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172-PT

PROFICIENCY TESTING 2016

AUJESZKY'S DISEASE VIRUS (ADV)

Detection of ADV gB- and gE-specific antibodies in serum by Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 27 JUNE 2016

DATE REPORT: 4 OCTOBER 2016

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of ADV gB- and/or ADV gE-specific antibodies in porcine serum by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be analyzed by means of an ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. ADV gB reference samples

Replicates of 6 reference serum samples of porcine origin, either free from detectable ADV gB-specific antibodies (n = 3; coded 'PT2016AUJELIgBNS1', 'PT2016AUJELIgBNS2', 'PT2016AUJELIgBNS3') or containing detectable ADV gB-specific antibodies (n = 3; coded 'PT2016AUJELIgBPS1', 'PT2016AUJELIgBPS2', 'PT2016AUJELIgBPS3'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2016AUJELIgBNS2, PT2016AUJELIgBNS3, PT2016AUJELIgBPS1, PT2016AUJELIgBPS3 and 4 aliquots of the reference serum samples PT2016AUJELIgBNS1 and PT2016AUJELIgBPS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 4).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification using the PrioCheck PRV gB antibody ELISA test from Prionics. The reference serum samples 'PT2016AUJELIgBNS1', 'PT2016AUJELIgBNS2', 'PT2016AUJELIgBNS3' were obtained from both uninfected and non-vaccinated animals. The reference serum sample PT2016AUJELIgBPS2 was obtained from an uninfected animal that was vaccinated twice with the live attenuated marker vaccine Suvaxyn Aujeszky 783+0/W (ADV attenuated NIA3-783 strain in mineral oil adjuvant). The reference serum sample PT2016AUJELIgBPS1 was a 1/32 dilution of a serum obtained from a naturally ADV-infected animal and the reference serum sample PT2016AUJELIgBPS3 was a 1/64 dilution of serum obtained from an experimentally ADV-infected animal.
Taken together, the reference serum samples 'PT2016AUJELIgBNS1', 'PT2016AUJELIgBNS2', 'PT2016AUJELIgBNS3' were considered as negative sera, and the reference serum samples 'PT2016AUJELIgBPS1', 'PT2016AUJELIgBPS2', 'PT2016AUJELIgBPS3' as positive sera in ADV gB ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the PrioCheck PRV gB antibody ELISA test from Prionics, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of ADV gB-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the PrioCheck PRV gB antibody ELISA test from Prionics.

III.2.2. ADV gE reference samples

Replicates of 6 reference serum samples of porcine origin, either free from detectable ADV gE-specific antibodies (n = 3; coded 'PT2016AUJELIgENS1', 'PT2016AUJELIgENS2', 'PT2016AUJELIgENS3') or containing detectable ADV gE-specific antibodies (n = 3; coded 'PT2016AUJELIgEPS1', 'PT2016AUJELIgEPS2', 'PT2016AUJELIgEPS3'), were used. In total, 180 aliquots were distributed to 9 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference serum samples PT2016AUJELIgENS2, and PT2016AUJELIgEPS3 and 4 aliquots of the reference serum samples PT2016AUJELIgENS1, PT2016AUJELIgENS3, PT2016AUJELIgEPS1 and PT2016AUJELIgEPS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 5).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification using the HerdChek PRV gpI antibody ELISA test from IDEXX. The reference serum samples 'PT2016AUJELIgENS2' and 'PT2016AUJELIgENS3' were obtained from both uninfected and non-vaccinated animals. Reference serum sample 'PT2016AUJELIgENS1' originated from an uninfected animal that was vaccinated twice with the live attenuated marker vaccine Suvaxyn Aujeszky 783+0/W (ADV attenuated NIA3-783 strain in mineral oil adjuvant). Reference serum samples 'PT2016AUJELIgEPS1' and 'PT2016AUJELIgEPS2' were respectively 4 and 32 fold dilutions of sera from 2 different naturally ADV infected animals. Reference serum sample 'PT2016AUJELIgEPS3' was a 16 fold dilution of serum from an experimentally ADV infected animal. Taken together, the reference serum samples 'PT2016AUJELIgENS1', 'PT2016AUJELIgENS2' and 'PT2016AUJELIgENS3' were considered as negative sera, whereas the reference serum samples 'PT2016AUJELIgEPS1', 'PT2016AUJELIgEPS2', 'PT2016AUJELIgEPS3' as positive sera in ADV gE ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek PRV gpI antibody ELISA test from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of ADV gE-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the HerdChek PRV gpI antibody ELISA test from IDEXX.

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference samples used for either the PT ADV gB or the PT ADV gE.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference samples is at least 95% for the PT ADV gB and 90% for the PT ADV gE.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the CODA-CERVA-Uccle.

IV.1. Transfer and start of the analyses of the reference samples

LAB3 until LAB8 participated in both the PT ADV gB and the PT ADV gE and hence received 40 aliquots of reference serum samples (20 for the PT ADV gB and 20 for the PT ADV gE). In contrast, LAB9 only participated in the PT ADV gE and received 20 aliquots of reference serum samples. LAB1 and LAB2 had been registered for both the PT ADV gB and the PT ADV gE but did'nt participate in the PT ADV gE.
The reference serum samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 27th of June 2016 (340 aliquots in total). LAB4, LAB5, LAB6 and LAB9 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 28th (LAB1, LAB2 and LAB3) or 29th (LAB7 and LAB8) of June 2016. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 28th of June and 14th of July 2016 (Table 1).

