REPORT ON THE VALIDATION OF THE LUMIVAST TRICHINELLA METHOD OF THE EUROIMMUN COMPANY

June 2018 – July 2024

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Introduction

When carrying out *Trichinella* testing in susceptible host species, the ISO 18743:2015/Amd 1:2023 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/Amd 1:2023); 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).

If a new method/apparatus/kit is to be used, prior to use it should be validated in accordance with the "Guidelines for the evaluation of new methods, reagents and apparatuses for the detection of *Trichinella* larvae in meat intended for human consumption", which was approved by the Standing Committee on Plants, Animals, Food and Feed, Section Biological Safety of the Food Chain of the DG SANTE on 1st April 2022 (herein referred to as "the Guidelines").

Evaluation rounds

On June 6th, 2018, the Euroimmun Medizinische Labordiagnostika AG (Seekamp 31, 23560 Lübeck, Germany), (herein referred to as "the Company") contacted the European Union Reference Laboratory for Parasites (EURL-P) to request information regarding the requirements for approval of a new test in the EU. The new test, *lumiVAST Trichinella*, would be suitable to detect *Trichinella* species (*T. spiralis*, *T. britovi*, *T. nativa,* and *T. pseudospiralis*) present in Europe in pig meat and it is based on the detection of specific *Trichinella* antigens by a highly sensitive immunoassay.

The Company sent the instruction manual, a description of the most important features of the method and data on the validation process carried out (Annex 1).

The *lumiVAST* (luminescent Veterinary Automated Standardised Technique) *Trichinella* is a method for the analysis of meat samples from pigs based on the automated detection of specific *Trichinella* antigens by a newly developed immunoassay based on chemiluminescence immunoassay (ChLIA). For this test each meat sample is shredded with PBS buffer in a high-quality knife mill resulting in the destruction of *Trichinella* larvae so that antigens are released. Following centrifugation, the supernatant (muscle tissue extract containing released excretory and secretory *Trichinella* antigens) is used for *Trichinella*-specific antigen detection. The process is fully automated with the Random Access Analyzer SuperFlex™ and involve the use of the lumiVAST *Trichinella* kit.

Following the positive evaluation of documents, the EURL-P submitted an agreement to the Company and contacted four National Reference Laboratories (NRLs) for *Trichinella* asking their availability for validating the *lumiVAST Trichinella*. These NRLs were selected among those that obtained a positive evaluation at the last proficiency testing (PT) on *Trichinella* detection in meat organized by the EURL-P.

The four participating NRLs were:

- NRL for *Trichinella* of Austria
- NRL for *Trichinella* of France
- NRL for *Trichinella* of Germany
- NRL for *Trichinella* of Poland

The Company supplied to each participating laboratory all the apparatuses and reagents required to test the samples, the instruction manuals and a video tutorial. Furthermore, technical assistance on setting up and managing all apparatuses involved in the new assay was provided by the National Company subsidiaries in each country to each participating laboratory.

According to the International Commission on Trichinellosis (Gajadhar et al., 2019): "For meat inspection it is necessary to ensure a test sensitivity which allows detection of the lowest number of larvae that may cause clinical symptoms in humans (Dupouy-Camet and Bruschi, 2007; Nöckler and Kapel, 2007). Results from digestion test validation studies in pork show that a 1 g sample size reliably allows for the detection of ≥ 3 larvae per g (lpg) in muscle tissue, whereas 3 and 5 g sample sizes can reliably detect ≥ 1.5 lpg and ≥ 1 lpg, respectively (Gamble, 1996; Forbes and Gajadhar, 1999). Therefore, it is recommended that positive samples within the PT panel should be spiked with 3–5 larvae, and that at least one of these samples contains 3 larvae".

The EURL-P, in agreement with the Company, organized a total of three RTs to evaluate the *lumiVAST Trichinella*. During the RTs, pig meat samples were spiked with three or five larvae of the *Trichinella* species present in Europe.

