



Rijksinstituut voor Volksgezondheid  
en Milieu  
*Ministerie van Volksgezondheid,  
Welzijn en Sport*

# Test sensitivity of a commercial serine protease digestion kit

Frits Franssen, Annette Johne,  
Joke van der Giessen, Karsten  
Nöckler and Anne Mayer-Scholl



# Introduction

Most *Trichinella*-labs in EU and non-EU countries use the EU Reference method EC 2015/1375

- Prone to pepsin shortage
- Fluctuating quality and prices



# Introduction

EC 2015/1375 Equivalent methods:

- A. Mechanically assisted pooled sample digestion method / sedimentation Technique
- B. Mechanically assisted pooled sample digestion method/'on filter isolation' technique
- C. Automatic digestion method for pooled samples of up to 35 g ('on filter isolation' technique)



# Introduction

EC 2015/1375 Equivalent methods only considered equivalent for the testing of meat of domestic swine:

D. Magnetic stirrer method for pooled sample digestion/'on filter isolation' and larva detection by a latex agglutination test

E. Artificial digestion test for in vitro detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® *Trichinella* AAD Kit



## M&M

PrioCHECK® Trichinella AAD Kit (T-AAD Kit) protocol manufacturer

EC 2015/1375 Magnetic stirrer method (MSM)

*T. spiralis* larvae (strain code ISS 003) were isolated from the meat of a domestic pig, with- and without capsule

*T. pseudospiralis* (strain code ISS 470) larvae were isolated from a mouse which was kindly provided by the EURL-P



## M&M

To compare the limit of detection of both MSM and the T-AAD kit:

Experiment I:

- 10 g minced pork spiked with 1, 3 or 10 *T. spiralis* muscle larvae (ML) free of capsule in five-fold (15 samples)
- 10 g minced pork spiked with 1, 3 or 10 *T. spiralis* encapsulated muscle larvae in five-fold (15 samples)
- 10 g minced pork spiked with 10 *T. spiralis* ML free of capsule muscle larvae (five-fold) as control for the digestion experiments using *T. spiralis* with capsule. (5 samples)

One series of 5 samples spiked with 10 free *T. spiralis* ML each, was used



# M&M

## Experiment II

15 samples consisting of 10 g minced pork spiked with 1, 3 or 10 *T. pseudospiralis* ML in 5-fold.

The protocol used for the production of proficiency test samples by the NRL for *Trichinella* (Germany) was followed (Johne et al., 2018).

For both methods, 10 g spiked pork samples were added to 90 g *Trichinella* negative pork and digested in 2 L volumes and the amount of residual, undigested meat from the sieve was weighed.



## M&M

Statistical analysis by Generalized Linear Modelling (GLM)

count  $\sim$  Binomial ( $p$ , spike),

Logit ( $p$ ) = method + meat.residue + species

$p=4.19 \times 10^{-14}$

$p=0.0645$

$p=9.54 \times 10^{-12}$

Model per species per spike level:

count  $\sim$  Binomial ( $p$ , spike),

logit( $p$ ) = method





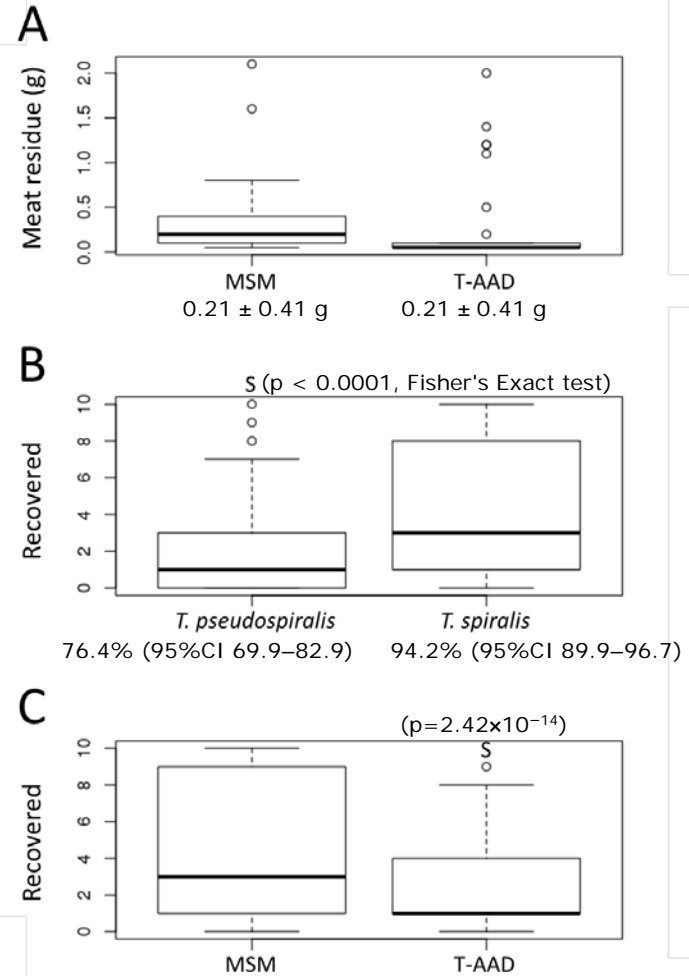
## Results – experimentally infected meat