IV.2. Dates at which results were returned to the CODA-CERVA-Uccle

Results from the participating laboratories were submitted to the CODA-CERVA-Uccle between 30th of June and 15th of July 2016. All participants hereby respected the deadline of 15th of July 2016 for submission of the results (Table 1).

Table 1. Overview of the dates on which (i) the reference serum samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the CODA-CERVA-Uccle.

Participating laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	28/06/2016	29/06/206	NR	14/07/2016
LAB2	28/06/2016	07/07/2016	NR	11/07/2016
LAB3	28/06/2016	29/06/2016	01/07/2016	06/07/2016
LAB4	27/06/2016	29/06/2016	07/07/2016	12/07/2016
LAB5	27/06/2016	28/06/2016	28/06/2016	30/06/2016
LAB6	27/06/2016	05/07/2016	29/06/2016	07/07/2016
LAB7	29/06/2016	14/07/2016	12/07/2016	15/07/2016
LAB8	29/06/2016	30/06/2016	30/06/2016	04/07/2016
LAB9	27/06/2016	NA	01/07/2016	06/07/2016

Legend: NA = not applicable; NR = no results

IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results except LAB1.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that:

- **(i)** For the detection of **ADV gB-specific antibodies**, 7 out of 8 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence obtained 100% of agreement (Table 2). Only LAB2 has misidentified 3 samples and hence obtained 85% of agreement.
- **(ii)** For the detection of **ADV gE-specific antibodies**, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence obtained 100% of agreement. (Table 3).

Table 2. Agreement between results obtained by the participating laboratories (LABNR) and the status of the **ADV gB** reference serum samples assigned by the Aujeszky reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of ADV gB reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
failure	0 (0.0)	3 (15.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	17 (85.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. Agreement between results obtained by the participating laboratories (LABNR) and the status of the **ADV gE** reference serum samples assigned by the Aujeszky reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of ADV gE reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	3	**4**	**5**	**6**	**7**	**8**	**9**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

IV.4.2. Variability among participating laboratories

For the detection of ADV gB-specific antibodies, LAB2 misclassified 3 aliquots in total: 2 aliquots of the reference serum PT2016AUJELIgBNS1 were reported as positive instead of negative and 1 aliquot of reference serum PT2016AUJELIgBPS3 was reported as negative instead of positive.

Since all participating laboratories reached 100% of agreement for the detection of both ADV gE antibodies in reference serum samples, no variability between qualitative laboratory results could be observed.

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 4 for the PT ADV gB and in Table 5 for the PT ADV gE.

Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **ADV gB** reference serum samples (SAMPLE), the positions of the ADV gB reference serum samples as placed in the block (LABPOSIT), and the status assigned by the Aujeszky reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016AUJELIgBPS2	POS	POS	1
2	1	2	PT2016AUJELIgBNS3	NEG	NEG	1
3	1	3	PT2016AUJELIgBNS2	NEG	NEG	1
4	1	4	PT2016AUJELIgBNS1	NEG	NEG	1
5	1	5	PT2016AUJELIgBPS1	POS	POS	1
6	1	6	PT2016AUJELIgBPS2	POS	POS	1
7	1	7	PT2016AUJELIgBNS1	NEG	NEG	1
8	1	8	PT2016AUJELIgBNS3	NEG	NEG	1
9	1	9	PT2016AUJELIgBNS2	NEG	NEG	1
10	1	10	PT2016AUJELIgBPS2	POS	POS	1
11	1	11	PT2016AUJELIgBNS1	NEG	NEG	1
12	1	12	PT2016AUJELIgBPS3	POS	POS	1
13	1	13	PT2016AUJELIgBPS1	POS	POS	1
14	1	14	PT2016AUJELIgBNS3	NEG	NEG	1
15	1	15	PT2016AUJELIgBPS3	POS	POS	1
16	1	16	PT2016AUJELIgBPS2	POS	POS	1
17	1	17	PT2016AUJELIgBPS3	POS	POS	1
18	1	18	PT2016AUJELIgBPS1	POS	POS	1
19	1	19	PT2016AUJELIgBNS2	NEG	NEG	1
20	1	20	PT2016AUJELIgBNS1	NEG	NEG	1
21	2	1	PT2016AUJELIgBNS1	NEG	NEG	1
22	2	2	PT2016AUJELIgBNS2	NEG	NEG	1
23	2	3	PT2016AUJELIgBNS3	NEG	NEG	1
24	2	4	PT2016AUJELIgBPS1	POS	POS	1
25	2	5	PT2016AUJELIgBPS2	POS	POS	1
26	2	6	PT2016AUJELIgBPS3	POS	POS	1
27	2	7	PT2016AUJELIgBNS1	**NEG**	**POS**	**0**
28	2	8	PT2016AUJELIgBPS1	POS	POS	1
29	2	9	PT2016AUJELIgBPS2	POS	POS	1
30	2	10	PT2016AUJELIgBNS3	NEG	NEG	1
31	2	11	PT2016AUJELIgBNS2	NEG	NEG	1
32	2	12	PT2016AUJELIgBPS3	**POS**	**NEG**	**0**
33	2	13	PT2016AUJELIgBNS1	**NEG**	**POS**	**0**
34	2	14	PT2016AUJELIgBPS1	POS	POS	1
35	2	15	PT2016AUJELIgBPS2	POS	POS	1
36	2	16	PT2016AUJELIgBPS3	POS	POS	1
37	2	17	PT2016AUJELIgBPS2	POS	POS	1
38	2	18	PT2016AUJELIgBNS1	NEG	NEG	1
39	2	19	PT2016AUJELIgBNS2	NEG	NEG	1
40	2	20	PT2016AUJELIgBNS3	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2016AUJELIgBPS2	POS	POS	1
42	3	2	PT2016AUJELIgBNS3	NEG	NEG	1
43	3	3	PT2016AUJELIgBNS2	NEG	NEG	1
44	3	4	PT2016AUJELIgBNS1	NEG	NEG	1
45	3	5	PT2016AUJELIgBPS1	POS	POS	1
46	3	6	PT2016AUJELIgBPS2	POS	POS	1
47	3	7	PT2016AUJELIgBNS1	NEG	NEG	1
48	3	8	PT2016AUJELIgBNS3	NEG	NEG	1
49	3	9	PT2016AUJELIgBNS2	NEG	NEG	1
50	3	10	PT2016AUJELIgBPS2	POS	POS	1
51	3	11	PT2016AUJELIgBNS1	NEG	NEG	1
52	3	12	PT2016AUJELIgBPS3	POS	POS	1
53	3	13	PT2016AUJELIgBPS1	POS	POS	1
54	3	14	PT2016AUJELIgBNS3	NEG	NEG	1
55	3	15	PT2016AUJELIgBPS3	POS	POS	1
56	3	16	PT2016AUJELIgBPS2	POS	POS	1
57	3	17	PT2016AUJELIgBPS3	POS	POS	1
58	3	18	PT2016AUJELIgBPS1	POS	POS	1
59	3	19	PT2016AUJELIgBNS2	NEG	NEG	1
60	3	20	PT2016AUJELIgBNS1	NEG	NEG	1
61	4	1	PT2016AUJELIgBNS1	NEG	NEG	1
62	4	2	PT2016AUJELIgBNS2	NEG	NEG	1
63	4	3	PT2016AUJELIgBNS3	NEG	NEG	1
64	4	4	PT2016AUJELIgBPS1	POS	POS	1
65	4	5	PT2016AUJELIgBPS2	POS	POS	1
66	4	6	PT2016AUJELIgBPS3	POS	POS	1
67	4	7	PT2016AUJELIgBNS1	NEG	NEG	1
68	4	8	PT2016AUJELIgBPS1	POS	POS	1
69	4	9	PT2016AUJELIgBPS2	POS	POS	1
70	4	10	PT2016AUJELIgBNS3	NEG	NEG	1
71	4	11	PT2016AUJELIgBNS2	NEG	NEG	1
72	4	12	PT2016AUJELIgBPS3	POS	POS	1
73	4	13	PT2016AUJELIgBNS1	NEG	NEG	1
74	4	14	PT2016AUJELIgBPS1	POS	POS	1
75	4	15	PT2016AUJELIgBPS2	POS	POS	1
76	4	16	PT2016AUJELIgBPS3	POS	POS	1
77	4	17	PT2016AUJELIgBPS2	POS	POS	1
78	4	18	PT2016AUJELIgBNS1	NEG	NEG	1
79	4	19	PT2016AUJELIgBNS2	NEG	NEG	1
80	4	20	PT2016AUJELIgBNS3	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2016AUJELIgBPS2	POS	POS	1
82	5	2	PT2016AUJELIgBNS3	NEG	NEG	1
83	5	3	PT2016AUJELIgBNS2	NEG	NEG	1
84	5	4	PT2016AUJELIgBNS1	NEG	NEG	1
85	5	5	PT2016AUJELIgBPS1	POS	POS	1
86	5	6	PT2016AUJELIgBPS2	POS	POS	1
87	5	7	PT2016AUJELIgBNS1	NEG	NEG	1
88	5	8	PT2016AUJELIgBNS3	NEG	NEG	1
89	5	9	PT2016AUJELIgBNS2	NEG	NEG	1
90	5	10	PT2016AUJELIgBPS2	POS	POS	1
91	5	11	PT2016AUJELIgBNS1	NEG	NEG	1
92	5	12	PT2016AUJELIgBPS3	POS	POS	1
93	5	13	PT2016AUJELIgBPS1	POS	POS	1
94	5	14	PT2016AUJELIgBNS3	NEG	NEG	1
95	5	15	PT2016AUJELIgBPS3	POS	POS	1
96	5	16	PT2016AUJELIgBPS2	POS	POS	1
97	5	17	PT2016AUJELIgBPS3	POS	POS	1
98	5	18	PT2016AUJELIgBPS1	POS	POS	1
99	5	19	PT2016AUJELIgBNS2	NEG	NEG	1
100	5	20	PT2016AUJELIgBNS1	NEG	NEG	1
