PT-05: "Detection of *Echinococcus* spp worms in the intestinal mucosa of the definitive host"

Procedure

PT items

Description. Each item consists of 13 ml homogenized intestinal mucosa plus ethanol 70% (ratio 2:1), spiked or not with worms of *Echinococcus* spp.

Item preparation.

Matrix. A pool of fox intestinal mucosa (previously stored at –80°C for 14 days) from *E. multilocularis* free-areas is mixed with ethanol 70% (ratio 2:1) to obtain an homogenate.

Parasites. The worms of *Echinococcus* spp are preserved at room temperature in ethanol 70%.

Worms are counted under a stereomicroscope (by two different technicians) and transferred to the homogenate mucosa samples by a micropipette. The tip of the micropipette is then observed under the stereomicroscope to verify that no worms are sticking onto the tip.

The PT items consist of 13 ml homogenate mucosa, spiked or not with worms and placed in 15 ml tubes. The tubes are then plugged, sealed with plastic paraffin film, and put in a plastic bag sealed under vacuum and labeled with an alphanumeric code.

Homogeneity check. Since items for the detection of *Echinococcus* spp. worms by SCT consists of individually spiked samples, homogeneity is ensured by an accurate control of the number of larvae spiked into each item made by two operators.

Preparation of packages. Each item is put in a plastic bag sealed under vacuum in order to assure their preservation. Each sample is labeled with a unique code without any indication of the level of contamination or any information on the identity of the testing laboratory. Each PT panel is put inside a polystyrene carton, after being sealed under vacuum in a larger bag. A number of ice packs are placed in the package in order to maintain the inside temperature between +4-15 °C during transport.

Stability check and quality control. The stability of the items in the package has been evaluated by ad hoc experiments made by EURLP. Worms preserved in mucosa containing 70% ethanol, and stored between +4-15°C maintain their morphology up to 21 days from the date of preparation.

Criteria for result evaluation

Results evaluation is performed only by qualitative approach: the results of the SCT are reported as "correct" (i.e., detection of one or more *Echinococcus* adult worm in spiked samples) or "incorrect" (false positive or false negative), irrespective of the number of worms in the item/s. Final evaluation is considered as "positive" if all samples are correctly identified.

Report

Within 10 working days after the due date to submit the results of samples analysis, the EURLP provides an Individual PT Report including the following information: i) the number of spiked worms per sample; ii) the number of worms detected by the laboratory in the sample; iii) qualitative evaluation; iv) final evaluation and v) recommendations based on the laboratory performance.

The Individual PT Report will be delivered as .pdf file via e-mail.

EURLP also provides the Final PT Report, including results obtained by all participants. The final report is published on the EURLP website 30 working days after the due date to submit the results of samples analysis and presented to the NRL during the annual workshop. To guarantee confidentiality, in the final report laboratories are identified by alphanumeric codes.
The PT Reports are retained by EURLP for 10 years.

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

Dr. Adriano Casulli; e-mail: adriano.casulli@iss.it