The first RT was done in May 2021 and involved the four NRLs and the EURL-P. The panel of samples provided by the EURL-P to each participant consisted in:

- 2 meat samples (100g) without any *Trichinella* larvae (negative samples)
- 1 meat sample (100g) spiked with 3 *T. spiralis* larvae
- 1 meat sample (100g) spiked with 5 *T. spiralis* larvae
- 1 meat sample (100g) spiked with 3 *T. nativa* larvae
- 1 meat sample (100g) spiked with 5 *T. nativa* larvae
- 1 meat sample (100g) spiked with 3 *T. britov*i larvae
- 1 meat sample (100g) spiked with 5 *T. britovi* larvae
- 1 meat sample (100g) spiked with 3 *T. pseudospiralis* larvae
- 1 meat sample (100g) spiked with 5 *T. pseudospiralis* larvae

Five false negatives (FN) samples (two spiked with 3 *T. pseudospiralis* larvae, one spiked with 5 *T. spiralis* larvae, one spiked with 5 *T. britovi* larvae and one spiked with 3 *T. nativa* larvae) out of a total of 50 samples, were reported. However, no false positive (FP) sample was reported. Three FNs were reported by a laboratory in which an in-depth training could not be done due to the COVID-19 pandemic and the consequent travel restrictions. Following the negative evaluation of the EURL-P, the Company agreed to optimize the sensitivity of the kit and to provide a more complete training to all participating NRLs. After that, the EURL-P agreed to organize a second 'provisional' RT.

The second RT was done in February 2022 and involved only four participants, the three NRLs in which FN results were reported and the EURL-P, and it focused mainly on the *Trichinella* pseudospiralis for which most FN were observed, whereas two *T. spiralis* samples were used as positive controls.

The panel of samples provided by the EURL-P to each participant during the second RT consisted in:

- 1 meat sample (100g) without any *Trichinella* larvae (negative sample)
- 1 meat sample (100g) spiked with 3 *T. spiralis* larvae
- 1 meat sample (100g) spiked with 5 *T. spiralis* larvae
- 4 meat samples (100g) spiked with 3 *T. pseudospiralis* larvae
- 4 meat samples (100g) spiked with 5 *T. pseudospiralis* larvae

Five FN (all spiked with 3 *T. pseudospiralis* larvae) out of a total of 44 samples were obtained. All but one participating laboratories provided at least one FN result. Therefore, the EURL-P asked the Company to further improve the method to reach an appropriate sensitivity for the correct identification of samples spiked with low number of *Trichinella* larvae.

The Company performed several tests with blades and containers made of different materials, and with different times of grinding to improve the efficacy of meat shredding, which was identified as the main responsible for the FNs obtained when testing meat samples spiked with 3 *Trichinella* larvae. Moreover, the Company optimized the cut-off setup improving the lumiVAST kit sensitivity. Once the efficacy of the *lumiVAST Trichinella* during all steps of the process was demonstrated by specific tests (meat samples spiked with low number of *Trichinella* larvae), which involved the EURL-P and one NRL, the EURL-P, in agreement with the Company, organized the third RT.

The third RT was done in July 2024 and involved the same laboratories that participated to the first RT (four NRLs and the EURL-P) and focused on samples spiked with low number of *Trichinella* larvae to definitively evaluate the accuracy (sensitivity and specificity) of the test.

Test samples provided by the EURL-P to each participant during the third RT were:

- 1 meat sample (100g) without *Trichinella* larvae (negative sample)
- 3 meat samples (100g) spiked with 3 *T. spiralis* larvae
- 3 meat samples (100g) spiked with 3 *T. nativa* larvae
- 3 meat samples (100g) spiked with 3 *T. pseudospiralis* larvae

In all RTs, the participating laboratories were asked to fill in a form, forwarded with the samples, to add comments and notes on the performance and on the user-friendliness of the test, as required by the Guidelines.

Sample forwarding

In the 1st and 2nd RT, samples consisted in 100g of pig minced meat, free of fat and fascia, spiked with three or five live *Trichinella* larvae collected after a short period of digestion of an infected mouse. In the third RT, samples consisted of 10 g of pig minced meat, spiked with three *Trichinella* larvae in muscle tissue, to be added to 90 g of pig diaphragm. This modification was done to better simulate the routine sample testing at slaughterhouses. In all RTs, samples were sealed in a plastic bag under vacuum, coded, and forwarded in a polystyrene box, with coolers to maintain a temperature of less than 10°C, by an international courier. The parcels containing samples were delivered within 24 hours.

Results

The evaluation of the assay was carried out according to the Guidelines endorsed on 1st April 2022. As concern section 4, "for the approval of the method, no false positive nor false negative samples shall be reported".
In the third 'definitive' RT, all participants correctly identified as positive or negative all the 50 samples tested.

