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081-PT

PROFICIENCY TESTING 2018

AUJESZKY'S DISEASE VIRUS (ADV)

DETECTION OF ADV GB- AND GE-SPECIFIC ANTIBODIES IN SERUM

BY ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS

SCIENSANO

DATE BEGIN PT: 4 JUNE 2018

DATE REPORT: 2 OCTOBER 2018

THIS REPORT REPLACES AND CANCELS THE PREVIOUS REPORT PT2018AUJSER

Reason : discussion regarding the assigned value of the serum sample PT2018AUJELIgBNS3

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 'PT2018AUJELIgBNS1', 'PT2018AUJELIgBNS2', 'PT2018AUJELIgBNS3') or containing detectable ADV gB-specific antibodies (n = 3; coded 'PT2018AUJELIgBPS1', 'PT2018AUJELIgBPS2', 'PT2018AUJELIgBPS3'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2018AUJELIgBNS1, PT2018AUJELIgBNS2, PT2018AUJELIgBPS2, PT2018AUJELIgBPS3 and 4 aliquots of the reference serum samples PT2018AUJELIgBNS3 and PT2018AUJELIgBPS1. 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 'PT2018AUJELIgBNS1' and 'PT2018AUJELIgBNS2' were obtained from both uninfected and non-vaccinated animals. The reference serum samples 'PT2018AUJELIgBNS3' and 'PT2018AUJELIgBPS1' were 1/256 and 1/64 dilutions, respectively, of a serum obtained from a naturally ADV-infected animal. The reference serum sample 'PT2018AUJELIgBPS2' 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 'PT2018AUJELIgBPS3' was obtained from a naturally ADV-infected animal.
Taken together, the reference serum samples 'PT2018AUJELIgBNS1', 'PT2018AUJELIgBNS2' and 'PT2018AUJELIgBNS3' were considered as negative sera, and the reference serum samples 'PT2018AUJELIgBPS1', 'PT2018AUJELIgBPS2' and 'PT2018AUJELIgBPS3' as positive sera in ADV gB ELISA. Since the S/P value of reference serum sample 'PT2018AUJELIgBPS1' was close to the limit of positivity in the PrioCheck PRV gB antibody ELISA test, positive, non-interpretable (NI) and negative results were accepted for this sample.

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 'PT2018AUJELIgENS1', 'PT2018AUJELIgENS2', 'PT2018AUJELIgENS3') or containing detectable ADV gE-specific antibodies (n = 3; coded 'PT2018AUJELIgEPS1', 'PT2018AUJELIgEPS2', 'PT2018AUJELIgEPS3'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2018AUJELIgENS1, PT2018AUJELIgENS2, PT2018AUJELIgENS3 and PT2018AUJELIgEPS3 and 4 aliquots of the reference serum samples PT2018AUJELIgEPS1 and PT2018AUJELIgEPS2. 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 'PT2018AUJELIgENS1', 'PT2018AUJELIgENS2' and 'PT2018AUJELIgENS3' were obtained from uninfected and non-vaccinated animals. Reference serum samples 'PT2018AUJELIgEPS1' and 'PT2018AUJELIgEPS3' consisted of a 1/32 dilution of serum and pure serum, respectively, obtained from an experimentally ADV-infected animal. 'PT2018AUJELIgEPS2' was an 8 fold dilution of a serum from a naturally ADV infected animal. Taken together, the reference serum samples 'PT2018AUJELIgENS1', 'PT2018AUJELIgENS2' and 'PT2018AUJELIgENS3' were considered as negative sera, whereas the reference serum samples 'PT2018AUJELIgEPS1', 'PT2018AUJELIgEPS2', 'PT2018AUJELIgEPS3' 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

LAB1 until LAB7 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, LAB8 only participated in the PT ADV gE and received 20 aliquots of reference serum samples.
The reference serum samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 4th of June 2018 (300 aliquots in total). LAB1, LAB2, and LAB8 acknowledged receipt of the samples on the same day, whereas LAB3, LAB4, LAB6 and LAB7 received the samples on 5th of June 2018 and LAB5 on 6th of June 2018. LAB5 reported that the reference serum samples were unfrozen upon receipt. A second shipment of samples was sent to them on 19th of June 2018 and received on 21st of June 2018.
Analyses were performed between 4th of June 2018 and 3th of July 2018 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 7th of June 2018 and 6th of July 2018 (Table 1). All participants, except LAB5, respected the deadline of 25th of June 2018 for submission of the results. LAB5 received more time to carry out the analyzes since conform samples only arrived on 21st of June 2018.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano.

Participating laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	04/06/2018	04/06/2018	04/06/2018	07/06/2018
LAB2	04/06/2018	11/06/2018	11/06/2018	18/06/2018
LAB3	05/06/2018	12/06/2018	18/06/2018	20/06/2018
LAB4	05/06/2018	07/06/2018	14/06/2018	25/06/2018
LAB4.2	05/06/2018	07/06/2018	18-19/06/2018	25/06/2018
LAB5	06/06/2018 (unfrozen) 21/06/2018	03/07/2018	03/07/2018	06/07/2018
LAB6	05/06/2018	19/06/2018	19/06/2018	20/06/2018
LAB7	05/06/2018	12/06/2018	13/06/2018	25/06/2018
LAB8	04/06/2018	NA	06/06/2018	13/06/2018

Legend: NA = not applicable

IV.3. Compliance with the procedure

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

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**, 5 out of 7 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). LAB3 and LAB4 misidentified 4 samples and hence obtained 80% of agreement.

(ii) For the detection of **ADV gE-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 3). LAB5 misidentified 5 samples and hence obtained 75% of agreement.

