

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

Instructions

At arrival in the lab, the package content and items conditions must be checked and reported in the **Form 1** (*MO/POPVI-07/04: Check package*)*

Test results must be submitted within the deadline reported in the PT request form. (use **Form 4** - *MO/POPVI-07/07: Results**- to submit the results); test results will not be accepted if forwarded after the deadline.

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 µl), 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 µl 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.

The applied method should be described in the **Form 3** (*MO/POPVI-07/06: Procedure**), whereas instruments, reagents and materials used to perform the test should be listed in the **Form 2** (*MO/POPVI-07/05: List of instruments, reagents and materials used to perform the test*). In case a published method is used, reference detail must be indicated as well as and any variation from the original procedure (in the dedicated column).

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

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**Note: all forms will be sent by e-mail to the participants on the same day of PT panel shipping*