needed in the precise morphological identification, especially in differentiating *Echinococcus* spp. from other closely resembling cestodes. Finally, for methods other than SCT, applicability to real samples (i.e., intact rather than homogenized mucosa) is advised.

General recommendations

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

<u>False negatives</u>

The negativity of a positive sample can depend on several factors such as:

- Exchange of PT items during analysis.
- Preparation and use of a too low-concentrated saline solution, that hampers the sedimentation.
- Insufficient time of sedimentation.
- Insufficient steps of sedimentation, hampering the clear microscopic observation of the sediment.
- Failure to recognize the worms by the operator.

<u>False positives</u>

The positiveness of a negative sample may result from:

- Exchange of PT items during analysis.
- Misidentification of worms naturally present in the matrix, different from *Echinococcus* spp.
- In this latter case, it is recommended to accurately evaluate size and morphology of the worms found.

Written and elaborated by

Verified and issued by

PTP person in charge
Dr. A. Santoro

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.
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