PT-07: "Molecular identification of Anisakid nematodes at the species level"

Procedure

PT items

Description. The panel of PT consists of 4 tubes: 2 tubes each containing a single fragment of Anisakidae L3 larva and 2 tubes each containing DNA extracted from a single Anisakidae L3 larva.

Sample preparation. L3 larvae are isolated from naturally highly infected fishes, and stored in 96% ethanol. Each larva is cut in several fragments, and any single fragment is individually transferred into a new tube, filled with 96% ethanol. For DNAs, an aliquot (10 µl) of DNA extracted from a single L3 larva is transferred into a new tube and sealed.

Homogeneity check. All larvae have been individually identified at species level by analyzing one of their fragments by the EURLP method "Identification at species level of parasites of the family Anisakidae by PCR/RFLP". The DNAs extracted from single larvae were also identified at species level by the method mentioned above. Homogeneity is ensured by providing to all participants aliquots of the same DNA preparations.

Preparation of packages. The 1.5 ml tubes are plugged and sealed with plastic paraffin film, individually identified with a code and sealed under vacuum in a plastic bag. Each PT panel is inserted in a polystyrene box together with ice packs in order to ensure a temperature between +4-15 °C during transport. To prevent shaking of the package content, insulating material (e.g. styrofoam chips) is added.

Stability check and quality control. The stability of the items in the package has been evaluated by ad hoc experiments carried out by EURLP. Larvae preserved in 96% ethanol, and stored between -20 and +30°C maintain their stability up to 5 years after the date of preparation. DNA stored below 15°C are stable for years.

Criteria for result evaluation

Results evaluation is qualitative, the participant must identify PT items at species level.

The PTP evaluates the result provided by the participant according to the sensitivity of the method applied. The participant is asked to correctly identify at least the species, for hybrid genomes (i.e. *A. simplex/A. pegreffi* hybrid genotype) the result will be evaluated based on the genetic marker used (i.e. mitochondrial vs nuclear markers). Likewise, the correct identification of the subspecies will be evaluated according to the resolution of the method applied.

Final evaluation is considered as "positive" if all PT items are correctly identified.

Report

The EURLP provides an Individual PT Report including the following information: i) species expected; ii) species identified by the laboratory; iii) final evaluation and iv) recommendation based on the laboratory performance. Moreover, when applicable, an updated summary of laboratory performance over successive PT rounds will be provided. The Individual PT Report will be sent as .pdf file via e-mail or fax.

The EURLP also provides the Final PT Report, including results obtained by all participants. The final report is published on the EURLP website and presented to the NRL during the annual workshop.

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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