



Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases



## **European Union Reference Laboratory for Parasites**

## 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.

## Protocol to be used for the analysis of samples

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, taking into account 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 µI), then is necessary to wait for ethanol evaporation before starting the DNA purification procedure;
- 2) to purify DNA from single larvae, we recommend to use kits based on magnetic beads that are more efficient compared to other systems. We suggest to use 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 to obtain non-specific PCR products.

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

Dr. Gianluca Marucci; e-mail: <a href="mailto:gianluca.marucci@iss.it">gianluca.marucci@iss.it</a>