

Istituto Superiore di Sanità

Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases



European Union Reference Laboratory for Parasites

PT-08: "Molecular identification of *Echinococcus* at the species level"

Procedure

PT items

<u>Description</u>. The PT panel consists of three tubes containing: DNA extracted from *Echinococcus granulosus* sensu lato (s.l.), DNA extracted from *Echinococcus multilocularis* and a negative control.

<u>Sample preparation</u>. Echinococcus granulosus s.l. metacestode larvae collected from animal and/or human hosts and *Echinococcus multilocularis* worms removed from the intestines of canids are stored in 70% ethanol and used for the extraction of high quality DNA using a commercial kit.

<u>Homogeneity check.</u> Metacestode larvae and worms were individually identified at species level through PCR and analysis of generated fragments. The method used is based on Trachsel et al. (2007) modified in the reaction mixture (0.2 μ M final concentration of each primer and 2μ L template DNA in 30 μ L final volume). Homogeneity is ensured by providing participants with aliquots of the same DNA preparations.

<u>Preparation of packages</u>. The tubes are plugged and sealed using plastic paraffin film, individually coded 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 movement of the package contents during transit, insulating material (e.g. styrofoam chips) is added.

<u>Stability check and quality control.</u> The stability of the items in the package was evaluated by ad hoc experiments carried out by EURLP. DNA material stored below 15°C is stable for at least 5 years.

Criteria for result evaluation

Results evaluation is qualitative; the participants are required to identify the two species belonging to the genus *Echinococcus* and the negative item. Final evaluation is considered "positive" if all species are successfully identified including the negative control.

Report

Within 10 working days the result submission of the PT analysis, the EURLP provides an Individual PT Report with the following information: i) species expected; ii) species identified by the participating 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 is provided. The Individual PT Report is sent as a .pdf file via e-mail.

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 at the annual National Reference Laboratories (NRLs) workshop.

To guarantee confidentiality, in the final report laboratories are identified by alphanumeric codes.

PT Reports are held by EURLP for 10 years.

For any information or queries related to PT participation, please contact:

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