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

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the **ADV gB** reference serum samples assigned by the Aujeszky reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. 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**
failure	0 (0)	0 (0)	**4 (20)**	**4 (20)**	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	**16 (80)**	**16 (80)**	20 (100)	20 (100)	20 (100)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the **ADV gE** reference serum samples assigned by the Aujeszky reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of ADV gE reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR								
	1	**2**	**3**	**4**	**4.2**	**5**	**6**	**7**	**8**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**5 (25)**	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	**15 (75)**	20 (100)	20 (100)	20 (100)

IV.4.2. Variability among participating laboratories

For the detection of ADV gB-specific antibodies no variability between LAB1, LAB2, LAB5, LAB6 and LAB7 could be observed since these participants correctly identified all reference serum samples. In contrast, LAB3 and LAB4 misclassified the 4 aliquots of the reference serum sample PT2018AUJELIgBNS3. The 4 aliquots were reported as positive instead of negative.

For the detection of ADV gE-specific antibodies no variability between LAB1, LAB2, LAB3, LAB4, LAB4.2, LAB6, LAB7 and LAB8 could be observed since these participants correctly identified all reference serum samples. In contrast, LAB5 misclassified 1 aliquot of the reference serum samples PT2018AUJELIgEPS3 reported as NI instead of positive and the 4 aliquots of the reference serum samples PT2017AUJELIgEPS1 reported as NI instead of positive.

