



---

**Guidelines for the validation of apparatuses for the detection of  
*Trichinella* larvae in meat samples by digestion**



## Index

1. Introduction .....	3
2. Procedures .....	3
3. Agreement .....	3
4. Evaluation and report of the results.....	4
ANNEX 1.....	5
ANNEX 2.....	6

## 1. Introduction

According to the EC regulation 2075/2005, 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, four 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; and 4. the automatic digestion method for pooled samples of up to 35 g.

Many small or medium laboratories performing the digestion test have the need to use an automatic digestion apparatus in stead of to use different apparatuses which should be used sequentially with the risk of environmental contamination and/or the loss of larvae. Private companies are producing new apparatuses for the automatic digestion of pooled samples of meat from *Trichinella* susceptible animals, but these apparatuses cannot be used by laboratories until they are validated by independent bodies and approved by the European Commission which should amend the current legislation.

The aim of these guidelines is to establish a unique protocol for the validation process of automatic digestion apparatuses.

## 2. Procedures

1. The company producing the automatic digestion apparatus should contact the Community Reference Laboratory for Parasites (CRLP), 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](mailto: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) and the type of muscle (e.g., tongue, diaphragm, masseter).
2. After the evaluation of the information provided by the Company, the CRLP will inform:
  - i. the Company on the procedures and detailed costs of the validation process;
  - ii. the DG SANCO 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 CRLP. In the agreement, the Company agree to:

1. supply five apparatuses to CRLP and to substitute at its expenses the apparatuses which do not work or which show some production defects;
2. supply the reagents required to test the respective amount of samples to be tested by these apparatuses;

3. declare what kind of meat, i.e. from which animals, can be digested;
4. declare the maximum amount in grams of meat that can be digested per time;
5. cover all the expenses which will be detailed from the CRLP, namely:
  - a. the forwarding of the apparatuses from the CRLP to the other four laboratories which will be selected by the CRLP, and back from the laboratories to the company;
  - b. the costs of the meat (e.g. pork, horse meat, wild boar meat, crocodile meat) required for examination
  - c. the preparation of 30 minced meat samples spiked with *Trichinella* larvae (10 samples with 3 larvae; 10 samples with 6 larvae; 10 samples with 10-20 larvae) for each of the three laboratories, for each host species for which the apparatus can be used (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;
  - d. the preparation of 10 samples without any larva for each type of muscle can be digested for each host species, to evaluate the capacity to clean the apparatus and to digest the different type of muscles, evaluating if the weight of undigested material is lower or equal to the upper limit imposed by the EC regulation 2075/2005;
  - e. the package of meat samples and forwarding costs from CRLP to the four laboratories performing the test.
6. the CRLP and the four participating laboratories are not responsible of damages of the apparatuses; in the case of breaking, the company should provide at its own expenses the apparatus repair or its substitution; all the forwarding expenses and packing of the apparatus will be charged to the company.

#### **4. Evaluation and report of the results**

1. each participating laboratory 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).
2. for the approval of the apparatus, at least 1 larva should be detected in the samples containing 3 larvae; at least 3 larvae in the samples containing 6 larvae; and at least 75% of the larvae in the samples containing 10-20 larvae;
3. for the approval of the apparatus, the digestion process is considered satisfactory if not more than 5% of the starting sample weight remains undigested for all type of muscles and hosts (Annex 3);
4. on the basis of the digestion results and comments, the CRLP will write a final document. The final document will be sent to the DG SANCO and 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 <sup>st</sup> washing of the apparatus	after the 2 <sup>nd</sup> 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	6					
12	6					
13	6					
14	6					
15	6					
16	6					
17	6					
18	6					
19	6					
20	6					
21	10-20					
22	10-20					
23	10-20					
24	10-20					
25	10-20					
26	10-20					
27	10-20					
28	10-20					
29	10-20					
30	10-20					

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**

**Example of the form for the report of the digestion results of meat samples to evaluate the capacity of digestion**

Laboratory code ..... pork  horse meat  other  \_\_\_\_\_ gram of minced meat \_\_\_\_

Sample code	Type of muscle	Weight of undigested material (g)	
		after digestion	after the 1 <sup>st</sup> washing of the apparatus
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

Date .....

Name and Surname

Signature