101	6	1	PT2016AUJELIgBNS1	NEG	NEG	1
102	6	2	PT2016AUJELIgBNS2	NEG	NEG	1
103	6	3	PT2016AUJELIgBNS3	NEG	NEG	1
104	6	4	PT2016AUJELIgBPS1	POS	POS	1
105	6	5	PT2016AUJELIgBPS2	POS	POS	1
106	6	6	PT2016AUJELIgBPS3	POS	POS	1
107	6	7	PT2016AUJELIgBNS1	NEG	NEG	1
108	6	8	PT2016AUJELIgBPS1	POS	POS	1
109	6	9	PT2016AUJELIgBPS2	POS	POS	1
110	6	10	PT2016AUJELIgBNS3	NEG	NEG	1
111	6	11	PT2016AUJELIgBNS2	NEG	NEG	1
112	6	12	PT2016AUJELIgBPS3	POS	POS	1
113	6	13	PT2016AUJELIgBNS1	NEG	NEG	1
114	6	14	PT2016AUJELIgBPS1	POS	POS	1
115	6	15	PT2016AUJELIgBPS2	POS	POS	1
116	6	16	PT2016AUJELIgBPS3	POS	POS	1
117	6	17	PT2016AUJELIgBPS2	POS	POS	1
118	6	18	PT2016AUJELIgBNS1	NEG	NEG	1
119	6	19	PT2016AUJELIgBNS2	NEG	NEG	1
120	6	20	PT2016AUJELIgBNS3	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2016AUJELIgBPS2	POS	POS	1
122	7	2	PT2016AUJELIgBNS3	NEG	NEG	1
123	7	3	PT2016AUJELIgBNS2	NEG	NEG	1
124	7	4	PT2016AUJELIgBNS1	NEG	NEG	1
125	7	5	PT2016AUJELIgBPS1	POS	POS	1
126	7	6	PT2016AUJELIgBPS2	POS	POS	1
127	7	7	PT2016AUJELIgBNS1	NEG	NEG	1
128	7	8	PT2016AUJELIgBNS3	NEG	NEG	1
129	7	9	PT2016AUJELIgBNS2	NEG	NEG	1
130	7	10	PT2016AUJELIgBPS2	POS	POS	1
131	7	11	PT2016AUJELIgBNS1	NEG	NEG	1
132	7	12	PT2016AUJELIgBPS3	POS	POS	1
133	7	13	PT2016AUJELIgBPS1	POS	POS	1
134	7	14	PT2016AUJELIgBNS3	NEG	NEG	1
135	7	15	PT2016AUJELIgBPS3	POS	POS	1
136	7	16	PT2016AUJELIgBPS2	POS	POS	1
137	7	17	PT2016AUJELIgBPS3	POS	POS	1
138	7	18	PT2016AUJELIgBPS1	POS	POS	1
139	7	19	PT2016AUJELIgBNS2	NEG	NEG	1
140	7	20	PT2016AUJELIgBNS1	NEG	NEG	1
141	8	1	PT2016AUJELIgBNS1	NEG	NEG	1
142	8	2	PT2016AUJELIgBNS2	NEG	NEG	1
143	8	3	PT2016AUJELIgBNS3	NEG	NEG	1
144	8	4	PT2016AUJELIgBPS1	POS	POS	1
145	8	5	PT2016AUJELIgBPS2	POS	POS	1
146	8	6	PT2016AUJELIgBPS3	POS	POS	1
147	8	7	PT2016AUJELIgBNS1	NEG	NEG	1
148	8	8	PT2016AUJELIgBPS1	POS	POS	1
149	8	9	PT2016AUJELIgBPS2	POS	POS	1
150	8	10	PT2016AUJELIgBNS3	NEG	NEG	1
151	8	11	PT2016AUJELIgBNS2	NEG	NEG	1
152	8	12	PT2016AUJELIgBPS3	POS	POS	1
153	8	13	PT2016AUJELIgBNS1	NEG	NEG	1
154	8	14	PT2016AUJELIgBPS1	POS	POS	1
155	8	15	PT2016AUJELIgBPS2	POS	POS	1
156	8	16	PT2016AUJELIgBPS3	POS	POS	1
157	8	17	PT2016AUJELIgBPS2	POS	POS	1
158	8	18	PT2016AUJELIgBNS1	NEG	NEG	1
159	8	19	PT2016AUJELIgBNS2	NEG	NEG	1
160	8	20	PT2016AUJELIgBNS3	NEG	NEG	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **ADV gE** reference serum samples (SAMPLE), the positions of the ADV gE reference serum samples as placed in the block (LABPOSIT), and the status assigned by the Aujeszky reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	3	1	PT2016AUJELIgENS1	NEG	NEG	1
2	3	2	PT2016AUJELIgEPS1	POS	POS	1
3	3	3	PT2016AUJELIgENS3	NEG	NEG	1
4	3	4	PT2016AUJELIgEPS3	POS	POS	1
5	3	5	PT2016AUJELIgENS2	NEG	NEG	1
6	3	6	PT2016AUJELIgEPS2	POS	POS	1
7	3	7	PT2016AUJELIgEPS3	POS	POS	1
8	3	8	PT2016AUJELIgEPS2	POS	POS	1
9	3	9	PT2016AUJELIgEPS1	POS	POS	1
10	3	10	PT2016AUJELIgENS1	NEG	NEG	1
11	3	11	PT2016AUJELIgENS3	NEG	NEG	1
12	3	12	PT2016AUJELIgEPS2	POS	POS	1
13	3	13	PT2016AUJELIgEPS1	POS	POS	1
14	3	14	PT2016AUJELIgENS2	NEG	NEG	1
15	3	15	PT2016AUJELIgENS3	NEG	NEG	1
16	3	16	PT2016AUJELIgEPS2	POS	POS	1
17	3	17	PT2016AUJELIgENS1	NEG	NEG	1
18	3	18	PT2016AUJELIgEPS1	POS	POS	1
19	3	19	PT2016AUJELIgENS1	NEG	NEG	1
20	3	20	PT2016AUJELIgENS3	NEG	NEG	1
21	4	1	PT2016AUJELIgENS2	NEG	NEG	1
22	4	2	PT2016AUJELIgEPS2	POS	POS	1
23	4	3	PT2016AUJELIgENS1	NEG	NEG	1
24	4	4	PT2016AUJELIgENS3	NEG	NEG	1
25	4	5	PT2016AUJELIgEPS3	POS	POS	1
26	4	6	PT2016AUJELIgEPS1	POS	POS	1
27	4	7	PT2016AUJELIgENS1	NEG	NEG	1
28	4	8	PT2016AUJELIgENS3	NEG	NEG	1
29	4	9	PT2016AUJELIgEPS1	POS	POS	1
30	4	10	PT2016AUJELIgEPS2	POS	POS	1
31	4	11	PT2016AUJELIgEPS3	POS	POS	1
32	4	12	PT2016AUJELIgENS3	NEG	NEG	1
33	4	13	PT2016AUJELIgEPS2	POS	POS	1
34	4	14	PT2016AUJELIgENS1	NEG	NEG	1
35	4	15	PT2016AUJELIgEPS2	POS	POS	1
36	4	16	PT2016AUJELIgENS2	NEG	NEG	1
37	4	17	PT2016AUJELIgEPS1	POS	POS	1
38	4	18	PT2016AUJELIgENS1	NEG	NEG	1
39	4	19	PT2016AUJELIgEPS1	POS	POS	1