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 internal identification of the **ADV gB** reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the Aujeszky reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive; DBS : doubtful; NI (non-interpretable)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2018AUJELIgBNS2	NEG	NEG	1
2	1	2	PT2018AUJELIgBPS1	DBS	POS	1
3	1	3	PT2018AUJELIgBNS3	NEG	NEG	1
4	1	4	PT2018AUJELIgBPS1	DBS	POS	1
5	1	5	PT2018AUJELIgBNS3	NEG	NEG	1
6	1	6	PT2018AUJELIgBNS1	NEG	NEG	1
7	1	7	PT2018AUJELIgBPS2	POS	POS	1
8	1	8	PT2018AUJELIgBNS3	NEG	NEG	1
9	1	9	PT2018AUJELIgBPS3	POS	POS	1
10	1	10	PT2018AUJELIgBNS2	NEG	NEG	1
11	1	11	PT2018AUJELIgBPS2	POS	POS	1
12	1	12	PT2018AUJELIgBPS1	DBS	POS	1
13	1	13	PT2018AUJELIgBNS1	NEG	NEG	1
14	1	14	PT2018AUJELIgBPS3	POS	POS	1
15	1	15	PT2018AUJELIgBNS2	NEG	NEG	1
16	1	16	PT2018AUJELIgBPS3	POS	POS	1
17	1	17	PT2018AUJELIgBNS1	NEG	NEG	1
18	1	18	PT2018AUJELIgBPS1	DBS	POS	1
19	1	19	PT2018AUJELIgBNS3	NEG	NEG	1
20	1	20	PT2018AUJELIgBPS2	POS	POS	1
21	2	1	PT2018AUJELIgBNS2	NEG	NEG	1
22	2	2	PT2018AUJELIgBNS1	NEG	NEG	1
23	2	3	PT2018AUJELIgBPS1	DBS	POS	1
24	2	4	PT2018AUJELIgBNS3	NEG	NEG	1
25	2	5	PT2018AUJELIgBPS3	POS	POS	1
26	2	6	PT2018AUJELIgBNS2	NEG	NEG	1
27	2	7	PT2018AUJELIgBNS1	NEG	NEG	1
28	2	8	PT2018AUJELIgBPS1	DBS	POS	1
29	2	9	PT2018AUJELIgBNS3	NEG	NEG	1
30	2	10	PT2018AUJELIgBPS1	DBS	POS	1
31	2	11	PT2018AUJELIgBPS2	POS	POS	1
32	2	12	PT2018AUJELIgBNS2	NEG	NEG	1
33	2	13	PT2018AUJELIgBNS3	NEG	NEG	1
34	2	14	PT2018AUJELIgBPS2	POS	POS	1
35	2	15	PT2018AUJELIgBPS3	POS	POS	1
36	2	16	PT2018AUJELIgBNS1	NEG	NEG	1
37	2	17	PT2018AUJELIgBPS1	DBS	POS	1
38	2	18	PT2018AUJELIgBNS3	NEG	NEG	1
39	2	19	PT2018AUJELIgBPS3	POS	POS	1
40	2	20	PT2018AUJELIgBPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018AUJELIgBNS2	NEG	NEG	1
42	3	2	PT2018AUJELIgBPS1	DBS	POS	1
43	3	3	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
44	3	4	PT2018AUJELIgBPS1	DBS	POS	1
45	3	5	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
46	3	6	PT2018AUJELIgBNS1	NEG	NEG	1
47	3	7	PT2018AUJELIgBPS2	POS	POS	1
48	3	8	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
49	3	9	PT2018AUJELIgBPS3	POS	POS	1
50	3	10	PT2018AUJELIgBNS2	NEG	NEG	1
51	3	11	PT2018AUJELIgBPS2	POS	POS	1
52	3	12	PT2018AUJELIgBPS1	DBS	POS	1
53	3	13	PT2018AUJELIgBNS1	NEG	NEG	1
54	3	14	PT2018AUJELIgBPS3	POS	POS	1
55	3	15	PT2018AUJELIgBNS2	NEG	NEG	1
56	3	16	PT2018AUJELIgBPS3	POS	POS	1
57	3	17	PT2018AUJELIgBNS1	NEG	NEG	1
58	3	18	PT2018AUJELIgBPS1	DBS	POS	1
59	3	19	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
60	3	20	PT2018AUJELIgBPS2	POS	POS	1
61	4	1	PT2018AUJELIgBNS2	NEG	NEG	1
62	4	2	PT2018AUJELIgBNS1	NEG	NEG	1
63	4	3	PT2018AUJELIgBPS1	DBS	POS	1
64	4	4	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
65	4	5	PT2018AUJELIgBPS3	POS	POS	1
66	4	6	PT2018AUJELIgBNS2	NEG	NEG	1
67	4	7	PT2018AUJELIgBNS1	NEG	NEG	1
68	4	8	PT2018AUJELIgBPS1	DBS	POS	1
69	4	9	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
70	4	10	PT2018AUJELIgBPS1	DBS	POS	1
71	4	11	PT2018AUJELIgBPS2	POS	POS	1
72	4	12	PT2018AUJELIgBNS2	NEG	NEG	1
73	4	13	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
74	4	14	PT2018AUJELIgBPS2	POS	POS	1
75	4	15	PT2018AUJELIgBPS3	POS	POS	1
76	4	16	PT2018AUJELIgBNS1	NEG	NEG	1
77	4	17	PT2018AUJELIgBPS1	DBS	POS	1
78	4	18	PT2018AUJELIgBNS3	**NEG**	**POS**	**0**
79	4	19	PT2018AUJELIgBPS3	POS	POS	1
80	4	20	PT2018AUJELIgBPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2018AUJELIgBNS2	NEG	NEG	1
82	5	2	PT2018AUJELIgBPS1	DBS	NI	1
83	5	3	PT2018AUJELIgBNS3	NEG	NEG	1
84	5	4	PT2018AUJELIgBPS1	DBS	NI	1
85	5	5	PT2018AUJELIgBNS3	NEG	NEG	1
86	5	6	PT2018AUJELIgBNS1	NEG	NEG	1
87	5	7	PT2018AUJELIgBPS2	POS	POS	1
88	5	8	PT2018AUJELIgBNS3	NEG	NEG	1
89	5	9	PT2018AUJELIgBPS3	POS	POS	1
90	5	10	PT2018AUJELIgBNS2	NEG	NEG	1
91	5	11	PT2018AUJELIgBPS2	POS	POS	1
92	5	12	PT2018AUJELIgBPS1	DBS	NI	1
93	5	13	PT2018AUJELIgBNS1	NEG	NEG	1
94	5	14	PT2018AUJELIgBPS3	POS	POS	1
95	5	15	PT2018AUJELIgBNS2	NEG	NEG	1
96	5	16	PT2018AUJELIgBPS3	POS	POS	1
97	5	17	PT2018AUJELIgBNS1	NEG	NEG	1
98	5	18	PT2018AUJELIgBPS1	DBS	NI	1
99	5	19	PT2018AUJELIgBNS3	NEG	NEG	1
100	5	20	PT2018AUJELIgBPS2	POS	POS	1
101	6	1	PT2018AUJELIgBNS2	NEG	NEG	1
102	6	2	PT2018AUJELIgBNS1	NEG	NEG	1
103	6	3	PT2018AUJELIgBPS1	DBS	POS	1
104	6	4	PT2018AUJELIgBNS3	NEG	NEG	1
105	6	5	PT2018AUJELIgBPS3	POS	POS	1
106	6	6	PT2018AUJELIgBNS2	NEG	NEG	1
107	6	7	PT2018AUJELIgBNS1	NEG	NEG	1
108	6	8	PT2018AUJELIgBPS1	DBS	POS	1
109	6	9	PT2018AUJELIgBNS3	NEG	NEG	1
110	6	10	PT2018AUJELIgBPS1	DBS	POS	1
111	6	11	PT2018AUJELIgBPS2	POS	POS	1
112	6	12	PT2018AUJELIgBNS2	NEG	NEG	1
113	6	13	PT2018AUJELIgBNS3	NEG	NEG	1
114	6	14	PT2018AUJELIgBPS2	POS	POS	1
115	6	15	PT2018AUJELIgBPS3	POS	POS	1
116	6	16	PT2018AUJELIgBNS1	NEG	NEG	1
117	6	17	PT2018AUJELIgBPS1	DBS	POS	1
118	6	18	PT2018AUJELIgBNS3	NEG	NEG	1
119	6	19	PT2018AUJELIgBPS3	POS	POS	1
120	6	20	PT2018AUJELIgBPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2018AUJELIgBNS2	NEG	NEG	1
122	7	2	PT2018AUJELIgBPS1	DBS	NEG	1
123	7	3	PT2018AUJELIgBNS3	NEG	NEG	1
124	7	4	PT2018AUJELIgBPS1	DBS	NEG	1
125	7	5	PT2018AUJELIgBNS3	NEG	NEG	1
126	7	6	PT2018AUJELIgBNS1	NEG	NEG	1
127	7	7	PT2018AUJELIgBPS2	POS	POS	1
128	7	8	PT2018AUJELIgBNS3	NEG	NEG	1
