DEPARTMENT
INFECTIOUS DISEASES

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Validation of the lumi-VAST kit, a new test for the detection of *Trichinella* infections in pigs

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Official diagnostic methods

ISO 18743:2015/Amd1:2023 (Magnetic stirrer method)

Mechanically assisted digestion method (Stomacher) with sedimentation or filtration technique

Automatic digestion method (Trichomatic 35™)

Magnetic stirrer + latex agglutination method (domestic swine)

Artificial digestion test by PrioCHECK® Trichinella AAD kit (domestic swine)

Validation of new methods

The EU regulation 2015/1375 (Annex 1) specifies the detection methods to be used during official controls for *Trichinella* in meat

New method/apparatus, prior to use, should be validated according to the EURLP guidelines approved by the DG SANTE (updated in 2022)

On June 6th, 2018, the Euroimmun Company contacted the EURL-P to request the validation for the lumiVAST Trichinella kit

The Company sent instruction manuals of kit and instruments, a description of the most important features of the method and the in-house validation data

How the *lumiVAST* method works

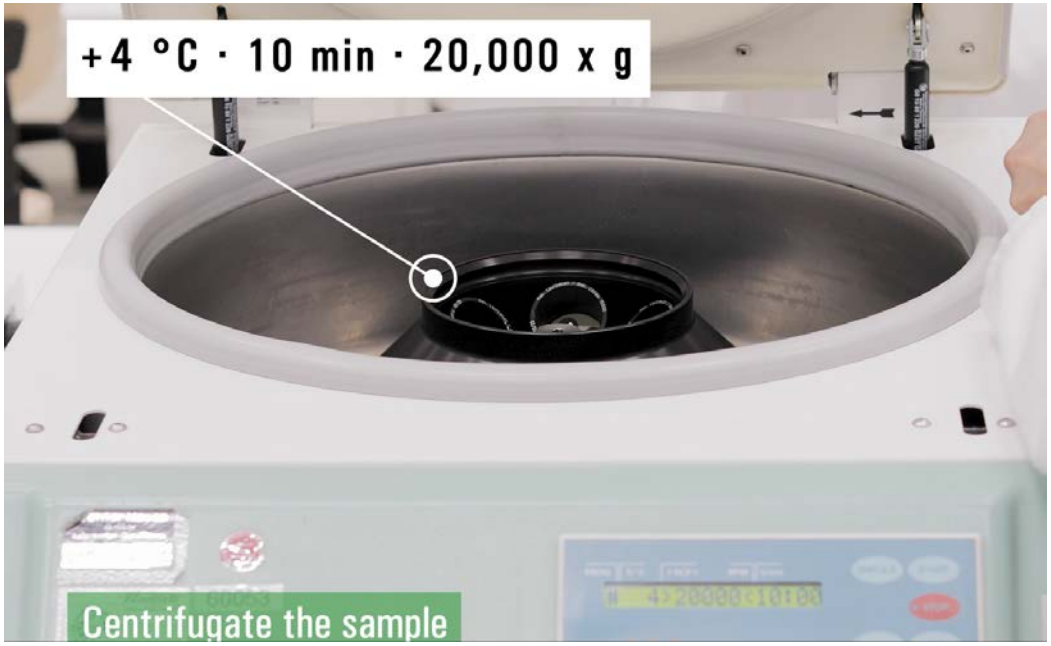
The analysis of meat samples is based on the detection of specific *Trichinella* antigens by an immunoassay based on chemiluminescence (ChLIA)

