Guidelines for the evaluation of new methods, reagents and apparatuses for the detection of *Trichinella* larvae in meat intended for human consumption

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1. Introduction

When carrying out *Trichinella* testing in susceptible host species, the ISO 18743:2015 or one of the approved equivalent methods laid down in Annex I, Chapter 1 of the Regulation (EU) 2015/1375, must be used. At present, six methods have been approved: 1) the magnetic stirrer method for pooled sample digestion, considered as the gold standard (ISO 18743:2015); 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, using the Trichomatic 35® blender; 5) the magnetic stirrer method for pooled sample digestion/'on filter isolation' and larva detection by a latex agglutination test, using the Trichin-L antigen test kit; and 6) the artificial digestion test for in vitro detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® Trichinella AAD Kit (the last two methods are considered equivalent only for testing meat from domestic swine).

The aim of these guidelines is to establish a procedure for the evaluation of new methods, reagents or apparatuses, for the detection of *Trichinella* larvae in meat intended for human consumption.

2. Procedures

a. The Company that wants to evaluate a new method, reagent or apparatus for the detection of *Trichinella* larvae in meat intended for human consumption, shall contact the European Union Reference Laboratory for Parasites (EURLP) and provide information on the apparatus, method or reagent including, if available, the user manual. The information provided shall necessarily include:
   i. data on the validation process carried out by the Company;
   ii. the amount of meat that can be tested per batch;
   iii. the type of meat that can be tested, i.e., the host species (e.g., pig, horse, wild boar).

b. After the positive evaluation of the information provided by the Company, the EURLP will:
   i. Inform the Company on the procedures and detailed costs of the evaluation process, or ask the Company to provide additional data and/or documents concerning its internal validation process;
   ii. Inform the DG SANTE about the Company's request;
   iii. Organize a ring trial (RT) involving the EURLP and four National Reference Laboratories (NRLs) to determine the performance of the new apparatus, method or reagent.

3. Agreement

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

a. Supply the appropriate number of the new apparatuses or reagents to the EURLP and to the NRLs selected for the RT, and to substitute at its expenses the materials which do not work or show some production defects;

b. Declare the animal species for which the new apparatus or method or reagent shall be evaluated;

c. Declare the maximum amount of meat (in grams) that can be analyzed per single test;

d. Provide a detailed protocol on the new method, or, in case of apparatus or reagent, instructions for use;

e. Cover all the expenses that will be detailed by the EURLP, including:

I. The forwarding of the material from the EURLP to the NRLs, and back from the laboratories to the Company at the end of the evaluation process (if applicable);

II. The costs of the meat samples required for the evaluation process, as determined by the EURLP;

III. The costs of packaging and shipping of meat samples from EURLP to the NRLs that will perform the test;

IV. The costs of the staff at the EURLP and at the NRLs that will work for the evaluation of the new apparatus or reagents;

f. The EURLP and the participating NRLs are not responsible for any damage of the material provided by the Company; in case of breaking, the Company shall provide at its own expenses the material repair or its substitution; the costs for packing and shipment of the material will be charged to the Company.

4. Evaluation procedures

The results of the analyzed samples will be evaluated qualitatively and quantitatively (when possible). The obtained data will be used to calculate the performance of the method, reagent or apparatus. The proportion of correctly identified samples using the new method shall be of 100%. Additional features such as execution time, easy to use etc., will be considered in the final evaluation report.

For all the methods that allow the direct observation of the *Trichinella* larvae, each laboratory participating to the RT must indicate in an *ad hoc* form (Annex 1):

1. the number of detected larvae;
2. the net weight of undigested material (if applicable);
3. comments on the use of the apparatuses and reagents required by the method (optional)

For the approval of the method, no false positive nor false negative samples shall be reported. In case of a method involving artificial digestion of the muscle tissue, the digestion process will be considered satisfactory if less than 5% of the starting meat sample net weight remains undigested for all type of muscles and host species.

The decontamination procedure of the process will be evaluated in terms of easiness, effectiveness, safety for the operator and environmental impact.

In case of diagnostic methods not allowing a direct observation of the *Trichinella* larvae, as in the case of tests using antibodies or molecular methods, instead of the number of larvae the participating laboratory will report in the form only positive or negative.

5. Evaluation report

On the basis of RT results and comments, the EURLP will prepare a final report on the evaluation process including sensitivity, specificity, reproducibility, repeatability, robustness, and user-friendliness, when applicable. The final report will be sent to the DG SANTE and to the Company. The method may only be used for official controls after inclusion in Annex I of Regulation (EU) 2015/1375. In case the new method, reagent or apparatus does not require an amendment of the Regulation, it can be immediately used based on a favorable evaluation report. In such case, the EURLP will inform the NRLs.

6. Extension of the evaluation

If a Company wants to extend to other matrices (i.e., meat of different host species) the use of a method, reagent or apparatus, already included among the official methods of detection accepted by the European Commission, a new evaluation process by RT is required. If available, the Company should provide validation data regarding the application of the method, reagent or apparatus to the new matrix.

After the positive evaluation of the information provided by the Company, the EURLP will inform:

1. The Company on the procedures and detailed costs of the evaluation process;
2. The DG SANTE about the Company's request;
3. The National Reference Laboratories (NRLs) that will be involved in the extension of the evaluation.

A new agreement will be signed between the legal representative of the Company and the legal representative of the Institution that hosts the EURLP. In the agreement, the Company agrees to:

a. Supply the appropriate number of the new apparatus or reagent to the EURLP and to the NRLs selected by the EURLP, and to substitute at its expenses the materials which do not work or which show some production defects;

b. Declare to which animal species and laboratory condition, the already positively assessed method, reagent or apparatus should be extended;

c. Provide information on any changes in the test protocol;

d. Cover all the expenses that will be detailed by the EURLP (as detailed in point 3e)

g. The EURLP and the NRLs are not responsible for damages of the material provided by the Company; in the case of breaking, the Company shall provide at its own expenses the material repair or its substitution; the costs for packing and shipment of the material will be charged to the Company.

7. Evaluation procedures of the extension

Same procedure as in point 4.

8. Evaluation report of the extension

Same procedure as in point 5.

ANNEX 1

Example of test results form for meat samples spiked or not with *Trichinella* larvae*

Laboratory code ….. pig meat □ horse meat □ other □ _____ gram of minced meat _

Sample code	Result (positive or negative)	N. of detected larvae	Net weight of undigested material	Note

*The form could change according to the apparatus or reagents under evaluation.

Comments on the use of the apparatus or reagents (e.g., robustness, user friendliness, procedure of decontamination)

Date ……

Name and Surname of the analyst
performing the tests
Signature

Name and Surname of the Supervisor
Signature