40	4	20	PT2016AUJELIgENS3	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2016AUJELIgENS1	NEG	NEG	1
42	5	2	PT2016AUJELIgEPS1	POS	POS	1
43	5	3	PT2016AUJELIgENS3	NEG	NEG	1
44	5	4	PT2016AUJELIgEPS3	POS	POS	1
45	5	5	PT2016AUJELIgENS2	NEG	NEG	1
46	5	6	PT2016AUJELIgEPS2	POS	POS	1
47	5	7	PT2016AUJELIgEPS3	POS	POS	1
48	5	8	PT2016AUJELIgEPS2	POS	POS	1
49	5	9	PT2016AUJELIgEPS1	POS	POS	1
50	5	10	PT2016AUJELIgENS1	NEG	NEG	1
51	5	11	PT2016AUJELIgENS3	NEG	NEG	1
52	5	12	PT2016AUJELIgEPS2	POS	POS	1
53	5	13	PT2016AUJELIgEPS1	POS	POS	1
54	5	14	PT2016AUJELIgENS2	NEG	NEG	1
55	5	15	PT2016AUJELIgENS3	NEG	NEG	1
56	5	16	PT2016AUJELIgEPS2	POS	POS	1
57	5	17	PT2016AUJELIgENS1	NEG	NEG	1
58	5	18	PT2016AUJELIgEPS1	POS	POS	1
59	5	19	PT2016AUJELIgENS1	NEG	NEG	1
60	5	20	PT2016AUJELIgENS3	NEG	NEG	1
61	6	1	PT2016AUJELIgENS2	NEG	NEG	1
62	6	2	PT2016AUJELIgEPS2	POS	POS	1
63	6	3	PT2016AUJELIgENS1	NEG	NEG	1
64	6	4	PT2016AUJELIgENS3	NEG	NEG	1
65	6	5	PT2016AUJELIgEPS3	POS	POS	1
66	6	6	PT2016AUJELIgEPS1	POS	POS	1
67	6	7	PT2016AUJELIgENS1	NEG	NEG	1
68	6	8	PT2016AUJELIgENS3	NEG	NEG	1
69	6	9	PT2016AUJELIgEPS1	POS	POS	1
70	6	10	PT2016AUJELIgEPS2	POS	POS	1
71	6	11	PT2016AUJELIgEPS3	POS	POS	1
72	6	12	PT2016AUJELIgENS3	NEG	NEG	1
73	6	13	PT2016AUJELIgEPS2	POS	POS	1
74	6	14	PT2016AUJELIgENS1	NEG	NEG	1
75	6	15	PT2016AUJELIgEPS2	POS	POS	1
76	6	16	PT2016AUJELIgENS2	NEG	NEG	1
77	6	17	PT2016AUJELIgEPS1	POS	POS	1
78	6	18	PT2016AUJELIgENS1	NEG	NEG	1
79	6	19	PT2016AUJELIgEPS1	POS	POS	1
80	6	20	PT2016AUJELIgENS3	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	7	1	PT2016AUJELIgENS1	NEG	NEG	1
82	7	2	PT2016AUJELIgEPS1	POS	POS	1
83	7	3	PT2016AUJELIgENS3	NEG	NEG	1
84	7	4	PT2016AUJELIgEPS3	POS	POS	1
85	7	5	PT2016AUJELIgENS2	NEG	NEG	1
86	7	6	PT2016AUJELIgEPS2	POS	POS	1
87	7	7	PT2016AUJELIgEPS3	POS	POS	1
88	7	8	PT2016AUJELIgEPS2	POS	POS	1
89	7	9	PT2016AUJELIgEPS1	POS	POS	1
90	7	10	PT2016AUJELIgENS1	NEG	NEG	1
91	7	11	PT2016AUJELIgENS3	NEG	NEG	1
92	7	12	PT2016AUJELIgEPS2	POS	POS	1
93	7	13	PT2016AUJELIgEPS1	POS	POS	1
94	7	14	PT2016AUJELIgENS2	NEG	NEG	1
95	7	15	PT2016AUJELIgENS3	NEG	NEG	1
96	7	16	PT2016AUJELIgEPS2	POS	POS	1
97	7	17	PT2016AUJELIgENS1	NEG	NEG	1
98	7	18	PT2016AUJELIgEPS1	POS	POS	1
99	7	19	PT2016AUJELIgENS1	NEG	NEG	1
100	7	20	PT2016AUJELIgENS3	NEG	NEG	1
101	8	1	PT2016AUJELIgENS2	NEG	NEG	1
102	8	2	PT2016AUJELIgEPS2	POS	POS	1
103	8	3	PT2016AUJELIgENS1	NEG	NEG	1
104	8	4	PT2016AUJELIgENS3	NEG	NEG	1
105	8	5	PT2016AUJELIgEPS3	POS	POS	1
106	8	6	PT2016AUJELIgEPS1	POS	POS	1
107	8	7	PT2016AUJELIgENS1	NEG	NEG	1
108	8	8	PT2016AUJELIgENS3	NEG	NEG	1
109	8	9	PT2016AUJELIgEPS1	POS	POS	1
110	8	10	PT2016AUJELIgEPS2	POS	POS	1
111	8	11	PT2016AUJELIgEPS3	POS	POS	1
112	8	12	PT2016AUJELIgENS3	NEG	NEG	1
113	8	13	PT2016AUJELIgEPS2	POS	POS	1
114	8	14	PT2016AUJELIgENS1	NEG	NEG	1
115	8	15	PT2016AUJELIgEPS2	POS	POS	1
116	8	16	PT2016AUJELIgENS2	NEG	NEG	1
117	8	17	PT2016AUJELIgEPS1	POS	POS	1
118	8	18	PT2016AUJELIgENS1	NEG	NEG	1
119	8	19	PT2016AUJELIgEPS1	POS	POS	1
120	8	20	PT2016AUJELIgENS3	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	9	1	PT2016AUJELIgENS1	NEG	NEG	1
122	9	2	PT2016AUJELIgEPS1	POS	POS	1
123	9	3	PT2016AUJELIgENS3	NEG	NEG	1
124	9	4	PT2016AUJELIgEPS3	POS	POS	1
125	9	5	PT2016AUJELIgENS2	NEG	NEG	1
126	9	6	PT2016AUJELIgEPS2	POS	POS	1
127	9	7	PT2016AUJELIgEPS3	POS	POS	1
128	9	8	PT2016AUJELIgEPS2	POS	POS	1
129	9	9	PT2016AUJELIgEPS1	POS	POS	1
130	9	10	PT2016AUJELIgENS1	NEG	NEG	1
131	9	11	PT2016AUJELIgENS3	NEG	NEG	1
132	9	12	PT2016AUJELIgEPS2	POS	POS	1
133	9	13	PT2016AUJELIgEPS1	POS	POS	1
134	9	14	PT2016AUJELIgENS2	NEG	NEG	1
135	9	15	PT2016AUJELIgENS3	NEG	NEG	1
136	9	16	PT2016AUJELIgEPS2	POS	POS	1
137	9	17	PT2016AUJELIgENS1	NEG	NEG	1
138	9	18	PT2016AUJELIgEPS1	POS	POS	1
139	9	19	PT2016AUJELIgENS1	NEG	NEG	1
140	9	20	PT2016AUJELIgENS3	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess performances of the participating laboratories when analyzing reference serum samples of porcine origin for the detection of ADV gB- and/or ADV gE-specific antibodies by ELISA.

