CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE
GROESELENBERG 99 – B 1180 BRUSSELS (UKKEL)
TEL: +32 (0)2 379 04 11
FAX : + 32 (0)2 379 06 70
HTTP: // WWW.CODA-CERVA.BE

172-PT

PROFICIENCY TESTING 2012

AUJESZKY'S DISEASE VIRUS (ADV)

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

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 27 AUGUST 2012

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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 = 2; coded 'PT2012AUJgBSERNS1' and 'PT2012AUJgBSERNS2') or containing detectable ADV gB-specific antibodies (n = 4; coded 'PT2012AUJgBSERPS1', 'PT2012AUJgBSERPS2', 'PT2012AUJgBSERPS3' and 'PT2012AUJgBSERPS4'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012AUJgBSERNS1, PT2012AUJgBSERNS2, PT2012AUJgBSERPS1 and PT2012AUJgBSERPS2, and 4 aliquots of the reference serum samples PT2012AUJgBSERPS3 and PT2012AUJgBSERPS4. 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 by the PrioCheck PRV gB antibody ELISA test from Prionics and a seroneutralisation assay (SN) (pre-verification). The reference serum samples PT2012AUJgBSERNS1 and PT2012AUJgBSERNS2 were obtained from both uninfected and non-vaccinated animals. The reference serum samples PT2012AUJgBSERPS1 and PT2012AUJgBSERPS2 were obtained from 2 uninfected animals that were vaccinated twice with the live attenuated marker vaccine Suvaxyn Aujeszky 783+0/W (ADV attenuated NIA3-783 strain in mineral oil adjuvant). Hereby, reference serum sample PT2012AUJgBSERPS2 was a 1/16 dilution of the original serum. The reference serum samples PT2012AUJgBSERPS3 and PT2012AUJgBSERPS4 were a 1/4 and a 1/32 dilution, respectively, of 2 different sera obtained from ADV-infected animals. For each reference serum sample, the same qualitative result was obtained with both test methods used. Taken together, the reference serum samples PT2012AUJgBSERNS1 and PT2012AUJgBSERNS2 were considered as negative sera, and the reference serum samples PT2012AUJgBSERPS1, PT2012AUJgBSERPS2, PT2012AUJgBSERPS3 and PT2012AUJgBSERPS4 as variably positive sera in SN but strong 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, all reference serum samples were tested once 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 'PT2012AUJgESERNS1', 'PT2012AUJgESERNS2' and 'PT2012AUJgESERNS3') or containing detectable ADV gE-specific antibodies (n = 3; coded 'PT2012AUJgESERPS1', 'PT2012AUJgESERPS2' and 'PT2012AUJgESERPS3'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012AUJgESERNS1, PT2012AUJgESERNS2, PT2012AUJgESERNS3 and PT2012AUJgESERPS3, and 4 aliquots of the reference serum samples PT2012AUJgESERPS1 and PT2012AUJgESERPS2. 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 by the HerdChek PRV gpI antibody ELISA test from IDEXX (pre-verification). The reference serum samples PT2012AUJgESERNS1 (=PT2012AUJgBSERNS1) and PT2012AUJgESERNS2 (=PT2012AUJgBSERNS2) were obtained from both uninfected and non-vaccinated animals, whereas the reference serum sample PT2012AUJgESERNS3 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 samples PT2012AUJgESERPS1, PT2012AUJgESERPS2 (=PT2012AUJgBSERPS3) and PT2012AUJgESERPS3 were a 1/8, a 1/4 and a 1/4 dilution, respectively, of 3 different sera obtained from ADV-infected animals. Taken together, the reference serum samples PT2012AUJgESERNS1, PT2012AUJgESERNS2 and PT2012AUJgESERNS3 were considered as negative sera, whereas the reference serum samples PT2012AUJgESERPS1, PT2012AUJgESERPS2 and PT2012AUJgESERPS3 as strong 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, all reference serum samples were tested once 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* (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by a participating laboratory is expressed as the percentage *success* (i.e., the reported result matches with the assigned status) obtained for the 20 aliquots of reference serum samples used for either the ADV gB or the ADV gE PT.

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 serum samples used for either PT is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the operational unit CVD-ERA of CODA-CERVA.

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

LAB1 until LAB6 participated in both the ADV gB and the ADV gE PT and received 40 aliquots of reference serum samples (20 for the ADV gB PT and 20 for the ADV gE PT). LAB7 only participated in the ADV gB PT, whereas LAB8 only participated in the ADV gE PT. These 2 participating laboratories hence 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 27th of August 2012 (280 aliquots in total). LAB4, LAB5, LAB6 and LAB8 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 28th of August 2012. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 27th of August and 8th of September 2012. LAB1 did not communicate the date of analysis for the ADV gB PT, whereas LAB4 did not communicate the date of analysis for the ADV gE PT (Table 1).

