



**Guidelines for the validation of new methods for the detection of
Trichinella larvae in meat samples**

Revision of 9 December, 2015



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1. Introduction

According to the Commission Implementing regulation (EU) 2015/1375 of 10 August 2015, all *Trichinella* susceptible animals intended for human consumption should be tested by one of the approved methods as reported in the Annex 1. At present, five methods have been approved: 1. the magnetic stirrer method for pooled sample digestion, considered as the gold standard; 2. the mechanically assisted pooled sample digestion method/sedimentation technique, using the stomacher lab-blender 3,500 thermo model; 3. the mechanically assisted pooled sample digestion method/'on filter isolation' technique, using the stomacher lab-blender 3,500 thermo model; 4. the automatic digestion method for pooled samples of up to 35 g; 5. magnetic stirrer method for pooled sample digestion/'on filter isolation' and larva detection by a latex agglutination test, considered equivalent only for the testing of meat of domestic swine; and 6. artificial digestion test for in vitro detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® *Trichinella* AAD Kit, considered equivalent only for the testing of meat of domestic swine.

The aim of these guidelines is to establish a protocol for the validation process of new methods for the detection of *Trichinella* larvae in meat samples.

2. Procedures

- a. The company which want to validate a new method for the detection of *Trichinella* larvae in meat samples should contact the European Union Reference Laboratory for Parasites (EURLP), at the Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy; Tel. +39 06 4990 2304; Fax +39 06 4990 3561; email: edoardo.pozio@iss.it, providing written information on the apparatus, reagents and user manual, including:
 - i. the amount of meat which can be digested per batch;
 - ii. the type of meat that can be digested, i.e. the host species (e.g., pig, horse, wild boar).
- b. After the evaluation of the information provided by the Company, the EURLP will inform:
 - i. the Company on the procedures and detailed costs of the validation process;
 - ii. the DG SANTE about the request of the Company.

3. Agreement

An agreement will be signed between the legal representative of the company and the legal representative of the Institution which hosts the EURLP. In the agreement, the Company agree to:

- a. supply five replicates of the new method to EURLP and to substitute at its expenses the materials which do not work or which show some production defects;
- b. supply the reagents required to test the respective amount of samples to be tested;

- c. declare for what animal species the new method should be validated;
- d. declare the maximum amount in grams of meat that can be tested per time;
- e. provide a detailed protocol on the new method;
- f. cover all the expenses which will be detailed from the EURLP, namely:
 - i. the forwarding of the material from the EURLP to the four National Reference Laboratories (NRLs) which will be selected by the EURLP among those which had shown the best performance at the last proficiency test, and back from the laboratories to the company at the end of the validation process;
 - ii. the costs of the meat (e.g. pork, horse meat, wild boar meat) required for examination.
 - iii. the preparation of 20 minced meat samples spiked with three encapsulated *Trichinella* larvae for each of the five laboratories, for each host species for which the new method should be validated (e.g., pork, horse meat). The weight of the minced meat (free of fat and fascia) is the maximum weight of meat which can be digested per time;
 - iv. the package of meat samples and forwarding costs from EURLP to the four NRLs performing the test;
 - v. the staff of the EURLP and NRLs which will work for the evaluation of the new method.
- g. the EURLP and the four participating NRLs will be not responsible of damages of the material provided by the company; in the case of breaking, the company should provide at its own expenses the material repair or its substitution; all the forwarding expenses and packing of the material will be charged to the company.