The process is fully automated with the Random Access Analyzer SuperFlex™

and involve the use of the lumiVAST *Trichinella* kit

Meat sample is shredded with PBS buffer in a knife mill…

…resulting in a meat homogenate containing *Trichinella* antigens

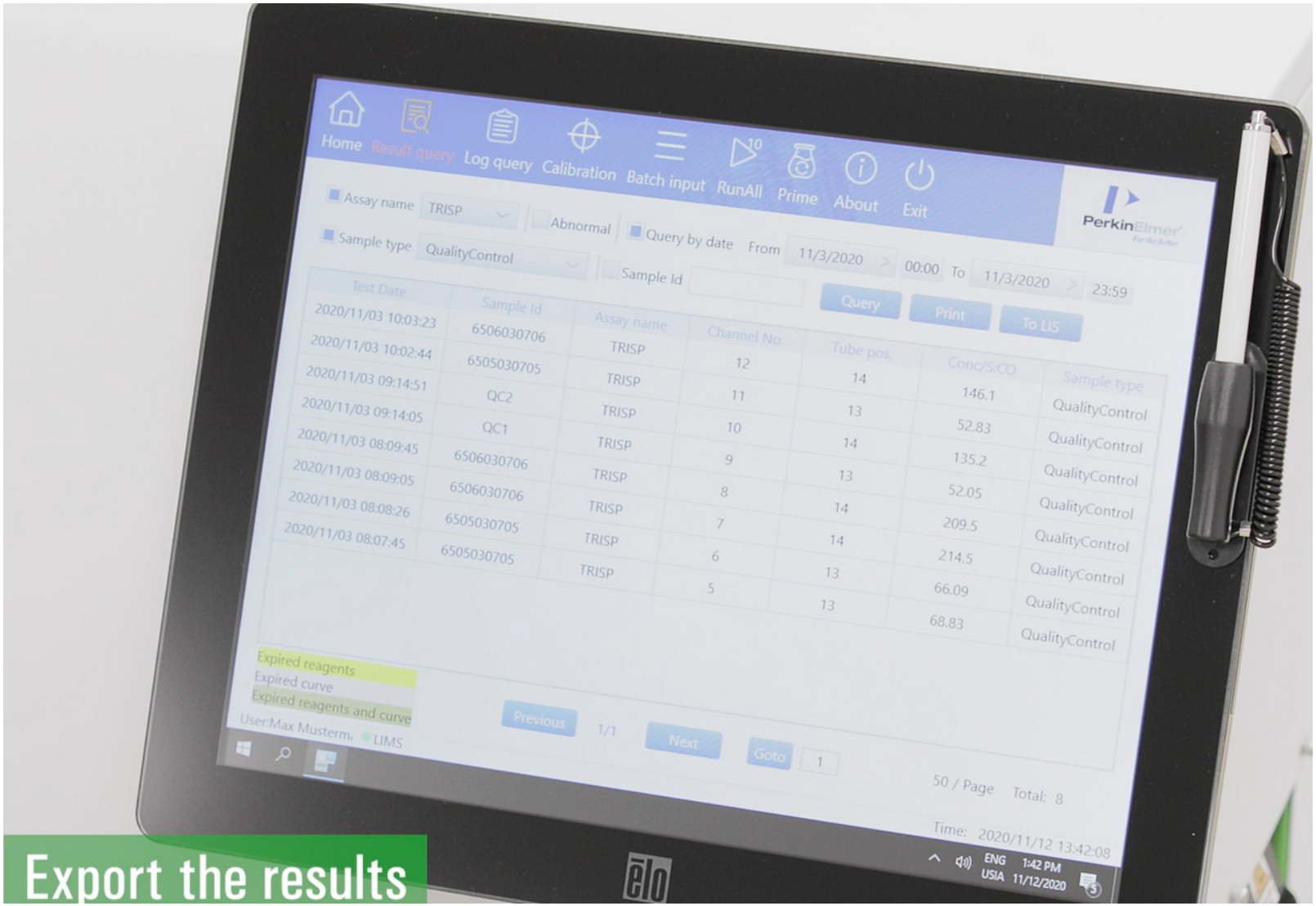


The meat homogenate is centrifuged

The supernatant (containing released *Trichinella* antigens) is collected…

… loaded in the Superflex and analysed using the cartridges included in the lumi-VAST kit

After about 35 min results are available



Export the results

Exported results

Model:SuperFlex S/N: 6110-0013

Test Date	Sample Id	Assay name	Channel No.	Tube pos.	Conc/S:CO	Sample type	Evaluation
2024/07/16 11:56:50	CHLIA 80	TRISP	12	10	1.847	TissueExtract	Positive
2024/07/16 11:56:11	CHLIA 80	TRISP	11	10	1.944	TissueExtract	Positive
2024/07/16 11:45:58	CHLIA 79	TRISP	10	9	2.489	TissueExtract	Positive
2024/07/16 11:45:19	CHLIA 79	TRISP	9	9	2.455	TissueExtract	Positive
2024/07/16 11:23:11	CHLIA 78	TRISP	8	8	1.904	TissueExtract	Positive
2024/07/16 11:22:32	CHLIA 78	TRISP	7	8	2.018	TissueExtract	Positive
2024/07/16 11:07:26	CHLIA 77	TRISP	6	7	4.003	TissueExtract	Positive
2024/07/16 11:06:48	CHLIA 77	TRISP	5	7	3.952	TissueExtract	Positive
2024/07/16 10:53:40	CHLIA 76	TRISP	2	6	4.485	TissueExtract	Positive
2024/07/16 10:53:00	CHLIA 76	TRISP	1	6	4.590	TissueExtract	Positive
2024/07/16 10:37:38	CHLIA 75	TRISP	12	5	6.068	TissueExtract	Positive
2024/07/16 10:36:59	CHLIA 75	TRISP	11	5	6.308	TissueExtract	Positive
2024/07/16 10:10:52	CHLIA 74	TRISP	10	4	9.898	TissueExtract	Positive
2024/07/16 10:10:12	CHLIA 74	TRISP	9	4	9.178	TissueExtract	Positive
2024/07/16 09:58:39	CHLIA 73	TRISP	8	3	5.687	TissueExtract	Positive
2024/07/16 09:57:59	CHLIA 73	TRISP	7	3	5.571	TissueExtract	Positive
2024/07/16 09:45:08	CHLIA 72	TRISP	6	2	2.506	TissueExtract	Positive
2024/07/16 09:44:29	CHLIA 72	TRISP	5	2	2.460	TissueExtract	Positive
2024/07/16 09:29:34	CHLIA 71	TRISP	2	1	0.611	TissueExtract	Negative
2024/07/16 09:28:54	CHLIA 71	TRISP	1	1	0.630	TissueExtract	Negative