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results from the participating laboratories were submitted to the operational unit CVD-ERA between 29th of August and 10th of September 2012. LAB2 hereby exceeded the deadline of 7th of September 2012 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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	28/08/2012	NOT PROVIDED	29/08/2012	03/09/2012
LAB2	28/08/2012	08/09/2012	08/09/2012	10/09/2012
LAB3	28/08/2012	30/08/2012	30/08/2012	30/08/2012
LAB4	27/08/2012	30/08/2012	NOT PROVIDED	03/09/2012
LAB5	27/08/2012	28/08/2012	28/08/2012	07/09/2012
LAB6	27/08/2012	28/08/2012	28/08/2012	30/08/2012
LAB7	28/08/2012	04/09/2012	NA	05/09/2012
LAB8	27/08/2012	NA	27/08/2012	29/08/2012

Legend: NA = not applicable

IV.3. Compliance with the procedure

Except LAB4, 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**, all 7 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples and hence obtained 100% of agreement (Table 2).
- **(ii)** For the detection of **ADV gE-specific antibodies**, all 7 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples and hence obtained 100% of agreement (Table 3).

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the **ADV gB** reference serum samples assigned by the ADV reference laboratory of CODA-CERVA. 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 (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)

Table 3. Agreement between results generated by the participating laboratories (LABNR) and the status of the **ADV gE** reference serum samples assigned by the ADV reference laboratory of CODA-CERVA. 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**	**5**	**6**	**8**
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 (including box plots) is shown for educational purposes in Annex 1 and Annex 2.

IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement for the detection of both ADV gB- and ADV gE-specific 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 ADV gB PT and in Table 5 for the ADV gE PT.

