

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 granulosus*, *Echinococcus multilocularis* and *Taenia* spp."

Procedure

PT items

<u>Description</u>. The PT panel consists of four tubes containing: DNA extracted from faecal material contaminated with <u>Echinococcus granulosus sensu lato</u> (s.l.), DNA extracted from faecal material contaminated with <u>Echinococcus multilocularis</u>, DNA extracted from faecal material contaminated with <u>Taenia</u> spp. and a negative control.

<u>Sample preparation</u>. Echinococcus granulosus s.l. metacestode larvae collected from animal and/or human hosts and *Echinococcus multilocularis* and *Taenia* spp. worms removed from the intestines of canids are stored in 70% ethanol. Parasitic material is homogenized with the faeces used for the extraction of DNA (coproDNA) using a commercial kit.

<u>Homogeneity check.</u> CoproDNA is individually identified 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 *Echinococcus* spp. at the species level, *Taenia* spp. and the negative item. Final evaluation is considered "positive" if all the items are successfully identified including the negative control.

Report

The EURLP provides an Individual PT Report with the following information: i) genus/species expected; ii) genus/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 to the NRL during the annual 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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