129	7	9	PT2018AUJELIgBPS3	POS	POS	1
130	7	10	PT2018AUJELIgBNS2	NEG	NEG	1
131	7	11	PT2018AUJELIgBPS2	POS	POS	1
132	7	12	PT2018AUJELIgBPS1	DBS	NEG	1
133	7	13	PT2018AUJELIgBNS1	NEG	NEG	1
134	7	14	PT2018AUJELIgBPS3	POS	POS	1
135	7	15	PT2018AUJELIgBNS2	NEG	NEG	1
136	7	16	PT2018AUJELIgBPS3	POS	POS	1
137	7	17	PT2018AUJELIgBNS1	NEG	NEG	1
138	7	18	PT2018AUJELIgBPS1	DBS	NEG	1
139	7	19	PT2018AUJELIgBNS3	NEG	NEG	1
140	7	20	PT2018AUJELIgBPS2	POS	POS	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the **ADV gE** reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the Aujeszky reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive, NI (non-interpretable).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2018AUJELIgEPS1	POS	POS	1
2	1	2	PT2018AUJELIgENS2	NEG	NEG	1
3	1	3	PT2018AUJELIgEPS2	POS	POS	1
4	1	4	PT2018AUJELIgENS3	NEG	NEG	1
5	1	5	PT2018AUJELIgENS1	NEG	NEG	1
6	1	6	PT2018AUJELIgENS3	NEG	NEG	1
7	1	7	PT2018AUJELIgEPS3	POS	POS	1
8	1	8	PT2018AUJELIgEPS2	POS	POS	1
9	1	9	PT2018AUJELIgEPS1	POS	POS	1
10	1	10	PT2018AUJELIgENS2	NEG	NEG	1
11	1	11	PT2018AUJELIgENS1	NEG	NEG	1
12	1	12	PT2018AUJELIgEPS2	POS	POS	1
13	1	13	PT2018AUJELIgEPS1	POS	POS	1
14	1	14	PT2018AUJELIgENS2	NEG	NEG	1
15	1	15	PT2018AUJELIgEPS3	POS	POS	1
16	1	16	PT2018AUJELIgENS1	NEG	NEG	1
17	1	17	PT2018AUJELIgEPS2	POS	POS	1
18	1	18	PT2018AUJELIgEPS3	POS	POS	1
19	1	19	PT2018AUJELIgENS3	NEG	NEG	1
20	1	20	PT2018AUJELIgEPS1	POS	POS	1
21	2	1	PT2018AUJELIgEPS2	POS	POS	1
22	2	2	PT2018AUJELIgENS2	NEG	NEG	1
23	2	3	PT2018AUJELIgENS3	NEG	NEG	1
24	2	4	PT2018AUJELIgENS1	NEG	NEG	1
25	2	5	PT2018AUJELIgEPS1	POS	POS	1
26	2	6	PT2018AUJELIgEPS2	POS	POS	1
27	2	7	PT2018AUJELIgEPS1	POS	POS	1
28	2	8	PT2018AUJELIgENS1	NEG	NEG	1
29	2	9	PT2018AUJELIgENS3	NEG	NEG	1
30	2	10	PT2018AUJELIgEPS2	POS	POS	1
31	2	11	PT2018AUJELIgENS2	NEG	NEG	1
32	2	12	PT2018AUJELIgEPS3	POS	POS	1
33	2	13	PT2018AUJELIgENS1	NEG	NEG	1
34	2	14	PT2018AUJELIgENS3	NEG	NEG	1
35	2	15	PT2018AUJELIgEPS2	POS	POS	1
36	2	16	PT2018AUJELIgEPS1	POS	POS	1
37	2	17	PT2018AUJELIgENS2	NEG	NEG	1
38	2	18	PT2018AUJELIgEPS3	POS	POS	1
39	2	19	PT2018AUJELIgEPS1	POS	POS	1
40	2	20	PT2018AUJELIgEPS3	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018AUJELIgEPS1	POS	POS	1
42	3	2	PT2018AUJELIgENS2	NEG	NEG	1
43	3	3	PT2018AUJELIgEPS2	POS	POS	1
44	3	4	PT2018AUJELIgENS3	NEG	NEG	1
45	3	5	PT2018AUJELIgENS1	NEG	NEG	1
46	3	6	PT2018AUJELIgENS3	NEG	NEG	1
47	3	7	PT2018AUJELIgEPS3	POS	POS	1
48	3	8	PT2018AUJELIgEPS2	POS	POS	1
49	3	9	PT2018AUJELIgEPS1	POS	POS	1
50	3	10	PT2018AUJELIgENS2	NEG	NEG	1
51	3	11	PT2018AUJELIgENS1	NEG	NEG	1
52	3	12	PT2018AUJELIgEPS2	POS	POS	1
53	3	13	PT2018AUJELIgEPS1	POS	POS	1
54	3	14	PT2018AUJELIgENS2	NEG	NEG	1
55	3	15	PT2018AUJELIgEPS3	POS	POS	1
56	3	16	PT2018AUJELIgENS1	NEG	NEG	1
57	3	17	PT2018AUJELIgEPS2	POS	POS	1
58	3	18	PT2018AUJELIgEPS3	POS	POS	1
59	3	19	PT2018AUJELIgENS3	NEG	NEG	1
60	3	20	PT2018AUJELIgEPS1	POS	POS	1
61	4	1	PT2018AUJELIgEPS2	POS	POS	1
62	4	2	PT2018AUJELIgENS2	NEG	NEG	1
63	4	3	PT2018AUJELIgENS3	NEG	NEG	1
64	4	4	PT2018AUJELIgENS1	NEG	NEG	1
65	4	5	PT2018AUJELIgEPS1	POS	POS	1
66	4	6	PT2018AUJELIgEPS2	POS	POS	1
67	4	7	PT2018AUJELIgEPS1	POS	POS	1
68	4	8	PT2018AUJELIgENS1	NEG	NEG	1
69	4	9	PT2018AUJELIgENS3	NEG	NEG	1
70	4	10	PT2018AUJELIgEPS2	POS	POS	1
71	4	11	PT2018AUJELIgENS2	NEG	NEG	1
72	4	12	PT2018AUJELIgEPS3	POS	POS	1
73	4	13	PT2018AUJELIgENS1	NEG	NEG	1
74	4	14	PT2018AUJELIgENS3	NEG	NEG	1
75	4	15	PT2018AUJELIgEPS2	POS	POS	1
76	4	16	PT2018AUJELIgEPS1	POS	POS	1
77	4	17	PT2018AUJELIgENS2	NEG	NEG	1
78	4	18	PT2018AUJELIgEPS3	POS	POS	1
79	4	19	PT2018AUJELIgEPS1	POS	POS	1
80	4	20	PT2018AUJELIgEPS3	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.2	1	PT2018AUJELIgEPS2	POS	POS	1
82	4.2	2	PT2018AUJELIgENS2	NEG	NEG	1
83	4.2	3	PT2018AUJELIgENS3	NEG	NEG	1
84	4.2	4	PT2018AUJELIgENS1	NEG	NEG	1
85	4.2	5	PT2018AUJELIgEPS1	POS	POS	1
86	4.2	6	PT2018AUJELIgEPS2	POS	POS	1
87	4.2	7	PT2018AUJELIgEPS1	POS	POS	1
88	4.2	8	PT2018AUJELIgENS1	NEG	NEG	1
89	4.2	9	PT2018AUJELIgENS3	NEG	NEG	1
90	4.2	10	PT2018AUJELIgEPS2	POS	POS	1
91	4.2	11	PT2018AUJELIgENS2	NEG	NEG	1
92	4.2	12	PT2018AUJELIgEPS3	POS	POS	1
93	4.2	13	PT2018AUJELIgENS1	NEG	NEG	1
94	4.2	14	PT2018AUJELIgENS3	NEG	NEG	1
95	4.2	15	PT2018AUJELIgEPS2	POS	POS	1
96	4.2	16	PT2018AUJELIgEPS1	POS	POS	1
97	4.2	17	PT2018AUJELIgENS2	NEG	NEG	1
98	4.2	18	PT2018AUJELIgEPS3	POS	POS	1
99	4.2	19	PT2018AUJELIgEPS1	POS	POS	1
100	4.2	20	PT2018AUJELIgEPS3	POS	POS	1
101	5	1	PT2018AUJELIgEPS1	**POS**	**NI**	**0**
102	5	2	PT2018AUJELIgENS2	NEG	NEG	1
103	5	3	PT2018AUJELIgEPS2	POS	POS	1
104	5	4	PT2018AUJELIgENS3	NEG	NEG	1
105	5	5	PT2018AUJELIgENS1	NEG	NEG	1
106	5	6	PT2018AUJELIgENS3	NEG	NEG	1
107	5	7	PT2018AUJELIgEPS3	**POS**	**NI**	**0**
108	5	8	PT2018AUJELIgEPS2	POS	POS	1
109	5	9	PT2018AUJELIgEPS1	**POS**	**NI**	**0**
110	5	10	PT2018AUJELIgENS2	NEG	NEG	1
111	5	11	PT2018AUJELIgENS1	NEG	NEG	1
112	5	12	PT2018AUJELIgEPS2	POS	POS	1
113	5	13	PT2018AUJELIgEPS1	**POS**	**NI**	**0**
114	5	14	PT2018AUJELIgENS2	NEG	NEG	1
115	5	15	PT2018AUJELIgEPS3	POS	POS	1
116	5	16	PT2018AUJELIgENS1	NEG	NEG	1
117	5	17	PT2018AUJELIgEPS2	POS	POS	1
118	5	18	PT2018AUJELIgEPS3	POS	POS	1
119	5	19	PT2018AUJELIgENS3	NEG	NEG	1
120	5	20	PT2018AUJELIgEPS1	**POS**	**NI**	**0**