Relationship between the results obtained by lumiVAST Trichinella and the Guidelines requirements

According to the Guidelines, no false positive nor false negative samples shall be reported during the testing of RT samples. The test successfully satisfied all the requirement of the Guidelines.

Strongness of the *lumiVAST Trichinella* method

- It is relatively easy to use;
- It is faster compared to methods based on digestion;
- Large part of the process is automated, which reduces the risk of human mistakes;
- The method does not rely on morphological identification of *Trichinella* larvae, therefore eliminating the need of trained technical staff.

Weakness of the *lumiVAST Trichinella* method

- The method requires specific apparatuses (as the Superflex™) that need regular maintenance.

Conclusions

According to the results of the validation rounds, the *lumiVAST Trichinella* is suitable to detect *Trichinella* spp. larvae in muscle samples of pigs. The use of this method is restricted to pork meat inspection only, as the kit has been not validated for other animal species.

References

Dupouy-Camet J, Bruschi F. Management and diagnosis of human trichinellosis. In J. Dupouy-Camet, K.D. Murrell (Eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis, World Organisation for Animal Health Press, Paris (2007), pp. 37-68.

Forbes LB, Gajadhar AA. A validated *Trichinella* digestion assay and an associated sampling and quality assurance system for use in testing pork and horse meat. J Food Prot. 1999 Nov;62(11):1308-13. doi: 10.4315/0362-028x-62.11.1308.

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Nöckler K, Kapel CMO. Detection of *Trichinella*, meat inspection and hygiene, legislation. In Dupouy-Camet J, Murrell KD (Eds.), FAO/WHO/OIE Guidelines for the Surveillance, Management, Prevention and Control of Trichinellosis (2007).

ANNEX 1

validation process carried out by Euroimmun company

EUROIMMUN
a PerkinElmer company

Date: 17.05.2023

Performance data for the assay lumiVAST Trichinella from EUROIMMUN

Sensitivity & Specificity

To collect sensitivity and specificity data the following samples were tested with the assay lumiVAST Trichinella from EUROIMMUN: (a) Negative samples made from muscle tissue, (b) negative reference samples made from minced meat, (c) positive samples made from muscle tissue spiked with larvae from different *Trichinella* species and (d) positive reference samples made from minced meat spiked with larvae from different *Trichinella* species.

The negative samples made from muscle tissue (*Trichinella* free) were bought in a local butcher shop. These samples consisted of muscle tissue from the diaphragm of pigs. The meat was tested negative with the gold standard (magnetic stirrer method). The negative reference samples originate from European Union Reference Laboratory for Parasites (EURLP) as well as from the local butcher shop.

All tested positive reference samples were spiked with larvae from different *Trichinella* species and originate from German Federal Institute for Risk Assessment (BfR) or EURLP, respectively.

The reference and muscle tissue samples were either frozen or fresh. Fresh means, that the samples were shredded, centrifugated and tested with lumiVAST Trichinella directly after receiving them. Frozen means, that the samples were shredded and centrifugated some time ago and that the resulting tissue extract was frozen at -80°C until testing with lumiVAST Trichinella.

For identification of larvae belonging to other *Trichinella* species than *T. spiralis,* 100 g negative muscle tissue from the diaphragm of pigs was spiked with a defined number of larvae from *T. britovi*, *T. nativa*, *T. papuae*, *T. pseudospiralis* and *T. zimbabwensis*. The larvae originate from EURLP.

The results are shown in the following table.

Sample					Result	
No.	***Trichinella* species**	**Number of larvae in 100 g sample**	**Fresh/ frozen**	**Muscle tissue/ minced meat**	**Result [Ratio]**	**Evaluation**
1	*Trichinella* free	0	fresh	muscle tissue	0.34	negative
2	*Trichinella* free	0	fresh	muscle tissue	0.33	negative
3	*Trichinella* free	0	fresh	muscle tissue	0.34	negative
4	*Trichinella* free	0	fresh	muscle tissue	0.42	negative
5	*Trichinella* free	0	fresh	muscle tissue	0.46	negative
6	*Trichinella* free	0	frozen	muscle tissue	0.49	negative
7	*Trichinella* free	0	frozen	muscle tissue	0.51	negative
8	*Trichinella* free	0	frozen	muscle tissue	0.46	negative
9	*Trichinella* free	0	frozen	muscle tissue	0.59	negative
10	*Trichinella* free	0	frozen	muscle tissue	0.57	negative