4. Evaluation and report of the results

- a. each participating laboratory (both EURLP and NRLs) will fill in a form with the digestion results and comments on the use of the apparatus (e.g., sensibility, reproducibility, robustness, user-friendly) (Annex 1).
- b. for the approval of the apparatus, at least 1 larva should be detected in the samples (see the Guidelines for the detection of *Trichinella* larvae at the slaughterhouse or connected laboratory in a Quality Assurance System, www.iss.it/binary/crlp/cont/Guideline%20Trichinella%20QAS.1185787912.pdf);
- c. the digestion process is considered satisfactory if no more than 5% of the starting sample weight remains undigested for all type of muscles and hosts (Annex 2);
- d. the easiness of the decontamination process will be also evaluated;
- e. on the basis of the digestion results and comments, the EURLP will prepare a final document with a positive or negative evaluation of the validation process. The final document will be sent to the DG SANTE and to the Company.

5. Extension of the validation

If a company which successfully validated a new method, wants to extend the method to other matrices, a new validation process should be organized. The procedure set out in points a. and b. of point 2 must be followed. In addition, the following procedure must be applied:

An agreement will be signed between the legal representative of the company and the legal representative of the Institution which hosts the EURLP. In the agreement, the Company agree to:

- a. supply three replicates of the new method to EURLP and to substitute at its expenses the materials which do not work or which show some production defects;
- b. supply the reagents required to test the respective amount of samples to be tested;
- c. declare to what animal species, or laboratory condition, the new method should be extended; provide any information on changes on the test protocol;
- d. cover all the expenses which will be detailed from the EURLP, namely:
 - I. the forwarding of the material from the EURLP to the two NRLs which will be selected by the EURLP among those which had shown the best performance at the last proficiency test, and back from the NRLs to the company at the end of the extension process;
 - II. the preparation of 20 minced meat samples spiked with three encapsulated *Trichinella* larvae for each of the three laboratories, for each host species for which the new method should be validated (e.g., pork, horse meat). The weight of the minced meat (free of fat and fascia) is the maximum weight of meat which can be digested per time;
 - III. the package of meat samples and forwarding costs from EURLP to the NRLs performing the test;
 - IV. the personnel costs of EURLP and NRLs for the evaluation of the new method;
 - V. the EURLP and the NRLs will be not responsible of damages of the material provided by the company; in the case of breaking, the company should provide at its own expenses the material repair or its substitution; all the forwarding expenses and packing of the material will be charged to the company.

ANNEX 1

Example of the form for the report of the digestion results of meat samples spiked with *Trichinella* larvae

Laboratory code pork horse meat other _____ gram of minced meat ____

| Sample code | N. of larvae in the sample | N. of recovered larvae | | | | Weight of undigested material |
|-------------|----------------------------|------------------------|--|--|---|-------------------------------|
| | | after digestion | after the 1 st washing of the apparatus | after the 2 nd washing of the apparatus | after additional washing of the apparatus | |
| 1 | 3 | | | | | |
| 2 | 3 | | | | | |
| 3 | 3 | | | | | |
| 4 | 3 | | | | | |
| 5 | 3 | | | | | |
| 6 | 3 | | | | | |
| 7 | 3 | | | | | |
| 8 | 3 | | | | | |
| 9 | 3 | | | | | |
| 10 | 3 | | | | | |
| 11 | 3 | | | | | |
| 12 | 3 | | | | | |
| 13 | 3 | | | | | |
| 14 | 3 | | | | | |
| 15 | 3 | | | | | |
| 16 | 3 | | | | | |
| 17 | 3 | | | | | |
| 18 | 3 | | | | | |
| 19 | 3 | | | | | |
| 20 | 3 | | | | | |

Larvae missed after digestion, should be detected in the sediment after washing the apparatus 1, 2, 3 or more times.

Date

Name and Surname

Signature

ANNEX 2

Form for the report of the digestion results of meat samples to evaluate the capacity of digestion

Laboratory, pork gram of minced meat 100

| Sample code | Type of muscle | Weight of undigested material (g) | |
|-------------|----------------|-----------------------------------|--|
| | | after digestion | after the 1 st washing of the apparatus |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |
| 15 | | | |
| 16 | | | |
| 17 | | | |
| 18 | | | |
| 19 | | | |
| 20 | | | |

Date

Name and Surname

Signature