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	6	1	PT2018AUJELIgEPS2	POS	POS	1
122	6	2	PT2018AUJELIgENS2	NEG	NEG	1
123	6	3	PT2018AUJELIgENS3	NEG	NEG	1
124	6	4	PT2018AUJELIgENS1	NEG	NEG	1
125	6	5	PT2018AUJELIgEPS1	POS	POS	1
126	6	6	PT2018AUJELIgEPS2	POS	POS	1
127	6	7	PT2018AUJELIgEPS1	POS	POS	1
128	6	8	PT2018AUJELIgENS1	NEG	NEG	1
129	6	9	PT2018AUJELIgENS3	NEG	NEG	1
130	6	10	PT2018AUJELIgEPS2	POS	POS	1
131	6	11	PT2018AUJELIgENS2	NEG	NEG	1
132	6	12	PT2018AUJELIgEPS3	POS	POS	1
133	6	13	PT2018AUJELIgENS1	NEG	NEG	1
134	6	14	PT2018AUJELIgENS3	NEG	NEG	1
135	6	15	PT2018AUJELIgEPS2	POS	POS	1
136	6	16	PT2018AUJELIgEPS1	POS	POS	1
137	6	17	PT2018AUJELIgENS2	NEG	NEG	1
138	6	18	PT2018AUJELIgEPS3	POS	POS	1
139	6	19	PT2018AUJELIgEPS1	POS	POS	1
140	6	20	PT2018AUJELIgEPS3	POS	POS	1
141	7	1	PT2018AUJELIgEPS1	POS	POS	1
142	7	2	PT2018AUJELIgENS2	NEG	NEG	1
143	7	3	PT2018AUJELIgEPS2	POS	POS	1
144	7	4	PT2018AUJELIgENS3	NEG	NEG	1
145	7	5	PT2018AUJELIgENS1	NEG	NEG	1
146	7	6	PT2018AUJELIgENS3	NEG	NEG	1
147	7	7	PT2018AUJELIgEPS3	POS	POS	1
148	7	8	PT2018AUJELIgEPS2	POS	POS	1
149	7	9	PT2018AUJELIgEPS1	POS	POS	1
150	7	10	PT2018AUJELIgENS2	NEG	NEG	1
151	7	11	PT2018AUJELIgENS1	NEG	NEG	1
152	7	12	PT2018AUJELIgEPS2	POS	POS	1
153	7	13	PT2018AUJELIgEPS1	POS	POS	1
154	7	14	PT2018AUJELIgENS2	NEG	NEG	1
155	7	15	PT2018AUJELIgEPS3	POS	POS	1
156	7	16	PT2018AUJELIgENS1	NEG	NEG	1
157	7	17	PT2018AUJELIgEPS2	POS	POS	1
158	7	18	PT2018AUJELIgEPS3	POS	POS	1
159	7	19	PT2018AUJELIgENS3	NEG	NEG	1
160	7	20	PT2018AUJELIgEPS1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	8	1	PT2018AUJELIgEPS2	POS	POS	1
162	8	2	PT2018AUJELIgENS2	NEG	NEG	1
163	8	3	PT2018AUJELIgENS3	NEG	NEG	1
164	8	4	PT2018AUJELIgENS1	NEG	NEG	1
165	8	5	PT2018AUJELIgEPS1	POS	POS	1
166	8	6	PT2018AUJELIgEPS2	POS	POS	1
167	8	7	PT2018AUJELIgEPS1	POS	POS	1
168	8	8	PT2018AUJELIgENS1	NEG	NEG	1
169	8	9	PT2018AUJELIgENS3	NEG	NEG	1
170	8	10	PT2018AUJELIgEPS2	POS	POS	1
171	8	11	PT2018AUJELIgENS2	NEG	NEG	1
172	8	12	PT2018AUJELIgEPS3	POS	POS	1
173	8	13	PT2018AUJELIgENS1	NEG	NEG	1
174	8	14	PT2018AUJELIgENS3	NEG	NEG	1
175	8	15	PT2018AUJELIgEPS2	POS	POS	1
176	8	16	PT2018AUJELIgEPS1	POS	POS	1
177	8	17	PT2018AUJELIgENS2	NEG	NEG	1
178	8	18	PT2018AUJELIgEPS3	POS	POS	1
179	8	19	PT2018AUJELIgEPS1	POS	POS	1
180	8	20	PT2018AUJELIgEPS3	POS	POS	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, 5 out of 7 participating laboratories (LAB1, LAB2, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB3 and LAB4 misclassified the 4 aliquots of the reference serum sample PT2018AUJELIgBNS3. Hereby, LAB3 and LAB4 only obtained 80% of agreement with the assigned status of the reference serum samples.

