

## **PT-03: “Identification of *Trichinella* larvae at the species level by a molecular method”**

### **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. 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.

### **Protocol to be used for the analysis of items**

The EURLP recommends performing the analysis using the EURLP method “Identification of *Trichinella* muscle stage larvae at species level by Multiplex-PCR”, however the participant can use any molecular method available in his/her laboratory, considering the following remarks:

- 1) if transfer of larvae from the original tube to a test tube is needed, it has to be done by taking larvae 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) to purify DNA from single larvae, we recommend using kits based on magnetic beads that are more efficient compared to other systems. We suggest using alternative DNA purification methods (based on silica-membrane), only on a pool of at least 6 larvae and to decrease the elution volume to 50-80 µl (if it is allowed by manufacturer) to obtain more concentrated DNA;
- 3) if the protocol used is multiplex PCR, every oligonucleotide must be present in the reaction mix at the same concentration;
- 4) the use of good quality Taq polymerase (e.g. hot start) reduces the risk of obtaining non-specific PCR products.

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

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