



**ADDENDUM TO THE REPORT ON THE VALIDATION OF
THE Trichin-L ANTIGEN TEST KIT OF THE BIO-RAD
COMPANY**

1. Introduction

To deeply investigate the specificity, sensibility and robustness of the Trichin-L Antigen Test Kit of the BIORAD Company, experiments were done at the EURLP in addition to those requested by the “Guidelines for the validation of apparatuses for the detection of *Trichinella* larvae in meat samples by digestion” (herein referred to as “the Guidelines”) approved by the DG SANCO.

2. Specificity

2.1 Specificity against different nematode and protozoan antigens

The specificity of the Trichin-L Antigen Test Kit was tested on nematodes belonging to four genera (*Ascaris*, *Trichuris*, *Toxocara*, *Anisakis*) since *Trichinella* share many antigens with other nematodes. Furthermore, the specificity of the Trichin-L Antigen Test Kit was tested with *Toxoplasma gondii*, a tissue protozoa. No cross reactions were observed. The results are shown in the ANNEX 1.

2.2 Specificity versus meat samples from slaughterhouses

Muscle samples of different weights from diaphragm pillars collected from backyard pigs in three slaughterhouses and tested as negative by digestion of 10 g of meat samples according to the method approved by the Commission regulation 2075/2005, were tested by the Trichin-L Antigen Test Kit. All the samples tested negative. The results are shown in the ANNEX 2.

3. Sensibility

3.1 Identification of larvae belonging to different *Trichinella* species

Meat ball samples were spiked with three or more *Trichinella* larvae, belonging to eight species of *Trichinella* recognised so far (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*). The Trichin-L Antigen Test Kit detected the larvae belonging to all the eight species. The results are shown in the ANNEX 3.

3.2 Identification of larvae present in undigested muscle tissues

Small pieces of mouse muscles of the size of a kernel, infected with 2-4 larvae of *Trichinella spiralis* were tested without digestion directly by the Trichin-L Antigen Test Kit. The results are shown in the ANNEX 4.

4. Robustness

4.1 Test of filters containing alive or dead larvae

Filters containing alive or dead *Trichinella spiralis* larvae after digestion, were stored at room temperature for different period of times (from 1 up to 10 h) and then they were tested by the Trichin-L Antigen Test Kit. The results are shown in the ANNEX 5.

ANNEX 1

Specificity against different nematode and protozoan antigens

Parasite	mg of crude antigens	Result
<i>Ascaris suum</i>	0.1	NEGATIVE
<i>Ascaris suum</i>	0.5	NEGATIVE
<i>Ascaris suum</i>	1.0	NEGATIVE
<i>Trichuris</i> sp.	0.1	NEGATIVE
<i>Trichuris</i> sp.	0.5	NEGATIVE
<i>Trichuris</i> sp.	1.0	NEGATIVE
<i>Toxocara canis</i>	0.1	NEGATIVE
<i>Toxocara canis</i>	0.5	NEGATIVE
<i>Toxocara canis</i>	1.0	NEGATIVE
<i>Anisakis pegreffii</i>	0.1	NEGATIVE
<i>Anisakis pegreffii</i>	0.5	NEGATIVE
<i>Anisakis pegreffii</i>	1.0	NEGATIVE
<i>Toxoplasma gondii</i>	0.1	NEGATIVE
<i>Toxoplasma gondii</i>	0.3	NEGATIVE
<i>Toxoplasma gondii</i>	0.5	NEGATIVE

