PT-03: "Identification of *Trichinella* larvae at the species level by a molecular method"

Instructions

PT ITEMS

Description. Each PT item consists of a 1.5ml tube containing *Trichinella* larvae belonging to different species. The PT panel consists of four tubes, each containing larvae from a different *Trichinella* species. Each tube contains larvae of the same species to be analyzed individually or as a pool, depending on the sensitivity of the analytical method and the experience of the technical staff.

Production. Larvae are obtained by artificial digestion of a mice carcass infected with a *Trichinella* reference strain, supplied by the International Trichinella Reference Center (ITRC). Artificial peptic digestion is performed according to the method described in ISO standard 18743:2015/Amd.1:2023.

Distribution. Ten larvae are counted under the stereomicroscope, transferred into the tube filled with 96% ethanol and checked to confirm the presence of the larvae. Due to the nature of the PT items, production and distribution are made some days before the shipping date and PT items are stored at room temperature until shipping.

Labeling. Each PT item is plugged and sealed with plastic film, labeled with a unique code not attributable to larvae species. The PT panel (consisting in 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.

Homogeneity check. Larvae of each species used in the PT belongs to the same *Trichinella* reference strain as certified by the ITRC. Homogeneity is further ensured through an accurate verification of larvae in the tube performed by two operators.

Packaging. Each PT panel (consisting in four PT items) is placed inside a polystyrene carton, ready for shipment. No temperature control is needed.

Stability and quality control. The stability of the PT items has been evaluated by *ad hoc* experiments carried out by EURL-P. Larvae preserved in 96% ethanol and stored between -20°C and +20°C maintain their stability for up to five years. Quality control requires that PT provider's staff analyse PT items before distribution. A subset of larvae from each of the four *Trichinella* species is tested to confirm their identity. Species identification is performed by EURL-P internal method, accredited according to ISO 17025 "Identification of *Trichinella* muscle stage larvae at species level by Multiplex-PCR".

SUBMIT A PARTICIPATION REQUEST

The PT provider (EURL-P) announces the PT via email addressed to potential participant. 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
- Materials and Methods used to analyze PT items
- Results

PT ITEMS STORAGE

- There are no specific conditions required for PT items storage
- 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 secondary envelope containing the PT panel.
2. PT items treatment:
   - treat PT items as routine samples
   - record the codes reported on the secondary envelop (participant code) and on each PT item (PT item identification code)
   - whenever transferring larvae from the original tube, use a small volume of ethanol (2-5µl). Wait for ethanol to evaporate completely before starting the DNA extraction
   - DNA extraction from single larvae is best performed using kits based on magnetic beads, which are generally more efficient than other systems. Alternative DNA purification methods (e.g., silica membrane-based kits) should be used only on pools of at least six larvae. In such cases, reduce the elution volume to 50–80 µL (if permitted by the manufacturer) to obtain more concentrated DNA
   - using high quality Taq polymerase (e.g. hot start) reduces the risk of obtaining non-specific PCR products.
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 code assigned, the PT item codes and the *Trichinella* larvae species of 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 email 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 larvae are properly identified at species level, 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, for each participant, 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 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, 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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