INTERNATIONAL STANDARD

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AMENDMENT 1
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Microbiology of the food chain — Detection of *Trichinella* larvae in meat by artificial digestion method

AMENDMENT 1: Method validation studies and performance characteristics

Microbiologie de la chaîne alimentaire — Recherche des larves de Trichinella *dans la viande par une méthode de digestion artificielle*

AMENDEMENT 1: Études de validation de la méthode et caractéristiques de performance

Reference number
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This document was prepared by Technical Committee ISO/TC 34, *Food products,* Subcommittee SC 9, *Microbiology,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Microbiology of the food chain — Detection of *Trichinella* larvae in meat by artificial digestion method

AMENDMENT 1: Method validation studies and performance characteristics

Clause 2, third reference

Replace the text with the following:

World Organisation for Animal Health (WOAH), Chapter 3.1.22 — "Trichinellosis (infection with *Trichinella* spp.)", *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 11th edition, 2022

3.1, Note 1 to entry

Replace the text with the following:

These larvae measure 0,70 mm to 1,10 mm in length and 0,02 mm to 0,04 mm in width.

After 3.3

Add the following text as 3.4:

3.4
level of detection
LOD_x
<qualitative methods> measured analyse concentration, obtained by a given measurement procedure, for which the probability of detection is x

[SOURCE: ISO 16140-1:2016, 2.35, modified — Example and note to entry deleted.]

4.1, first and second line

Replace the text with the following:

(see WOAH, Chapter 3.1.22 — "Trichinellosis (infection with *Trichinella* spp.)", *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 11th edition, 2022)

4.1, after the paragraph

Add the following text as a new paragraph:

The performance data for the detection of *Trichinella* spp. in unprocessed raw pig meat samples have been calculated from an interlaboratory study (see Annex E).

4.2, after the paragraph

Add the following text as a new paragraph:

In case predilection sites are not available, it is recommended to collect at least twice the minimum amount.

4.8, second sentence

Replace the text with the following:

Knowledge of the basic morphological characteristics of *Trichinella* larvae, including size (0,70 mm to 1,10 mm in length and 0,02 mm to 0,04 mm in width) and shape is required to examine the sediment (see Figure C.1 and Figure C.2).

5.3

Replace the text with the following:

Pepsin (Powdered or granular: 1: 10 000 NF, 1: 12 500 BP, 2 000 FIP; liquid: 660 U/ml).

5.3

Add the following text after the note:

Liquid pepsin shall be brought to room temperature before use.

5.4

Replace the text with the following:

Ethanol (70 % to 90 % ethyl alcohol) **or similar disinfectant with proven efficacy.**

5.5

Delete subclause 5.5.

6.10

Replace the text with the following:

Funnels (glass, plastic, or steel), of an appropriate size to accommodate the sieve.

6.17, last paragraph

Replace the text with the following:

Non-disposable equipment shall be regularly cleaned by rinsing with hot water, to ensure removal of traces of fat and tissues from previous analyses. In the case of positive results in the previous analysis, see the decontamination process described in Clause 12.

Clause 7, last paragraph

Replace the text with the following:

Muscle samples should be tested as soon as possible. To prevent the muscles from decomposing, the samples should be stored at 2 °C to 8 °C until they are examined. The samples shall not be frozen.

Clause 8, last sentence

Replace the text with the following and add the following text:

For pools with a lower total muscle mass (up to 50 g), the digest fluid volume and ingredients may be adjusted accordingly to a minimum of 1 l. One litre conical glass separatory funnels and 2 l beakers can be used.

9.2

Replace the text with the following:

For blending/grinding, a small amount of digest fluid or tap water (45 °C ± 2 °C) can be added to the meat in the blender/grinder to facilitate homogenization. Blending/grinding should be continued until the meat is chopped or minced thoroughly but shall not last so long as to harm the larvae.

9.3, point a)

Replace the text with the following:

add 16 ml ± 0,5 ml of 25 % hydrochloric acid (see Clause 5) to a glass beaker containing 2 l of tap water preheated to 45 °C ± 2 °C;

9.3, point c)

Replace the text with the following:

add 10 g ± 0,2 g of powdered or granular pepsin (1: 10 000 NF) or 30 ml ± 0,5 ml of liquid pepsin (660 U/ml).

