

PT-04: "Detection of Anisakidae L3 larvae in fish fillets"**Instructions****PT ITEMS**

Description. Each PT item consists of a fish fillet sandwich spiked or not with capsule-free Anisakidae L3 larvae (e.g., *Anisakis pegreffi*, *Anisakis simplex*).

Production. Matrix consists of fillet sandwich from Anisakidae-free fish, belonging to Salmonidae family (e.g. trout, *Salmo trutta*). Analyte consists of L3 Anisakidae live larvae free of their capsule. Larvae are collected from the coelomic cavity of highly infected fish (e.g., silver scabbardfish, family Trichiuridae),

Distribution. A predefined number of L3 larvae are carefully transferred by tweezers inside an incision made on the fish fillet of each sandwich. Each PT item is placed in a vacuum plastic bag. In case of negative PT item, the fish sandwich is incised but not spiked with larvae and directly placed in a vacuum plastic bag and vacuum sealed.

Labeling. Each PT item is vacuum sealed to preserve fish freshness and ensure larvae survival. Each plastic bag is labeled with a unique code with no indication of contamination.

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

Packaging. Each PT panel (consisting of three PT items) is placed inside a polystyrene carton, after being vacuum sealed in a larger plastic bag labeled with the code assigned to participant. Several ice packs are inserted 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. Capsule-free larvae remain viable up to seven days from the date of preparation. Quality control requires that PT provider's staff analyse 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 ISO 23036-2:2021 - Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method.

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 the 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

- 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 envelop (participant code) and on the plastic bag of each PT item (PT item identification code)
 - handle PT items according to safety procedures required for infectious biological material, i.e. wearing personal protective equipment (coat and gloves)
3. **Analysis:** each participant is requested to apply any method suitable for the purpose of the scheme, preferably the one used routinely, taking in account that two ISO standards are available (ISO 23036-1:2021 Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 1: UV-press method and ISO 23036-2:2021 - Microbiology of the food chain — Methods for the detection of Anisakidae L3 larvae in fish and fishery products — Part 2: Artificial digestion method)
4. **Results:** each participant is requested to indicate the assigned code, th PT item codes and the number of larvae recovered in each PT item. It is not allowed to submit results after the due date.
Results have to be reported in the on-line form addressed 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 EURLP 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 EURLP 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 properly identifies PT items spiked or not with larvae and "incorrect" in case of false positive or false negative identification. Results have to be expressed indicating the number of larvae 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 "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 number of larvae spiked per PT item; ii) the number of larvae detected by the participant in each 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 EURLP 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:** EURLP 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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