11	*Trichinella* free	0	fresh	minced meat	0.56	negative
12	*Trichinella* free	0	fresh	minced meat	0.48	negative
13	*Trichinella* free	0	fresh	minced meat	0.61	negative
14	*Trichinella* free	0	fresh	minced meat	0.56	negative
15	*Trichinella* free	0	fresh	minced meat	0.49	negative
16	*Trichinella* free	0	fresh	minced meat	0.52	negative
17	*Trichinella* free	0	fresh	minced meat	0.52	negative
18	*Trichinella* free	0	fresh	minced meat	0.57	negative
19	*Trichinella* free	0	fresh	minced meat	0.56	negative
20	*Trichinella* free	0	fresh	minced meat	0.59	negative
21	*T. spiralis*	1	frozen	muscle tissue	1.99	positive
22	*T. spiralis*	1	frozen	muscle tissue	2.41	positive
23	*T. spiralis*	1	fresh	minced meat	3.81	positive
24	*T. spiralis*	1	fresh	minced meat	4.09	positive
25	*T. spiralis*	1	fresh	minced meat	8.70	positive
26	*T. spiralis*	1	frozen	minced meat	5.74	positive
27	*T. spiralis*	3	fresh	muscle tissue	6.90	positive
28	*T. spiralis*	3	fresh	muscle tissue	2.56	positive
29	*T. spiralis*	3	frozen	muscle tissue	3.22	positive
30	*T. spiralis*	3	frozen	muscle tissue	5.17	positive
31	*T. spiralis*	3	frozen	muscle tissue	2.56	positive
32	*T. spiralis*	3	frozen	muscle tissue	1.88	positive
33	*T. spiralis*	3	frozen	muscle tissue	1.93	positive
34	*T. spiralis*	3	frozen	muscle tissue	2.51	positive
35	*T. spiralis*	3	frozen	muscle tissue	3.21	positive
36	*T. spiralis*	3	frozen	muscle tissue	3.61	positive
37	*T. spiralis*	3	fresh	minced meat	9.00	positive
38	*T. spiralis*	3	fresh	minced meat	4.81	positive
39	*T. spiralis*	3	fresh	minced meat	5.50	positive
40	*T. spiralis*	3	fresh	minced meat	5.64	positive
41	*T. spiralis*	3	frozen	minced meat	8.46	positive
42	*T. spiralis*	3	frozen	minced meat	9.36	positive
43	*T. spiralis*	5	fresh	muscle tissue	7.59	positive
44	*T. spiralis*	5	fresh	muscle tissue	5.44	positive
45	*T. spiralis*	5	fresh	minced meat	14.07	positive
46	*T. spiralis*	5	fresh	minced meat	10.32	positive
47	*T. spiralis*	5	fresh	minced meat	15.50	positive
48	*T. pseudospiralis*	3	fresh	muscle tissue	1.40	positive
49	*T. pseudospiralis*	3	fresh	minced meat	1.11	positive
50	*T. pseudospiralis*	3	fresh	minced meat	1.58	positive
51	*T. pseudospiralis*	3	fresh	minced meat	1.47	positive
52	*T. pseudospiralis*	3	fresh	minced meat	1.59	positive
53	*T. pseudospiralis*	3	fresh	minced meat	1.51	positive
54	*T. pseudospiralis*	3	fresh	minced meat	1.39	positive
55	*T. pseudospiralis*	3	fresh	minced meat	1.25	positive
56	*T. pseudospiralis*	3	fresh	minced meat	1.26	positive