User: Customer

Print Date: 2024-07-16 12:23:31

Cut-off = 1

Validation planning

4 NRLs + EURL-P

EURL-P and NRLs received the lumiVAST kit and the instruments (Superflex and knife mill)

Euroimmun company planned the visit, in each lab of a specialist, for installation, calibration and training on the use of the Superflex

EURL-P prepared the sample panels to be tested by NRLs

Ring trial 1 (May 2021)

Matrix: 100g minced pork spiked with larvae free of capsule

Sample panel tested by each participant

Sample type	N. of samples
Negative	2
T. spiralis 3 larvae	1
T. spiralis 5 larvae	1
T. nativa 3 larvae	1
T. nativa 5 larvae	1
T. britovi 3 larvae	1
T. britovi 5 larvae	1
T. pseudospiralis 3 larvae	1
T. pseudospiralis 5 larvae	1
TOT	10

RT-1 results

Laboratory	False positives	False nagatives	Final evaluation	Note
1	0	0	Positive	
2	0	0	Positive	
3	0	1	Negative	Sample spiked with 3 *T. pseudospiralis* larvae tested negative
4	0	1	Negative	Sample spiked with 3 *T. pseudospiralis* larvae tested negative
5*	0	3	Negative	Samples spiked with *T. spiralis* (5L), *T. nativa* (3L) and *T. britovi* (3L) tested negative

*proper calibration of Superflex and in-depth training not possible due to COVID-19 pandemic travel restrictions, and lumiVAST kit received in delay (no correct temperature maintained during the shipping)

RT-1 follow-up

With exception of lab 5, which failed because of more general problems, negative results involved only samples spiked with 3 larvae of *T. pseudospiralis*

According to the company, a second RT focusing on *T. pseudospiralis* was organized

The lumiVAST kit was optimized by the company and a more complete training and instrument setup was provided to lab 5

Ring Trial-2 (February 2022)

Matrix: 100g minced pork spiked with larvae free of capsule

Sample panel tested by each participant

Sample type	N. of samples
Negative	1
T. spiralis 3 larvae	1
T. spiralis 5 larvae	1
T. pseudospiralis 3 larvae	4
T. pseudospiralis 5 larvae	4
TOT	11

RT-2 results

Three replicates (3 tubes with the same meat omogenate) for each sample were tested in parallel

Laboratory	False positives	False nagatives	Final evaluation	Note
1	0	2	Negative	Conflicting results among replicates
3	0	1	Negative	Conflicting results among replicates
4	0	0	Positive	
5	0	1	Negative	

Samples that tested negative or showed conflicting results were spiked with 3 *T. pseudospiralis* larvae

RT-2 follow-up

Sometimes the amount of antigen was not enough to be detected by the kit

The sample **meat homogenization was not always effective to obtain enough antigens from** samples spiked with **three *T. pseudospiralis* larvae**

The company moved to a **more powerful knife mill**, changed the **blades**, tested different container lids and different **grinding time**

Several tests were made by Euroimmun, with the support of EURL-P **to increase the efficacy** of *lumiVAST Trichinella* **versus meat samples spiked with low number of *Trichinella* larvae**

Pre-test involving Euroimmun, EURL-P and one NRL confirmed the effectiveness of the changes made to the method

The EURL-P, in agreement with the Company, organized the third RT

Ring trial 3 (July 2024)

Matrix: 10g minced pork spiked with encapsulated larvae added to 90g of swine diaphragm