	MSM	T-AAD	P-value
<i>T. spiralis</i>	88.0 ± 6.5 (n=5)	61.0 ± 15.3 (n=5)	p=0.0194*
<i>T. pseudospiralis</i>	440.2 ± 56.5 (n=5)	106.2 ± 49.3, n=5)	p=0.0001*

\* Paired t-Test



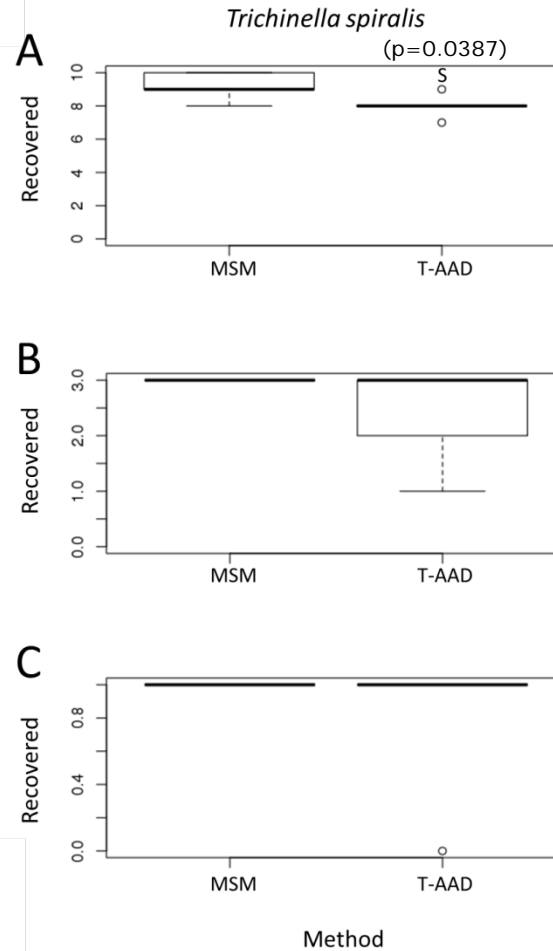
# Results – spiked samples





( $P=0.0024$ ) and

## Results – spiked samples





# Results

Performance of MSM and T-AAD using spiked pork samples.

Table 1.	test	n (samples)	Spiked <sup>a</sup>	Recovered <sup>a</sup>	Exact count	False neg.	% recovery	95%CI
Overall	MSM	50	260	235	34	2	90.4%	86.2–93.4
<i>T. spiralis</i> overall	MSM	35	190	179	25	0	94.2%	89.9–96.7
<i>T. spiralis</i> with capsule	MSM	15	70	67	12	0	95.7%	88.1–98.5
<i>T. spiralis</i> free larvae	MSM	20 <sup>b</sup>	120	112	13	0	93.3%	87.4–96.6
<i>T. pseudospiralis</i>	MSM	15	70	56	9	2	80.0%	69.2–87.7
Overall	T-AAD	45	210	121	15	10	57.6%	50.9–64.1
<i>T. spiralis</i> overall	T-AAD	30	140	107	15	1	76.4%	68.8–82.7
<i>T. spiralis</i> with capsule	T-AAD	15	70	51	8	0	72.9%	61.5–81.9
<i>T. spiralis</i> free larvae	T-AAD	15	70	56	7	1	80.0%	69.2–87.7
<i>T. pseudospiralis</i>	T-AAD	15	70	14	0	9	20.0%	12.3–30.8

Exact count: number of samples for which reported larval count was equal to spike. False neg.: number of samples that were reported false negative.

<sup>a</sup> Larval counts.

<sup>b</sup> One extra series of 5 samples spiked with 10 free *T. spiralis* ML each, was used as control for the digestion experiments using *T. spiralis* with capsule. Average and median for both series of 5 samples spiked with 10 free *Trichinella* larvae were equal at 9.2 and 9 larvae respectively.



## Discussion

- Konecsni et al., 2017\* (pig diaphragm samples spiked with 5 *T. spiralis* larvae), recovered on average  $74 \pm 10\%$  with T-AAD, and  $90\% \pm 11\%$  of spiked *Trichinella* larvae using MSM.  
(\* Veterinary Parasitology 243: 267–271)
- Gajadhar et al., 2018# (diaphragm, tongue, masseter, and loin spiked with 3 – 25 *T. spiralis* larvae) recovery at 3 larvae spike:  $\geq 86\%$  with T-AAD and  $> 80\%$  with MSM, at 4 and 5 larvae it was the opposite.  
(# Food and Waterborne Parasitology 10: 6-13)



## Discussion

- T-AAD test kit is comparable to the gold standard method for the qualitative detection of *T. spiralis*, but not for *T. pseudospiralis* in pigs.
- Accurate determination of the number of *Trichinella* larvae is not possible using the T-AAD test, which hampers on site quality control.
- Depending on the pass criteria of the proficiency test provider, the participants are at risk of failing due to shortcomings in the quantitative results.



## Take home message

- Usually, there is no prerequisite to validate a standard method which is used within the intended scope (ISO/IEC 17025:2017)
- We strongly recommend validating the T-AAD method on site prior to introduction into routine diagnostic laboratories.
- However, this will not alleviate the poor test sensitivity of the T-AAD for the detection of *T. pseudospiralis*.



# Acknowledgements



Annette Johne  
Karsten Nöckler  
Anne Mayer-Scholl



Joke van der Giessen

# QUESTIONS?