ANNEX 2

Specificity versus meat samples from slaughterhouses

Meat weight (g)	Digestion result	BioRad assay results
83	<i>Trichinella</i> free	NEGATIVE
71	<i>Trichinella</i> free	NEGATIVE
68	<i>Trichinella</i> free	NEGATIVE
63	<i>Trichinella</i> free	NEGATIVE
98	<i>Trichinella</i> free	NEGATIVE
101	<i>Trichinella</i> free	NEGATIVE
72	<i>Trichinella</i> free	NEGATIVE
57	<i>Trichinella</i> free	NEGATIVE
67	<i>Trichinella</i> free	NEGATIVE
95	<i>Trichinella</i> free	NEGATIVE
60	<i>Trichinella</i> free	NEGATIVE
48	<i>Trichinella</i> free	NEGATIVE
90	<i>Trichinella</i> free	NEGATIVE
93	<i>Trichinella</i> free	NEGATIVE
99	<i>Trichinella</i> free	NEGATIVE
100	<i>Trichinella</i> free	NEGATIVE
104	<i>Trichinella</i> free	NEGATIVE
36	<i>Trichinella</i> free	NEGATIVE
88	<i>Trichinella</i> free	NEGATIVE
77	<i>Trichinella</i> free	NEGATIVE
100	<i>Trichinella</i> free	NEGATIVE
78	<i>Trichinella</i> free	NEGATIVE
100	<i>Trichinella</i> free	NEGATIVE
100	<i>Trichinella</i> free	NEGATIVE
93	<i>Trichinella</i> free	NEGATIVE
61	<i>Trichinella</i> free	NEGATIVE
90	<i>Trichinella</i> free	NEGATIVE
48	<i>Trichinella</i> free	NEGATIVE
90	<i>Trichinella</i> free	NEGATIVE
77	<i>Trichinella</i> free	NEGATIVE
102	<i>Trichinella</i> free	NEGATIVE
60	<i>Trichinella</i> free	NEGATIVE
80	<i>Trichinella</i> free	NEGATIVE
68	<i>Trichinella</i> free	NEGATIVE
100	<i>Trichinella</i> free	NEGATIVE
83	<i>Trichinella</i> free	NEGATIVE
70	<i>Trichinella</i> free	NEGATIVE

Meat weight (g)	Digestion result	BioRad assay results
100	<i>Trichinella free</i>	NEGATIVE
68	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE
63	<i>Trichinella free</i>	NEGATIVE
74	<i>Trichinella free</i>	NEGATIVE
102	<i>Trichinella free</i>	NEGATIVE
67	<i>Trichinella free</i>	NEGATIVE
88	<i>Trichinella free</i>	NEGATIVE
71	<i>Trichinella free</i>	NEGATIVE
98	<i>Trichinella free</i>	NEGATIVE
87	<i>Trichinella free</i>	NEGATIVE
93	<i>Trichinella free</i>	NEGATIVE
91	<i>Trichinella free</i>	NEGATIVE
83	<i>Trichinella free</i>	NEGATIVE
74	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE
109	<i>Trichinella free</i>	NEGATIVE
97	<i>Trichinella free</i>	NEGATIVE
98	<i>Trichinella free</i>	NEGATIVE
58	<i>Trichinella free</i>	NEGATIVE
64	<i>Trichinella free</i>	NEGATIVE
49	<i>Trichinella free</i>	NEGATIVE
53	<i>Trichinella free</i>	NEGATIVE
101	<i>Trichinella free</i>	NEGATIVE
95	<i>Trichinella free</i>	NEGATIVE
54	<i>Trichinella free</i>	NEGATIVE
63	<i>Trichinella free</i>	NEGATIVE
71	<i>Trichinella free</i>	NEGATIVE
68	<i>Trichinella free</i>	NEGATIVE
73	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE
99	<i>Trichinella free</i>	NEGATIVE
58	<i>Trichinella free</i>	NEGATIVE
61	<i>Trichinella free</i>	NEGATIVE
93	<i>Trichinella free</i>	NEGATIVE
89	<i>Trichinella free</i>	NEGATIVE
94	<i>Trichinella free</i>	NEGATIVE
97	<i>Trichinella free</i>	NEGATIVE
55	<i>Trichinella free</i>	NEGATIVE
63	<i>Trichinella free</i>	NEGATIVE

Meat weight (g)	Digestion result	BioRad assay results
95	<i>Trichinella free</i>	NEGATIVE
92	<i>Trichinella free</i>	NEGATIVE
90	<i>Trichinella free</i>	NEGATIVE
53	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE
72	<i>Trichinella free</i>	NEGATIVE
84	<i>Trichinella free</i>	NEGATIVE
90	<i>Trichinella free</i>	NEGATIVE
88	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE
66	<i>Trichinella free</i>	NEGATIVE
96	<i>Trichinella free</i>	NEGATIVE
100	<i>Trichinella free</i>	NEGATIVE