9.7, NOTE 3

Add the following text after the last sentence:

If necessary, the washing step can be repeated.

9.7, point e)

Replace the text with the following:

It is recommended to leave the final 20 ml of digest fluid in the Petri dish to stand for 20 s to 30 s for any larvae to settle before microscopic examination.

9.8, point c)

Replace the text with the following:

Examine the digest fluid in the Petri dish grid by grid with a stereomicroscope or trichinoscope at a 10 X to 20 X magnification for at least 5 min, ensuring the whole Petri dish is examined or until a larva is detected. Examination shall be done systematically, taking care to avoid movement of fluid in the Petri dish.

Clause 11

Replace the text with the following:

Results shall be expressed as "detected" or "not detected" *Trichinella* larvae in the test portion.

Clause 12

Replace the text with the following:

Fluids (digestive fluids, supernatant fluids, rinses, etc.) as well as glassware and other equipment, which can be contaminated with *Trichinella* larvae, shall be decontaminated by heating to 70 °C for at least 1 min, or by chemical alternative methods (e.g. ethanol at final concentration 70 % for at least 1 min) before disposal or cleaning.

Clause A.1, second paragraph

Replace the text with the following:

For pigs, muscle samples shall be taken from the diaphragm pillars (crus) or from the masseter. In the absence of predilection site muscles, a greater amount of muscle sample (at least twice the minimum recommended amount) shall be taken from other striated muscles near bones or tendons.

Clause B.1, first paragraph

Add the following text at the end of first paragraph:

The testing of frozen samples is unreliable, dependent on the species of *Trichinella* infecting, as well as the time and temperature of freezing. The method cannot be used to rule out the presence of dead larvae, which do not survive digestion and should be used in surveillance studies only.

Annex E

Add the following text as a new Annex E.

Annex E
(informative)

Method validation studies and performance characteristics

The performance data for detection of *Trichinella* spp. in unprocessed raw pig meat samples were calculated from an interlaboratory study organized in 2022 by the EURL for parasites[15]. The samples tested in the study were raw minced pig meat. The samples were tested at two different levels of contamination, plus a negative control. The study was part of the annual proficiency test round funded by the European Commission[15].

The method submitted to the interlaboratory studies was that of ISO 18743:2015, also adopted in the EU regulation 2020/1478[16], for the detection of *Trichinella* in raw meat samples intended for human consumption from the primary production stage. This method has been incorporated in this document.

The values of the performance characteristics derived from the interlaboratory study are shown in Table E.1. All data obtained by collaborators have been included from the calculations as no identified technical reasons were identified (deviations to the protocol).

The level of detection (LOD) has been evaluated considering the number of larvae reported by participating collaborators for the test portion contaminated with three *Trichinella* larvae (see Table E.1).

NOTE The number of recovered larvae depends on multiple factors (e.g. correct rinsing of apparatus used for digestion and filtering) and not only on time spent in reading the plate containing the digestion sediment. No correlation has been observed between an extended reading time (mean time for full plate reading ranged between 3 min to 15 min) and an increased accuracy in larvae detection (i.e. number of counted larvae).

Table E.1 — Results of data analysis obtained with raw minced pig meat samples [food category: raw meat and ready to-cook meat products (except poultry)]

Parameter	Pig meat		
	Blank	TS3[a]	TS5[a]
Number of participating collaborators	31	31	31
Number of samples per collaborator	1	1	1
Number of collaborators retained after evaluation of data	31	31	31
Number of samples retained after evaluation of data	31	31	31
Test portion size in g	100	100	100
Specificity in %	100	—	—
Sensitivity per level in %	—	100	100
LOD in larvae/test portion	—	≥ 3[b]	

[a] Pig meatball samples were artificially contaminated with reference material with the following strain and levels: *Trichinella spiralis* (TS) at a level of 3 L1 larvae/test portion and a level of 5 L1 larvae/test portion.

[b] 55 % of participating collaborators detected 3 out of 3 larvae per test portion; 25 % of participating collaborators detected 2 out of 3 larvae per test portion; 20 % of participating collaborators detected 1 out of 3 larvae per test portion.