For the detection of ADV gB-specific antibodies in reference serum samples, 7 out of 8 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB2 misclassified 3 aliquots: 2 aliquots (out of 4) of the reference serum sample PT2016AUJELIgBNS1 and 1 aliquot (out of 3) of the reference serum sample PT2016AUJELIgBPS3. Hereby, LAB2 only obtained 85% of agreement with the assigned status of the reference serum samples.

LAB1, LAB2, LAB3, LAB4, LAB5, LAB6, and LAB8 used ADV gB antibody ELISA kits from 3 different commercial producers. LAB7 used a home-made ADV gB antibody ELISA kits (batch 15/3/16). Also different batches from the same ELISA kit were used. Hereby, Biochek batch FS6272 (LAB2) and batch FS6397 (LAB1), Idexx batch DL520 (LAB3) and batch CL393 (LAB8), Prionics batch Z150201L (LAB4, LAB5 and LAB6).
In addition, LAB8 and LAB7 performed a long incubation protocol, whereas LAB2, LAB3, LAB4, LAB5 and LAB6 used a short incubation protocol (LAB1 did not provide information about the used incubation protocol).

For the detection of ADV gE-specific antibodies in reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement).

All participants, except LAB7, used the ADV gE antibody ELISA kit from the same commercial kit producer. Hereby, different batches from the same ELISA kit were used: IDEXX 5 batches: LL537 (LAB3), CL468 (LAB4 and LAB5), AM779 (LAB6), CL472 (LAB8) and FL837(LAB9). LAB7 used a home-made ADV gE antibody ELISA (batch 30/6/16). Furthermore, LAB8 and LAB7 performed a long incubation protocol, whereas LAB3, LAB4, LAB5 and LAB9 used a short incubation protocol (LAB6 did not provide information about the used incubation protocol).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 95% (PT ADV gB) or at least 90% (PT ADV gE) of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the Aujeszky reference laboratory of CODA-CERVA-Uccle (see III.3.3.). Consequently, 7 out 8 participants to the PT ADV gB achieved a satisfactory performance for the detection of ADV gB-specific antibodies in porcine serum samples. LAB2 did not achieve a satisfactory performance for the detection of ADV gB-specific antibodies in serum samples.
For the PT ADVgE , all laboratories achieved a satisfactory performance for the detection of ADV gE-specific antibodies in porcine serum samples.