ANNEX 3

Identification of larvae belonging to different *Trichinella* species

Sample code	<i>Trichinella</i> species	n° larvae	Results
1	<i>T. spiralis</i>	5	positive
2	<i>T. spiralis</i>	5	positive
3	<i>T. spiralis</i>	5	positive
4	<i>T. spiralis</i>	5	positive
5	<i>T. spiralis</i>	5	positive
6	<i>T. spiralis</i>	5	positive
7	<i>T. spiralis</i>	5	positive
8	<i>T. spiralis</i>	5	positive
9	<i>T. spiralis</i>	3	positive
10	<i>T. spiralis</i>	3	positive
11	<i>T. spiralis</i>	3	positive
12	<i>T. spiralis</i>	3	positive
13	<i>T. spiralis</i>	3	positive
14	<i>T. spiralis</i>	3	positive
15	<i>T. spiralis</i>	3	positive
16	<i>T. spiralis</i>	3	positive
17	<i>T. spiralis</i>	3	positive
18	<i>T. spiralis</i>	1	positive
19	<i>T. spiralis</i>	1	positive
20	<i>T. spiralis</i>	1	positive
21	<i>T. spiralis</i>	1	positive
22	<i>T. spiralis</i>	1	positive
23	<i>T. spiralis</i>	1	positive
24	<i>T. spiralis</i>	1	NEGATIVE
25	<i>T. spiralis</i>	1	positive
26	<i>T. pseudospiralis</i>	5	positive
27	<i>T. pseudospiralis</i>	5	positive
28	<i>T. pseudospiralis</i>	5	positive
29	<i>T. pseudospiralis</i>	5	positive
30	<i>T. pseudospiralis</i>	5	positive
31	<i>T. pseudospiralis</i>	3	NEGATIVE
32	<i>T. pseudospiralis</i>	3	positive
33	<i>T. pseudospiralis</i>	3	positive
34	<i>T. pseudospiralis</i>	3	positive

Sample code	<i>Trichinella</i> species	n° larvae	Results
35	<i>T. pseudospiralis</i>	3	positive
36	<i>T. pseudospiralis</i>	3	positive
37	<i>T. pseudospiralis</i>	1	positive
38	<i>T. pseudospiralis</i>	1	NEGATIVE
39	<i>T. pseudospiralis</i>	1	positive
40	<i>T. pseudospiralis</i>	1	positive
41	<i>T. pseudospiralis</i>	1	positive
42	<i>T. britovi</i>	5	positive
43	<i>T. britovi</i>	5	positive
44	<i>T. britovi</i>	5	positive
45	<i>T. britovi</i>	5	positive
46	<i>T. britovi</i>	5	positive
47	<i>T. britovi</i>	3	positive
48	<i>T. britovi</i>	3	positive
49	<i>T. britovi</i>	3	positive
50	<i>T. britovi</i>	3	positive
51	<i>T. britovi</i>	3	positive
52	<i>T. britovi</i>	3	positive
53	<i>T. britovi</i>	3	positive
54	<i>T. britovi</i>	1	positive
55	<i>T. britovi</i>	1	positive
56	<i>T. britovi</i>	1	NEGATIVE
57	<i>T. britovi</i>	1	positive
58	<i>T. britovi</i>	1	positive
59	<i>T. britovi</i>	1	positive
60	<i>T. britovi</i>	1	positive
61	<i>T. spiralis</i>	1	positive
62	<i>T. spiralis</i>	1	NEGATIVE
63	<i>T. spiralis</i>	1	positive
64	<i>T. spiralis</i>	1	NEGATIVE
65	<i>T. spiralis</i>	1	positive
66	<i>T. spiralis</i>	3	positive
67	<i>T. spiralis</i>	3	positive
68	<i>T. spiralis</i>	3	positive
69	<i>T. spiralis</i>	3	positive
70	<i>T. spiralis</i>	3	positive
71	<i>T. nativa</i>	1	positive
72	<i>T. nativa</i>	1	positive
73	<i>T. nativa</i>	1	positive