Since reference serum sample PT2018AUJELIgBNS3 represents a 1/256 dilution of a serum from a naturally pseudorabies virus infected pig, it is possible that it still contains antibodies against the gB protein and that these are detected by the ELISA tests used by LAB3 and LAB4 (using Idexx and Hipra kits). This would indicate that these test are more sensitive than the PrioCheck PRV gB antibody ELISA test that was used to determine the status of the reference samples. This seems supported by the fact that LAB3 and LAB4 also scored higher normalized values for sample PT2018AUJELIgBPS1 (Figure 1), a 1/64 dilution of the same serum, than obtained with the PrioCheck PRV gB antibody ELISA test.

LAB1, LAB2, LAB3, LAB4, LAB6, and LAB7 used ADV gB antibody ELISA kits from 4 different commercial producers : Thermofisher Scientific, Biochek, Hipra and Idexx. LAB5 used a home-made ADV gB antibody ELISA kit. Also different batches from the same ELISA kit were used. Hereby, Thermofisher Scientific batch Z161201L and batch Z170401L.

For the detection of ADV gE-specific antibodies in reference serum samples, 7 out of 8 participating laboratories (LAB1, LAB2, LAB3, LAB4, LAB4.2, LAB6, LAB7 and LAB8) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB5 misclassified 1 aliquot of the reference serum samples PT2018AUJELIgEPS3 and the 4 aliquots of the reference serum samples PT2017AUJELIgEPS1. Hereby, LAB5 only obtained 75% of agreement with the assigned status of the reference serum samples.

LAB1, LAB2, LAB3, LAB4, LAB4.2, LAB6, LAB7 and LAB8 used ADV gE antibody ELISA kits from 4 different commercial producers: Thermofisher Scientific, Biochek, Hipra and Idexx. LAB5 used a home-made ADV gE antibody ELISA kit. Also different batches from the same ELISA kit were used. Hereby, Idexx batch BP878, batch BP882 and batch HN102. Furthermore, LAB4 performed a short incubation protocol and LAB4.2 a long incubation protocol with the same kit.

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 the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). **Following this procedure**, 5 out 7 participants to the PT ADV gB achieved a satisfactory performance for the detection of ADV gB-specific antibodies in porcine serum samples. Although LAB3 and LAB4 did not achieve a satisfactory **performance following this procedure, it seems highly likely that they used tests with a higher sensitivity than the test used for the determination of the reference status of the samples (see discussion section). It is therefore considered that also LAB3 and LAB4 performed satisfactory** for the detection of ADV gB-specific antibodies in serum samples.
For the PT ADVgE, 7 out of 8 participants achieved a satisfactory performance for the detection of ADV gE-specific antibodies in porcine serum samples. LAB5 did not achieve a satisfactory performance for the detection of ADV gE-specific antibodies in serum samples.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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

BioChek B.V. (Reeuwijk, The Nederlands)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

HIPRA Scientific SLU (Girona, Spain)

Laboratoire de Médecine Véterinaire de l'Etat (LMVE) (Dudelange, Grand Duché de Luxembourg)

Sciensano (Ukkel, Belgium)

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

Thermo Fisher Scientific Prionics AG (Schlieren, Switzerland)

Annex 1: Quantitative data analysis (Box plots)

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R (box plots).

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.

The box plots for the laboratories participating in the PT ADV gB are shown in Figures 1 and 2 and the box plot for the laboratories participating in the PT ADV gE is shown in Figure 3.

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.

The quantitative data analyses of the PT ADV gB were performed for LAB1, LAB2, LAB3, LAB4 and LAB6 on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory (Figure 1). In contrast the quantitative data analyses for LAB5 and LAB7 were performed on the raw OD values (Figure 2).