Coordinator proficiency tests
Katia Knapen

Appendix

Names of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (Ploufragan-Plouzané, France)

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)

BioChek Netherlands (Reeuwijk, The Netherlands)

Bio-Chek UK (Hounslow, United Kingdom)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE) (Maisons-Alfort, France)

State Veterinary and Food Institute, Veterinyry Institute (Zvolen, Slovakia)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Uccle, Belgium)

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software R. All quantitative data analyses were performed on normalized data, namely the percentages blocking calculated according to the instructions of the PT provider: [1- (OD_{Sample} / mean $OD_{Negative\ Kit\ Controls}$)] x 100.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

Box plots of the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory were made using the statistical software R. The box plots for the (sub)laboratories participating in the PT ADV gB and the PT ADV gE are shown in Figure 1 and Figure 2, respectively.

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25)) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

Figure 1 (Detection of ADV gB-specific antibodies in serum by ELISA)

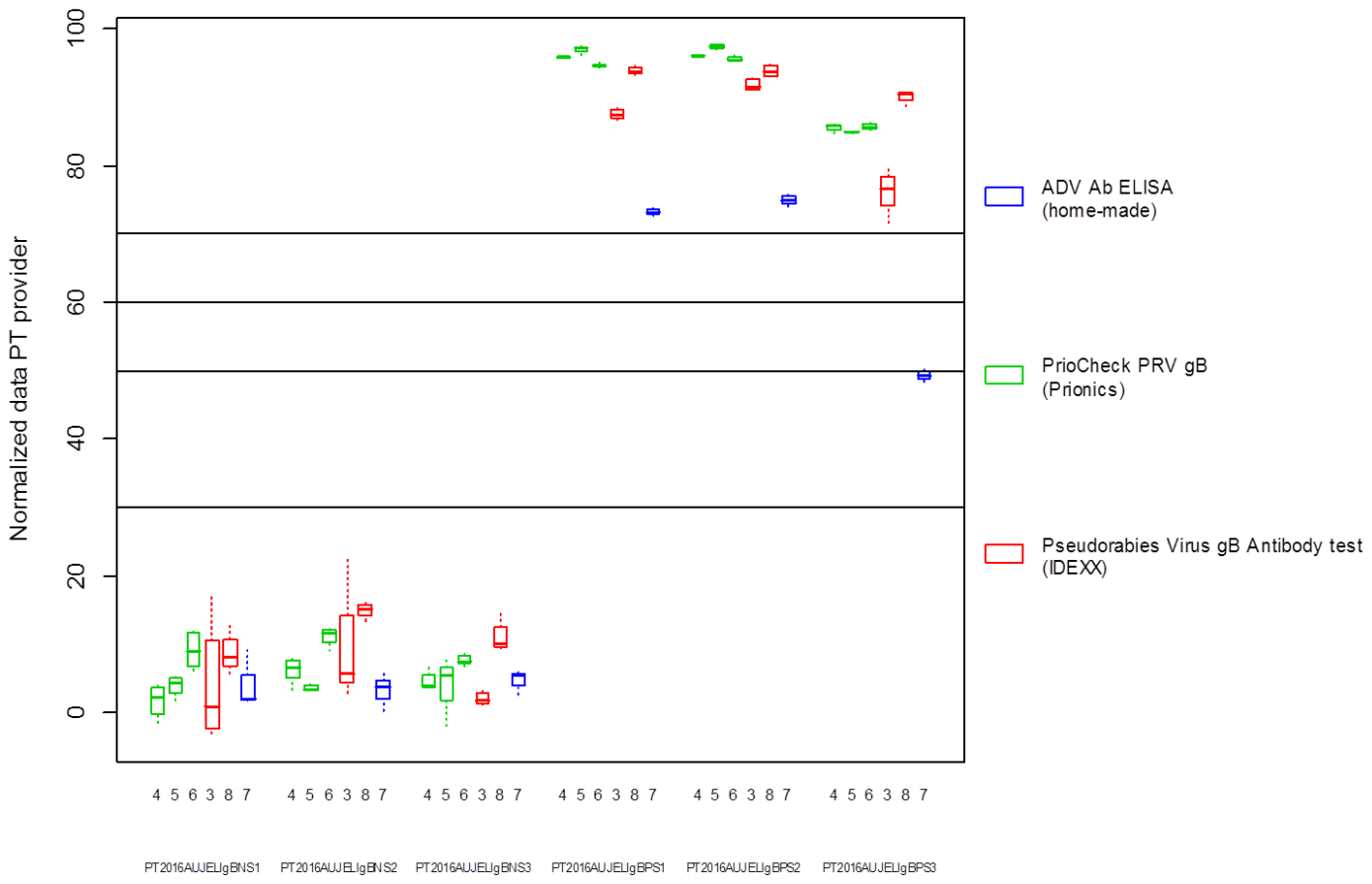

Figure 1. Box plots showing the percentage blocking per ADV gB reference serum sample and per participating (sub)laboratory. LAB3, LAB4, LAB5, LAB6, and LAB8 used ADV gB antibody ELISA kits from 2 different commercial producers. LAB7 used a home-made ADV gB antibody ELISA kits (batch 15/3/16). Also different batches from the same ELISA kit were used. Hereby, Idexx batch DL520 (LAB3) and batch CL393 (LAB8), Prionics batch Z150201L (LAB4, LAB5 and LAB6). In addition, LAB8 and LAB7 performed a long incubation protocol, whereas LAB3, LAB4, LAB5 and LAB6 used a short incubation protocol.Cut-off values for

the ADV gB antibody ELISA kit from IDEXX (70 or 60-50%), Prionics (50%) and the home-made ADV gB antibody ELISA kit (30-50%) are shown by horizontal lines.

