PT: "Detection of Anisakidae L3 larvae in fish fillets"

Procedure

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*).

Item preparation.
Matrix: PT items are made by fillet sandwich from Anisakidae-free fish, belonging to Salmonidae family (e.g. trout, *Salmo trutta*).
Parasites. L3 Anisakidae live larvae free of their capsule are used for the preparation of PT items. Larvae are collected from the coelomic cavity of highly infected fish (e.g., silver scabbardfish, family Trichiuridae), and identified at the genus level by microscopy. A known number of L3 larvae are carefully transferred by tweezers in the middle of each fish sandwich. The fish sandwich is then put individually in a plastic bag sealed under vacuum, identified by a numeric code stuck on the plastic bag and stored at +4°C.

Homogeneity check. Since proficiency items for the detection of Anisakidae larvae are made by individually spiked samples, homogeneity is ensured by accurate counting the number of larvae spiked into each sample, made by two operators.

Preparation of packages. In order to allow the preservation of fish freshness and assure larvae survival, each sample is put in a plastic bag sealed under vacuum. Each sample is labeled with a unique code without any indication of the level of contamination or any information on the identity of the testing laboratory. Each PT panel is put inside a polystyrene carton, after being sealed under vacuum in a larger bag. A number of ice packs are placed in the package in order to maintain the inside temperature between +4-15 °C during transport.

Stability check and quality control. The stability of the samples in the package has been evaluated by ad hoc experiments made by EURLP. Larval samples sealed under vacuum and stored between +4-15°C were viable up to 7 days from the date of preparation.

Criteria for result evaluation

The results are considered "correct" if the laboratory identify properly samples spiked or not with Anisakidae larvae, or "incorrect" in case of false positive or false negative result. The participating laboratory has also to indicate the number of larvae detected in each sample, the exact counting allowing a precise evaluation of laboratory and technician performance.
The EURLP, due to the low number of samples, cannot apply any statistical parameter for result evaluation. The final evaluation is "positive" if the results of all samples are correct or "negative" if at least one result is incorrect.

Report

Within 10 days after the due date to submit the results of samples analysis, the EURLP provides an Individual PT Report including the following information: i) number of spiked larvae per sample; ii) number of larvae detected by the laboratory in each sample; iii)) final evaluation and iv) recommendation based on the laboratory performance. Moreover, when applicable, an updated summary of laboratory performance over successive PT rounds is provided. The Individual PT Report will be delivered as .pdf file via e-mail or fax.
EURLP also provides the Final PT Report, including results obtained by all participants. The final report is presented to the NRLs during the annual workshop and subsequently published on the EURLP website.

To guarantee confidentiality, in the final report laboratories are identified by alphanumeric codes.

The PT Reports are retained by EURLP for 10 years.

For any information or problem related to the PT participation, please address to:

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