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

Instructions

PT ITEMS

Description. The PT panel consists of four 1.5 mL tubes, three of which with DNA extracted from faecal material contaminated with: i) *Echinococcus granulosus sensu lato (s.l.)*, ii) *Echinococcus multilocularis*, iii) *Taenia* spp. and iv) a negative control.

Production. *Echinococcus granulosus* metacestode larvae, collected from animal and/or human hosts, and *Echinococcus multilocularis* and *Taenia* spp. worms recovered from the intestines of definitive hosts, are stored in 70% ethanol. Each parasitic specimen is homogenized with the faecal material and subjected to DNA extraction (coproDNA) using a commercial kit. For the negative item, DNA extracted from organismsother than *Echinococcus* spp. and *Taenia* spp. is used.

Distribution. An aliquot (10ul) of copro-DNA, either from *Echinococcus* or *Taenia*, or DNA extracted from other species, is distributed into each tube. Due to the nature of the PT items, production and distribution are made some days before shipping date and PT items are stored at temperature below +15°C.

Labeling. Each PT item is plugged and sealed with plastic film, labeled with a unique code not attributable to its content.

Homogeneity check. Homogeneity is ensured by providing participants with aliquots of the same DNA preparations.

Packaging. The PT panel (consisting of four PT items) is placed in a secondary container (e.g. 50mL tube) to ensure that they remain grouped and protected during handling and transport. The secondary tube is labelled with participant code. The PT panel is placed inside a polystyrene carton, ready for shipment. Several ice packs are arranged in the package to maintain internal temperature below +15°C.

Stability and quality control. The stability of the PT items has been evaluated by *ad hoc* experiments carried out by PT provider (EURL-P). DNA material stored below +15°C is stable for at least five years. Quality control requires that PT provider's staff analyses PT items before distribution. CoproDNA is individually identified through PCR and analysis of generated fragments. The method used is based on Trachsel et al. (2007) modified in the reaction mixture (0.2 µM final concentration of each primer and 2 µL template DNA in 30 µL final volume).

SUBMIT A PARTICIPATION REQUEST

The PT provider (EURL-P) announces the PT via e-mail addressed to potential participants. The email provides information on: PT items shipping date, PT scheme scheduled, the link to the participation form and the deadline, a summary of all deadline dates for the different phases of the PT scheme and the link to the PT provider's website for further information on PT scheme.

Request to participate has to be submitted by completing the participation form (PT request form) within the due date indicated in the form and in the email.

At the time of PT items shipment, the PT provider sends participants a link to the on-line form where participant is requested to provide the following information:

- Package content and its condition of preservation
- Timing of analysis
- Methods used to analyze PT items
- Results

PT ITEMS STORAGE

- Store PT items refrigerated at a temperature below +15°C until the analysis is performed
- Check the packaging and its contents for correctness and completeness: four PT items properly labeled and sealed
- Record the verification of the packaging upon arrival in the on-line form (see point 3)
- In case of discrepancy, fill in the claim form available at EURL-P website: https://www.iss.it/documents/20126/6678302/Claim-Appeal-rev-1.pdf/f9bba72e-3032-88fd-9d05-2b04ad45d1f1?t=1716802008339 or contact via e-mail the person in charge of the PT

PT ITEMS ANALYSIS

1. The code assigned to participant is reported both on a paper sheet contained in the package and on the tube containing the PT panel.
2. PT items treatment:
   - Treat PT items as routine samples
   - Record the codes reported on the tube (participant code) and on each PT item (PT item identification code)
3. Analysis: each participant is requested to apply any molecular method suitable for the purpose of the scheme, preferably the one used routinely.
4. Results: each participant is requested to indicate the assigned code, PT item codes and the identified species of each PT item. Identification yields four possible outcomes: *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia* spp., or negative. It is not allowed to submit results after the due date.

   Results have to be reported using the on-line form sent via e-mail to participants by PT provider on the same day the PT items are shipped.

   In the on-line form, participant has to indicate full reference if a published method is applied and any relevant deviation from the chosen method.
5. Deadlines:
   - Shipping is usually performed on Monday
   - PT items should be delivered within 48-72 hours
   - The deadline for results submission is indicated in the annual timetable available on the EURL-P website (https://www.iss.it/en/eurlp-prove-valutative-interlaboratorio), in the PT request form and in the results form
   - The final PT report is published on the EURL-P website according to the annual timetable available online (https://www.iss.it/en/eurlp-prove-valutative-interlaboratorio)
6. Perform evaluation: Result evaluation is qualitative. A result is considered “correct” if parasite species in the PT item and the negative PT item are correctly identified, and “incorrect” in case of wrong identification. Results have to be expressed reporting the species assigned to each PT item. Due to the nature of PT items, no statistical parameters are applied for result evaluation. Each result is evaluated against the value assigned by the PT provider. The final evaluation is “positive” if all PT items are correctly identified, otherwise it is “negative”.
7. Report: PT provider drafts the Final PT Report, including information on i) the expected species per PT item; ii) the species identified by the participant per PT item; iii) the final evaluation and iv) recommendation based on participant performance and an updated summary of participant performance over successive PT rounds. To ensure confidentiality each participant is identified by an alphanumeric code changed annually. The final PT report is published on the EURL-P website (https://www.iss.it/en/rapporti-finali-prove-valutative-interlaboratorio) and presented to the National Reference Laboratories during the annual workshop.
8. Follow up: in case of negative result, the PT provider contacts the participant who fails the PT and sends a link to a form where the participant is asked to analyze the causes of failure and indicate the corrective actions planned or implemented.
9. Confidentiality: EURL-P retains the PT Reports for 10 years. PT provider staff signs a confidential agreement regarding acquired data. In the final PT report, each participant is identified by alphanumeric code. The PT provider reserves the right to provide participant’s PT results to competent authority and accreditation body, upon request. In such cases, the PT provider will inform the participant in writing.

For any information or problem related to the web site access, please address:

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