Comments :

- To normalized the data the PT provider used to calculate the percentages blocking the formula [1- (ODSample / mean OD Negative Kit Controls)] x 100. This formula did not allow to take into accound the data provided by LAB1 and LAB2.

- The figure with the normalized data shows that LAB7 obtained somewhat lower values for the 3 positive samples than the other laboratories and that one sample (PT2016AUJELIgBPS3) entered the doubtful range. LAB7 however identified this sample correctly as positive by using their own calculation method.

Figure 2 (Detection of ADV gE-specific antibodies in serum by ELISA)

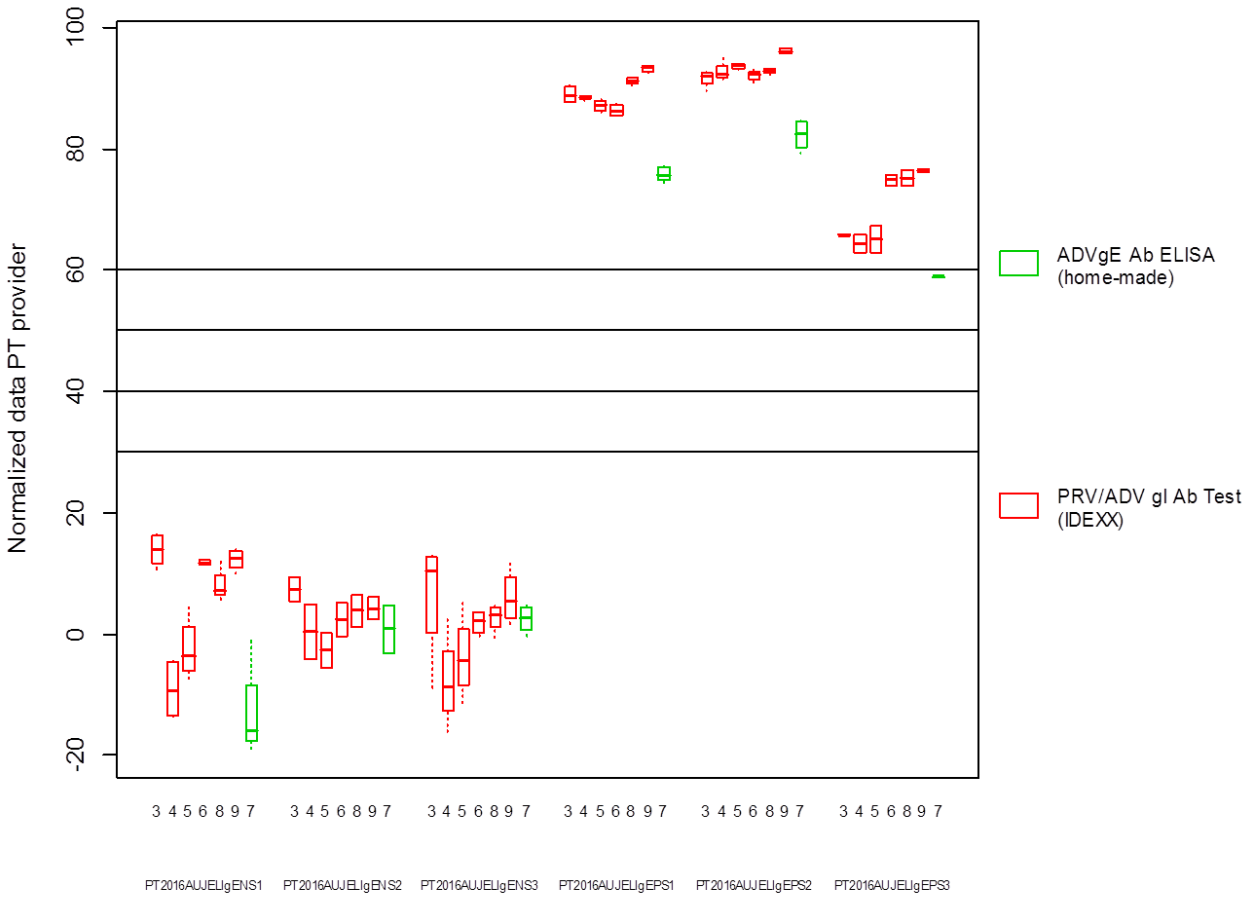

Figure 2. Box plots showing the percentage blocking per ADV gE reference serum sample and per participating laboratory. All participants, except LAB7, used the ADV gE antibody ELISA kit from the same commercial kit producer. Hereby, different batches from the same ELISA kit were used: IDEXX 5 batches: LL537 (LAB3), CL468 (LAB4 and LAB5), AM779 (LAB6), CL472 (LAB8) and FL837(LAB9). LAB7 used a home-made ADV gE antibody ELISA (batch 30/6/16). Furthermore, LAB8 and LAB7 performed a long incubation protocol, whereas LAB3, LAB4, LAB5 and LAB9 used a short incubation protocol (LAB6 did not provide information about the used incubation protocol). Cut-off values for the ADV gE antibody ELISA kit from IDEXX (30-40%) and the home-made ADV gE antibody ELISA kit (50-60%) are shown by horizontal lines.

Comment : *The figure with the normalized data shows that LAB7 obtained somewhat lower values for the 3 positive samples than the other laboratories and that one sample (PT2016AUJELIgEPS3) entered the doubtful range. LAB7 however identified this sample correctly as positive by using their own calculation method.*