Sample panel tested by each participant

Sample type	N. of samples
Negative	1
T. spiralis 3 larvae	3
T. nativa 3 larvae	3
T. pseudospiralis 3 larvae	3
TOT	10

RT-3 results

Two replicates for each sample were tested

Laboratory	False positives	False nagatives	Final evaluation
1	0	0	Positive
2	0	0	Positive
3	0	0	Positive
4	0	0	Positive
5	0	0	Positive

RT-3 results

All the 50 samples were correctly identified as positive or negative

No conflicting results between replicates were observed

The company demonstrated to have solved the problem of low sensitivity linked to samples spiked with three *T. pseudospiralis* larvae

Non-European *Trichinella* species

The lumiVAST kit has been successfully tested by the company on meat samples spiked with 3 and 5 larvae of the non-encapsulated species *T. papuae* and *T. zimbabwensis*

Cross reactivity

EUROIMMUN
a PerkinElmer company

Date: 17.05.2023

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

No cross reactivity reported

Strongness of the *lumiVAST*

- Relatively easy to use
- Faster compared to methods based on digestion
- Large part of the process is automated (less risk of human mistakes)
- Does not require morphological identification of the parasite (no need of specific training)

Weakness of the *lumiVAST*

- It requires specific instruments (Superflex™) that need regular maintenance and calibration

Conclusions

According to validation results, 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 not been validated for other animal species

Additional remarks

The Superflex need to be regularly calibrated as specified in the instruction manual

11. Calibration and recalibration

The results of the lumiVAST Trichinella are calculated using a standard curve. This standard curve is lot-dependent. It must be assigned to the instrument for each new test kit lot before the test is started (calibration). The technical information for this calibration is stored in a barcode on the quality control certificate and must be read with the handheld barcode scanner of the SuperFlex. The quality control certificate can also be downloaded from the customer portal. The calibration procedure is described in the SuperFlex short instructions and instructions for use.

Afterwards, the standard curve must be checked and adjusted, if necessary (recalibration). For this adjustment of the standard curve, the calibrator included in the test set (CAL S1) is used. The recalibration is an automated procedure and managed by the SuperFlex. The procedure is described in the SuperFlex short instructions and instructions for use.

Calibration is required when

- a new test kit lot is used.

Recalibration is required when

- a calibration has been performed,
- the calibration interval of 28 days has expired,
- the last recalibration failed,
- the quality control is repeatedly outside the valid range, or
- a service or repair has been performed on the instrument.

Additional remarks

The lumiVAST does not allow:

- To evaluate parasite load
- To identify the *Trichinella* species

Is then necessary to specify in the reg. 2015/1375 (as done for latex agglutination test) that the method can be used up to the identification of the single positive animal. At that time confirmation must be done by a method (e.g. magnetic stirrer), which allows to recover the larvae


THANK YOU
FOR YOUR
Attention!

Non-European Trichinella species

- ***T. murrelli*** (North America and the Vancouver region of Canada): **never documented in naturally infected pigs**, and **experimentally demonstrated very low level of infectivity for pigs**
- ***Trichinella* T6** (North America): isolated in several species of wild carnivores, **poor infectivity for domestic swine**
- ***T. nelsoni* (**Easter Africa and Sud Africa): often found in sylvatic suids (bush pigs) but **never reported in domestic pigs**
- ***Trichinella* T8** (Namibia and Sud Africa) only seven reports of infections in wild animals, never identified in human infections, **unknown infectivity in pig**
- ***Trichinella* T9** (Hokkaido Island, Japan): isolated in several species of wild carnivores and omnivores, **never reported infections in swine**
- ***T. papuae*** (Papua, New Guinea, Thailand and Australia): **isolated** from farmed crocodiles and **from wild and domestic pigs** (tested by lumiVAST)
- ***T. zimbabwensis*** (Zimbabwe, South Africa, Ethiopia and Mozambique): isolated in farmed and wild crocodiles, monitor lizards and mammalian carnivores, **experimentally demonstrated high infectivity for a wide spectrum of mammalian hosts including pigs** and rats (tested by lumiVAST)
- ***T. patagoniensis:*** (Argentina) isolated from mountain lions, experimentally shown to have **poor infectivity for pigs** and rodents
- ***T. chanchalensis,*** (North-western Canada): isolated from wolverines, **not evidences of its infectivity in pigs**

(POZIO E. & ZARLENGA D.S. (2021). Chapter 3: Taxonomy of the Trichinella genus. In: Trichinella and Trichinellosis, Bruschi F., ed. Academic Press, Amsterdam, Netherlands, pp: 35–76)