

PT-07: “Molecular identification of anisakid nematodes at the species level”

Instructions

The same day of items shipping, the participant receives a link to an on-line form where the following information must be reported:

- Package content and its condition of preservation
- Materials and Methods used to analyze PT samples
- Results

The on-line form remains active until the due date (specified in the PT request form), after this date, results will not be accepted.

At arrival in the lab, the packaging and its contents must be checked for correctness and completeness.

Protocol to be used for the analysis of samples

The EURLP recommends to perform the analysis using one of the EURLP accredited methods: “Identification at species level of parasites of the family Anisakidae by PCR/RFLP” or, “Identification of Anisakidae larvae at the species level by multiplex PCR”. However, any other suitable molecular method performed by the participant laboratory is acceptable. The following remarks should be anyhow taken into account:

- 1) if transfer of the larva fragment from the original tube to a test tube is needed, it has to be done by taking larva in a small volume of ethanol (2-5 μ l), then it is necessary to wait for ethanol evaporation before starting the DNA purification procedure;
- 2) for DNA purification, it is recommended to use kits based on magnetic beads that are more efficient compared to other systems. If a different method (e.g. kits based on silica-membrane) is used, the elution volume (if allowed by manufacturer) should be reduced to 50-80 μ l resulting in a more concentrated DNA;
- 3) if multiplex PCR is applied, the quality of oligonucleotide synthesis may affect the efficiency of PCR reaction (especially for primer APEf);
- 4) the use of good quality Taq polymerase (e.g. hot start) reduces the risk to obtain non-specific PCR products;
- 5) in case a published method is used, reference detail must be indicated in the on-line form as well as any variation from the original procedure.

Any information concerning the PT participation, should be addressed to:

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