Sample code	<i>Trichinella</i> species	n° larvae	Results
74	<i>T. nativa</i>	1	NEGATIVE
75	<i>T. nativa</i>	1	positive
76	<i>T. nativa</i>	1	positive
77	<i>T. nativa</i>	3	positive
78	<i>T. nativa</i>	3	positive
79	<i>T. nativa</i>	3	positive
80	<i>T. nativa</i>	3	positive
81	<i>T. nativa</i>	3	positive
82	<i>T. nativa</i>	5	positive
83	<i>T. nativa</i>	5	positive
84	<i>T. nativa</i>	5	positive
85	<i>T. nativa</i>	5	positive
86	<i>T. nativa</i>	5	positive
87	<i>T. murrelli</i>	1	positive
88	<i>T. murrelli</i>	1	NEGATIVE
89	<i>T. murrelli</i>	1	NEGATIVE
90	<i>T. murrelli</i>	1	positive
91	<i>T. murrelli</i>	1	positive
92	<i>T. murrelli</i>	1	positive
93	<i>T. murrelli</i>	3	positive
95	<i>T. murrelli</i>	3	positive
95	<i>T. murrelli</i>	3	positive
96	<i>T. murrelli</i>	3	positive
97	<i>T. murrelli</i>	3	positive
98	<i>T. murrelli</i>	5	positive
99	<i>T. murrelli</i>	5	positive
100	<i>T. murrelli</i>	5	positive
101	<i>T. murrelli</i>	5	positive
102	<i>T. murrelli</i>	5	positive
103	<i>T. papuae</i>	1	NEGATIVE
104	<i>T. papuae</i>	1	positive
105	<i>T. papuae</i>	1	positive
106	<i>T. papuae</i>	1	positive
107	<i>T. papuae</i>	1	positive
108	<i>T. papuae</i>	1	positive
109	<i>T. papuae</i>	3	positive
110	<i>T. papuae</i>	3	positive
111	<i>T. papuae</i>	3	positive
112	<i>T. papuae</i>	3	positive

Sample code	<i>Trichinella</i> species	n° larvae	Results
113	<i>T. papuae</i>	3	positive
114	<i>T. papuae</i>	5	positive
115	<i>T. papuae</i>	5	positive
116	<i>T. papuae</i>	5	positive
117	<i>T. papuae</i>	5	positive
118	<i>T. papuae</i>	5	positive
119	<i>T. nelsoni</i>	1	positive
120	<i>T. nelsoni</i>	1	NEGATIVE
121	<i>T. nelsoni</i>	1	positive
122	<i>T. nelsoni</i>	1	positive
123	<i>T. nelsoni</i>	1	positive
124	<i>T. nelsoni</i>	1	NEGATIVE
125	<i>T. nelsoni</i>	3	positive
126	<i>T. nelsoni</i>	3	positive
127	<i>T. nelsoni</i>	3	positive
128	<i>T. nelsoni</i>	3	positive
129	<i>T. nelsoni</i>	3	positive
130	<i>T. nelsoni</i>	5	positive
131	<i>T. nelsoni</i>	5	positive
132	<i>T. nelsoni</i>	5	positive
133	<i>T. nelsoni</i>	5	positive
134	<i>T. nelsoni</i>	5	positive
135	<i>T. zimbabwensis</i>	1	positive
136	<i>T. zimbabwensis</i>	1	positive
137	<i>T. zimbabwensis</i>	1	positive
138	<i>T. zimbabwensis</i>	1	positive
139	<i>T. zimbabwensis</i>	1	positive
140	<i>T. zimbabwensis</i>	1	positive
141	<i>T. zimbabwensis</i>	3	positive
142	<i>T. zimbabwensis</i>	3	positive
143	<i>T. zimbabwensis</i>	3	positive
144	<i>T. zimbabwensis</i>	3	positive
145	<i>T. zimbabwensis</i>	3	positive
146	<i>T. zimbabwensis</i>	5	positive
147	<i>T. zimbabwensis</i>	5	positive
148	<i>T. zimbabwensis</i>	5	positive
149	<i>T. zimbabwensis</i>	5	positive
150	<i>T. zimbabwensis</i>	5	positive

ANNEX 4

Identification of larvae present in undigested muscle tissues

Meat weight (mg)	N. of larvae detected by trichinoscope	BioRad assay results
125	2	positive
232	1	positive
178	4	positive
185	4	positive
153	2	positive
206	3	positive
147	2	positive
198	2	positive
214	3	positive
131	4	positive

ANNEX 5

Test of filters containing alive or dead larvae

N. of larvae on the filter	Larval status	Time (h) between digestion and agglutination test	BioRad assay results
3	alive	1	positive
2	dead	1	positive
3	alive	2	positive
3	dead	2	positive
3	alive	3	positive
3	dead	3	positive
4	alive	4	positive
3	dead	4	positive
3	alive	5	positive
2	dead	5	positive
3	alive	6	positive
3	dead	6	positive
4	alive	7	positive
5	dead	7	positive
2	alive	8	positive
2	dead	8	positive
3	alive	9	positive
3	dead	9	positive
2	alive	10	positive
3	dead	10	positive