57	*T. pseudospiralis*	3	fresh	minced meat	1.94	positive
58	*T. pseudospiralis*	3	frozen	minced meat	2.64	positive
59	*T. pseudospiralis*	3	frozen	minced meat	3.63	positive
60	*T. pseudospiralis*	3	frozen	minced meat	2.71	positive
61	*T. pseudospiralis*	3	frozen	minced meat	2.31	positive
62	*T. pseudospiralis*	3	frozen	minced meat	2.28	positive
63	*T. pseudospiralis*	3	frozen	minced meat	3.02	positive
64	*T. pseudospiralis*	3	frozen	minced meat	1.74	positive
65	*T. pseudospiralis*	5	fresh	muscle tissue	1.64	positive
66	*T. pseudospiralis*	5	fresh	muscle tissue	1.32	positive
67	*T. nativa*	3	fresh	muscle tissue	1.35	positive
68	*T. nativa*	3	frozen	minced meat	4.81	positive
69	*T. nativa*	3	frozen	minced meat	4.52	positive
70	*T. nativa*	3	frozen	minced meat	3.71	positive
71	*T. nativa*	3	frozen	minced meat	4.25	positive
72	*T. nativa*	3	fresh	muscle tissue	1.16	positive
73	*T. nativa*	5	fresh	muscle tissue	1.97	positive
74	*T. nativa*	5	fresh	muscle tissue	1.25	positive
75	*T. britovi*	3	fresh	muscle tissue	2.08	positive
76	*T. britovi*	3	fresh	muscle tissue	5.99	positive
77	*T. britovi*	5	fresh	muscle tissue	4.41	positive
78	*T. britovi*	5	fresh	muscle tissue	7.20	positive
79	*T. papuae*	3	fresh	muscle tissue	2.34	positive
80	*T. papuae*	3	fresh	muscle tissue	2.00	positive
81	*T. papuae*	5	fresh	muscle tissue	2.14	positive
82	*T. papuae*	5	fresh	muscle tissue	1.77	positive
83	*T. zimbabwensis*	3	fresh	muscle tissue	1.11	positive
84	*T. zimbabwensis*	3	fresh	muscle tissue	1.03	positive
85	*T. zimbabwensis*	5	fresh	muscle tissue	1.56	positive
86	*T. zimbabwensis*	5	fresh	muscle tissue	1.23	positive

The overall accuracy of lumiVAST Trichinella is 100% (Table 1). The specificity of lumiVAST Trichinella is 100%.

n = 86		**Muscle tissue samples (spiked/not spiked with different *Trichinella* species) and positive/negative reference samples**	
		positive	negative
lumiVAST Trichinella	positive	66	0
	negative	0	20

Sensitivity: 100%
Specificity: 100%

LumiVAST Trichinella has a sensitivity of 100% and a specificity of 100%.

Cross reactivity

The cross reactivity was tested against different nematode and protozoan antigens. Pigs can be a host for these tested parasites and thus, a cross reaction with lumiVAST Trichinella should be excluded. For each sample 100 g muscle tissue was spiked with 1 mg crude antigen from the different parasites.

Sample			Result	
No.	***Parasite***	**Crude antigen**	**Result [Ratio]**	**Evaluation**
1	*Toxocara canis*	1 mg	0.43	negative
2			0.42	negative
3	*Ascaris lumbricoides*	1 mg	0.44	negative
4			0.43	negative
5	*Stongyloides papillosus*	1 mg	0.40	negative
6			0.35	negative
7	*Strongyloides ratti*	1 mg	0.44	negative
8			0.37	negative
9	*Trichuris suis*	1 mg	0.48	negative
10			0.43	negative
11	*Toxoplasma gondii*	1 mg	0.41	negative
12			0.41	negative
13	*Toxocara cati*	1 mg	0.42	negative
14			0.41	negative
15	*Trypanosoma cruzi*	1 mg	0.43	negative
16			0.39	negative
17	*Ascaris suum*	1 mg	0.42	negative
18			0.46	negative

LumiVAST Trichinella shows no cross reactions with the tested parasites.

Reproducibility

The reproducibility was tested by Intra-Assay Variation and Batch-to-Batch Variation. As sample material muscle tissue extract and crude antigen from *T. spiralis* were used.

The following table shows mean value, standard deviation (SD) and coefficient of variation (CV). Three lots were tested for Batch-to-Batch Variation.

	Intra-Assay Variation				
Sample	Mean value [Ratio]	within-run		between-runs	
		SD	CV [%]	SD	CV [%]
1	2.7	0.1	4.2	0.2	5.7
2	3.9	0.2	4.0	0.2	4.4
3	403.9	22.4	5.6	20.2	5.0
4	44.2	1.5	3.4	3.9	9.1
5	86.2	2.8	3.1	2.7	3.1
6	414.9	18.8	4.3	32.5	7.8

	Batch-to-Batch Variation		
Sample	Mean value [Ratio]	between-batch	
		SD	CV [%]
1	2.7	0.1	3.5
2	3.9	0.2	4.5
3	401.7	25.9	6.5
4	45.4	1.5	3.4
5	86.5	3.1	3.5
6	424.0	18.2	4.3

The reproducibility of lumiVAST Trichinella is given.