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

Instructions

PT ITEMS

Description. Each PT item consists of a 15 mL tube containing 13 mL homogenized intestinal mucosa plus ethanol 70% (ratio 2:1), spiked or not with worms of *Echinococcus* spp.

Production. Matrix consists of a pool of carnivore intestinal mucosa (previously stored at –80°C at least one week) from *E. multilocularis* free-areas mixed with ethanol 70% (ratio 2:1) to obtain a homogenate. Analyte consists of *Echinococcus* spp. worm.

Distribution. For positive items, a predefined number of *Echinococcus* spp worms are counted under a stereomicroscope and transferred by a micropipette to the 15 mL tube. The tip of the micropipette is then checked under the stereomicroscope to confirm that all worms have been transferred. Homogenate is subsequently added until the total volume reaches 13 mL. For negative samples, 13 mL of homogenate are added directly to the tube.

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

Homogeneity check. Since proficiency PT items are individually spiked, homogeneity is ensured by accurately counting the number of worms spiked into each PT item, made by two operators.

Packaging. Each PT panel (consisting in three PT items) is placed in a secondary container (a vacuum-sealed plastic bag) to ensure that they remain grouped and protected during handling and transport. The secondary container is labelled with participant code. The PT panel is placed inside a polystyrene carton. Several ice packs are arranged in the package to maintain internal temperature between +4°C and +15 °C during shipping.

Stability and quality control. The stability of PT items in the package has been evaluated by *ad hoc* experiments made by PT provider (EURL-P) on vacuum sealed samples stored between +4°C and +15°C. *Echinococcus* spp. worms remain viable up to 21 days from the date of preparation. Quality control requires that PT provider's staff analyses PT items produced at the same time as those sent to participant and analysed on the deadline date for submitting results. The analysis is performed with an internal procedure based on Sedimentation and Counting Tehnique (SCT) available in WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern (ed. Eckert, J., Gemmell, M. A., Meslin, F.-X. & Pawloski, Z. S.) pp. 265. World Organization for Animal Health, Paris, France, 2001. ISBN 92-9044-522-X. &8364;40.

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.

Participation request 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 between +4°C and +15°C until the analysis is performed
- Check the packaging and its contents for correctness and completeness: three PT items properly labeled, vacuum sealed, internal temperature of the package between +4°C and +15°C
- 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 secondary envelope containing the PT panel.
2. PT items treatment:
   - Treat PT items as routine samples
   - Record the codes reported on the secondary envelope (participant code) and on each PT item (PT item identification code)
   - Perform the analysis within seven days after PT items delivery
   - If the Sedimentation and Counting Technique is used, extend the sedimentation time to 30-45 minutes as the PT item is different (denser) than the natural samples (opened intestine)
   - Handle PT items according to safety procedures required for infectious biological material, i.e. wearing personal protective equipment (coat, mask and gloves).
3. Analysis: each participant is requested to apply any method suitable for the purpose of the scheme, preferably the one used routinely, considering that the reference method for this analysis is the Sedimentation and Counting Technique.
4. Results: each participant is requested to indicate the assigned code, the PT item codes and the number of *Echinococcus* spp. worms recovered in each PT item. 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 in the EURL-P website (https://www.iss.it/en/eurlp-prove-valutative-interlaboratorio), in the PT request form and in 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. Result is considered “correct” if the participant correctly identifies whether PT item is spiked or not with *Echinococcus* spp. worms and “incorrect” in case of false positive or false negative identification. Participants have to indicate the number of *Echinococcus* spp. worms detected in each PT item, allowing the PT provider a more accurate evaluation of participant performance. 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 classified as “positive” if all PT items are correctly identified, otherwise it is “negative”.
7. Report: PT provider drafts the Final PT Report including, for each participant, information on: i) number of worms spiked per PT item; ii) the number of worms detected by the participant in each PT item; iii) the final evaluation and iv) recommendation based on participants performance and an updated summary of participants 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. The report is also sent to the European Commission (DG SANTE) within the time frame established by the latter.
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 PT results to competent authority and accreditation body, upon request. In such cases, the PT provider informs the participant in writing.

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

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