PT-01: "Artificial digestion to detect *Trichinella* larvae in meat samples according to the Regulation EU 2020/1478 and Annex III Reg UE 2015/1375"

Procedure

Test samples

Description. The EURLP provides test samples to be used for different purposes: training of laboratory personnel, validation of test methods, auditing activities or proficiency testing (PT). Each sample item consists of a meat ball made by 35 or 100 g of minced meat (pork, horse), spiked or not with free, partially-encapsulated or encapsulated *Trichinella spiralis* larvae.

Sample preparation. Naked, partially-encapsulated or encapsulated *Trichinella spiralis* live larvae are used for the preparation of proficiency panels, to allow an exact counting of the initial number of larvae in each sample. The known number of larvae allows a precise evaluation of laboratory and technician performance. Live larvae are obtained through artificial peptic digestion of mice carcasses experimentally infected. The artificial peptic digestion is performed according to a modified procedure of the method described in ISO standard 18743:2015 (included in Reg. EU 2020/1478). Proficiency testing items are made by minced meat without fat and fascia from the animal species that is the most routinely analyzed in the labs, e.g. pork from pigs or horse meat. In the middle of each meat ball, a hollow is made to house the larvae. The larvae are counted under a stereo-microscope onto a watch glass (by two different technicians) and transferred to the meat ball by rinsing the watch glass with 150 µL of PBS. The watch glass is then observed under the stereo-microscope to verify if some larvae are left sticking onto the glass. The meat balls are then "closed" and put individually in a plastic bag sealed under vacuum and identified by a number.

Homogeneity check. Since proficiency items for the detection of *Trichinella* larvae by artificial peptic digestion are made individually, homogeneity is ensured by an accurate control of the number of larvae spiked into each sample made by two operators.

Preparation of packages. In order to allow the preservation of meat freshness and larvae survival, each sample is put in a plastic bag sealed under vacuum. 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. All bags containing the meat balls are inserted in a larger bag sealed under vacuum, which is put inside a polystyrene carton, ready for shipment. A number of ice packs are placed in the package in order to maintain the inside a temperature between 4 and 15°C during transportation.

Stability check and quality control. The stability of the samples in the package has been evaluated by ad hoc experiments made by EURLP on vacuum sealed samples stored between +4 and +15°C. Naked larvae remain viable up to 5 days, while encapsulated larvae remain viable up to 20 days from the date of preparation.

Criteria for the result evaluation

The Regulation EU 2020/1478 refer to ISO standard 18743:2015 describing standard methods to detect *Trichinella* larvae in meat. Basing on this ISO standard, the results of artificial digestion methods have to be expressed only qualitatively, i.e. as positive if *Trichinella* spp. larvae are found in the sample, or negative if no larvae are present in the sample.

Report

The EURLP provides the PT Individual Report to the participant lab, based only on qualitative result according to the ISO standard 18743:2015, included in Regulation EU 2020/1478. The PT Individual Report is available within 7 working days after the due date for sending the test results. Furthermore, in order to allow laboratories to know the sensitivity of their SOPs in applying the method, a summary including the number of larvae spiked in each sample, the number of larvae recovered by the laboratory, and the difference (∆) between expected and observed number of larvae. The EURLP provides also recommendations on the basis of the PT outcome. The PT Final Report, including results of all participating labs, is published in the EURLP web site.

To guarantee confidentiality, the PT individual report is available only to the participant and in the PT 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 web site access, please address to:

Dr. Gianluca Marucci e-mail: gianluca.marucci@iss.it