The quantitative data analyses of the PT ADV gE were performed on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory (Figure 3).

Detection of ADV gB-specific antibodies in serum by ELISA

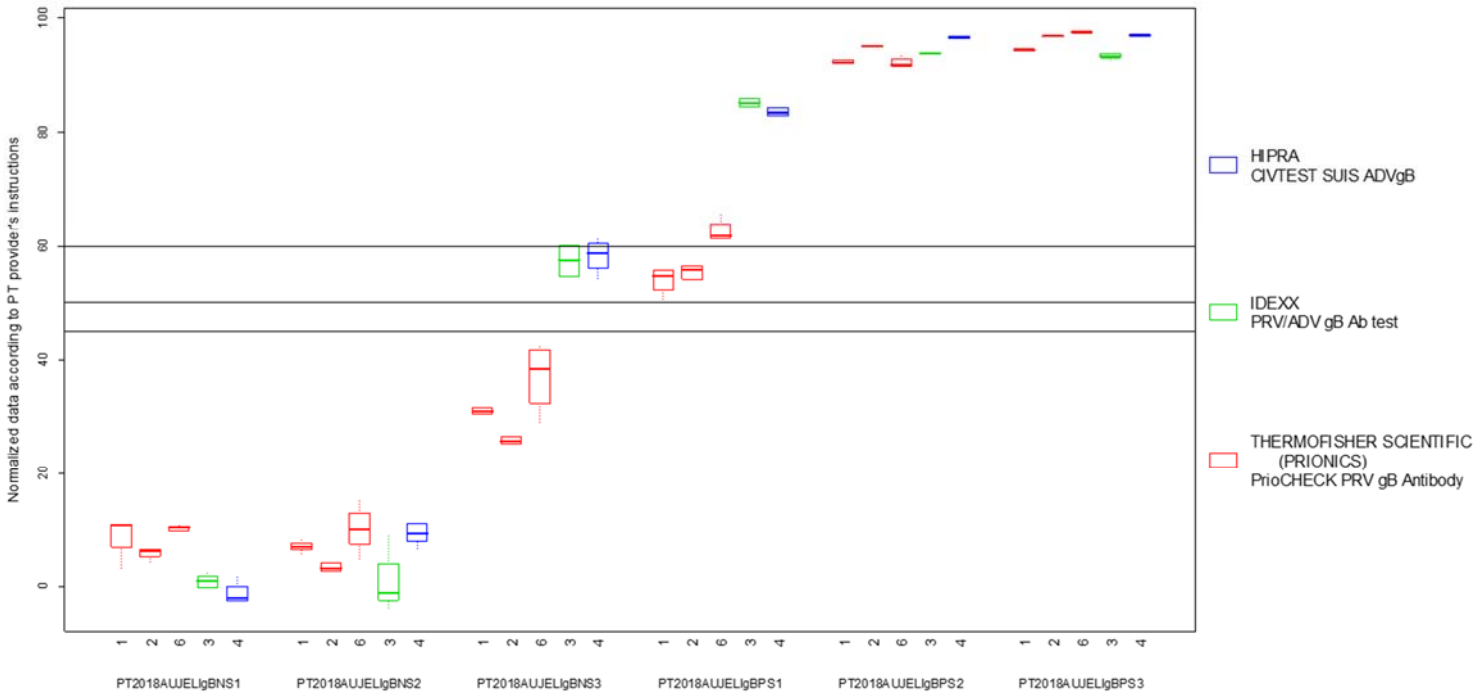
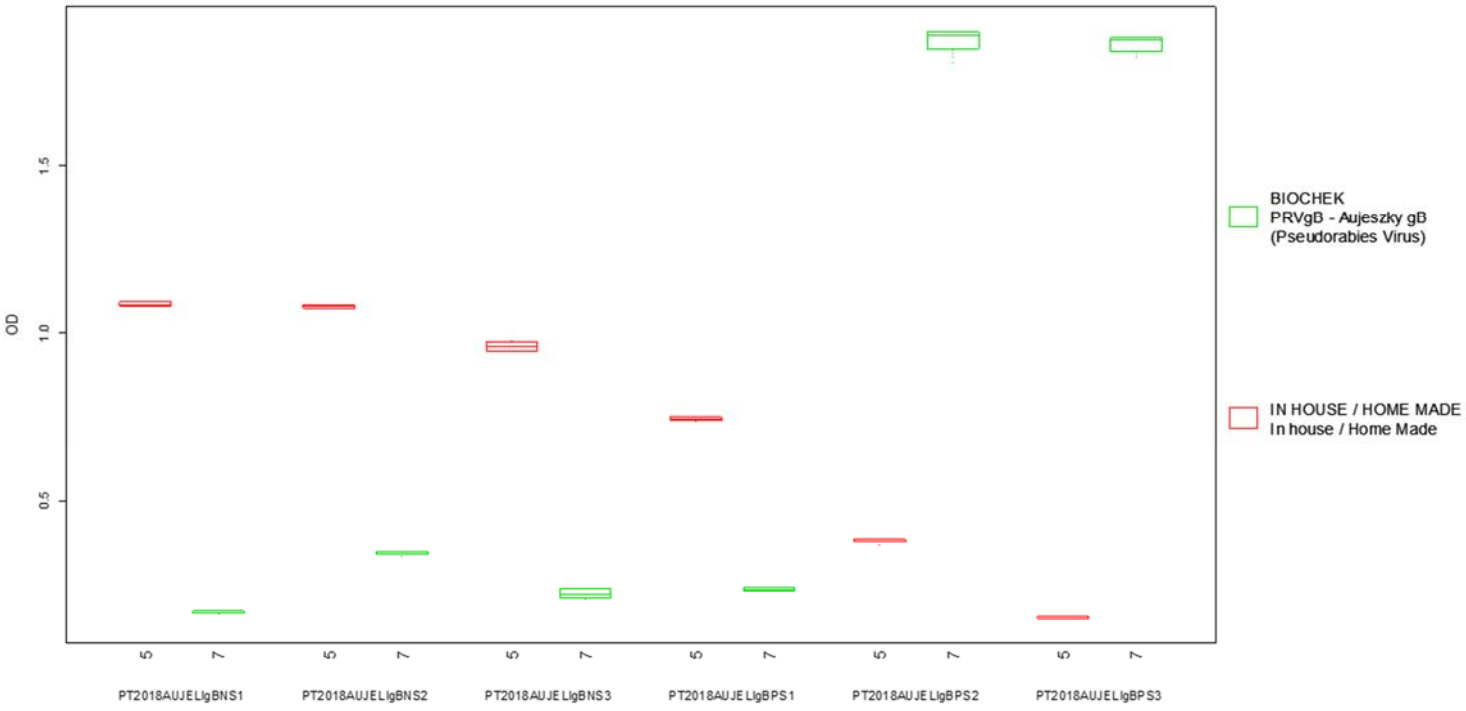


Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory except for LAB5 and LAB7. LAB1, LAB2, LAB3, LAB4 and LAB6 used ADV gB antibody ELISA kits from 3 different commercial producers : Thermofisher Scientific, Hipra and Idexx. Cut-off values (Hipra 45-50, Thermofisher Scientific 50 and Idexx 50-60) are shown by horizontal lines.

Figure 2. Box plots showing the raw OD values per reference serum and per participating laboratory for LAB5 and LAB7. LAB7 used an ADV gB antibody ELISA kit from Biochek and LAB5 used a home-made ADV gB antibody ELISA kit.



Detection of ADV gE-specific antibodies in serum by ELISA

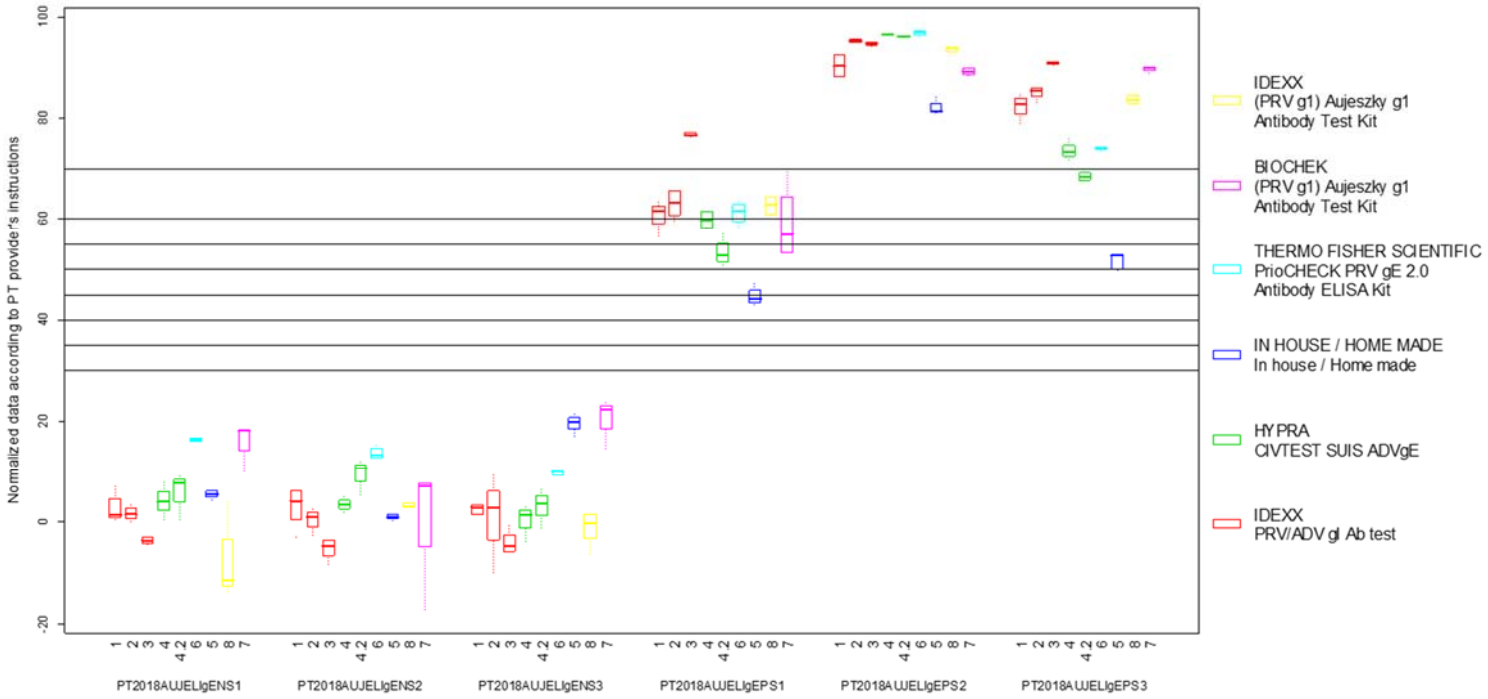


Figure 3. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory. LAB1, LAB2, LAB3, LAB4, LAB4.2, LAB6, LAB7 and LAB8 used ADV gE antibody ELISA kits from 4 different commercial producers: Thermofisher Scientific, Biochek, Hipra and Idexx. LAB5 used a home-made ADV gE antibody ELISA kit. Cut-off value (Idexx 30-40 and 60-70, Thermofisher Scientific 35, Hipra 40-45, In House 50-60 and Biochek 55) are shown by a horizontal line.