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 ADV reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012AUJgBSERNS2	NEG	NEG	1
2	1	2	PT2012AUJgBSERPS3	POS	POS	1
3	1	3	PT2012AUJgBSERPS1	POS	POS	1
4	1	4	PT2012AUJgBSERPS4	POS	POS	1
5	1	5	PT2012AUJgBSERPS4	POS	POS	1
6	1	6	PT2012AUJgBSERPS1	POS	POS	1
7	1	7	PT2012AUJgBSERPS2	POS	POS	1
8	1	8	PT2012AUJgBSERPS3	POS	POS	1
9	1	9	PT2012AUJgBSERNS1	NEG	NEG	1
10	1	10	PT2012AUJgBSERPS4	POS	POS	1
11	1	11	PT2012AUJgBSERNS1	NEG	NEG	1
12	1	12	PT2012AUJgBSERNS2	NEG	NEG	1
13	1	13	PT2012AUJgBSERNS1	NEG	NEG	1
14	1	14	PT2012AUJgBSERPS2	POS	POS	1
15	1	15	PT2012AUJgBSERPS1	POS	POS	1
16	1	16	PT2012AUJgBSERPS3	POS	POS	1
17	1	17	PT2012AUJgBSERPS2	POS	POS	1
18	1	18	PT2012AUJgBSERNS2	NEG	NEG	1
19	1	19	PT2012AUJgBSERPS3	POS	POS	1
20	1	20	PT2012AUJgBSERPS4	POS	POS	1
21	2	1	PT2012AUJgBSERNS2	NEG	NEG	1
22	2	2	PT2012AUJgBSERPS3	POS	POS	1
23	2	3	PT2012AUJgBSERPS4	POS	POS	1
24	2	4	PT2012AUJgBSERNS2	NEG	NEG	1
25	2	5	PT2012AUJgBSERPS3	POS	POS	1
26	2	6	PT2012AUJgBSERPS1	POS	POS	1
27	2	7	PT2012AUJgBSERPS4	POS	POS	1
28	2	8	PT2012AUJgBSERPS4	POS	POS	1
29	2	9	PT2012AUJgBSERPS1	POS	POS	1
30	2	10	PT2012AUJgBSERPS2	POS	POS	1
31	2	11	PT2012AUJgBSERPS3	POS	POS	1
32	2	12	PT2012AUJgBSERNS1	NEG	NEG	1
33	2	13	PT2012AUJgBSERPS4	POS	POS	1
34	2	14	PT2012AUJgBSERNS1	NEG	NEG	1
35	2	15	PT2012AUJgBSERNS2	NEG	NEG	1
36	2	16	PT2012AUJgBSERNS1	NEG	NEG	1
37	2	17	PT2012AUJgBSERPS2	POS	POS	1
38	2	18	PT2012AUJgBSERPS1	POS	POS	1
39	2	19	PT2012AUJgBSERPS3	POS	POS	1
40	2	20	PT2012AUJgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012AUJgBSERPS1	POS	POS	1
42	3	2	PT2012AUJgBSERPS3	POS	POS	1
43	3	3	PT2012AUJgBSERPS2	POS	POS	1
44	3	4	PT2012AUJgBSERNS2	NEG	NEG	1
45	3	5	PT2012AUJgBSERPS3	POS	POS	1
46	3	6	PT2012AUJgBSERPS4	POS	POS	1
47	3	7	PT2012AUJgBSERNS2	NEG	NEG	1
48	3	8	PT2012AUJgBSERPS3	POS	POS	1
49	3	9	PT2012AUJgBSERPS1	POS	POS	1
50	3	10	PT2012AUJgBSERPS4	POS	POS	1
51	3	11	PT2012AUJgBSERPS4	POS	POS	1
52	3	12	PT2012AUJgBSERPS1	POS	POS	1
53	3	13	PT2012AUJgBSERPS2	POS	POS	1
54	3	14	PT2012AUJgBSERPS3	POS	POS	1
55	3	15	PT2012AUJgBSERNS1	NEG	NEG	1
56	3	16	PT2012AUJgBSERPS4	POS	POS	1
57	3	17	PT2012AUJgBSERNS1	NEG	NEG	1
58	3	18	PT2012AUJgBSERNS2	NEG	NEG	1
59	3	19	PT2012AUJgBSERNS1	NEG	NEG	1
60	3	20	PT2012AUJgBSERPS2	POS	POS	1
61	4	1	PT2012AUJgBSERNS2	NEG	NEG	1
62	4	2	PT2012AUJgBSERNS1	NEG	NEG	1
63	4	3	PT2012AUJgBSERPS2	POS	POS	1
64	4	4	PT2012AUJgBSERPS1	POS	POS	1
65	4	5	PT2012AUJgBSERPS3	POS	POS	1
66	4	6	PT2012AUJgBSERPS2	POS	POS	1
67	4	7	PT2012AUJgBSERNS2	NEG	NEG	1
68	4	8	PT2012AUJgBSERPS3	POS	POS	1
69	4	9	PT2012AUJgBSERPS4	POS	POS	1
70	4	10	PT2012AUJgBSERNS2	NEG	NEG	1
71	4	11	PT2012AUJgBSERPS3	POS	POS	1
72	4	12	PT2012AUJgBSERPS1	POS	POS	1
73	4	13	PT2012AUJgBSERPS4	POS	POS	1
74	4	14	PT2012AUJgBSERPS4	POS	POS	1
75	4	15	PT2012AUJgBSERPS1	POS	POS	1
76	4	16	PT2012AUJgBSERPS2	POS	POS	1
77	4	17	PT2012AUJgBSERPS3	POS	POS	1
78	4	18	PT2012AUJgBSERNS1	NEG	NEG	1
79	4	19	PT2012AUJgBSERPS4	POS	POS	1
80	4	20	PT2012AUJgBSERNS1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2012AUJgBSERNS1	NEG	NEG	1
82	5	2	PT2012AUJgBSERPS4	POS	POS	1
83	5	3	PT2012AUJgBSERNS1	NEG	NEG	1
84	5	4	PT2012AUJgBSERNS2	NEG	NEG	1
85	5	5	PT2012AUJgBSERNS1	NEG	NEG	1
86	5	6	PT2012AUJgBSERPS2	POS	POS	1
87	5	7	PT2012AUJgBSERPS1	POS	POS	1
88	5	8	PT2012AUJgBSERPS3	POS	POS	1
89	5	9	PT2012AUJgBSERPS2	POS	POS	1
90	5	10	PT2012AUJgBSERNS2	NEG	NEG	1
91	5	11	PT2012AUJgBSERPS3	POS	POS	1
92	5	12	PT2012AUJgBSERPS4	POS	POS	1
93	5	13	PT2012AUJgBSERNS2	NEG	NEG	1
94	5	14	PT2012AUJgBSERPS3	POS	POS	1
95	5	15	PT2012AUJgBSERPS1	POS	POS	1
96	5	16	PT2012AUJgBSERPS4	POS	POS	1
97	5	17	PT2012AUJgBSERPS4	POS	POS	1
98	5	18	PT2012AUJgBSERPS1	POS	POS	1
99	5	19	PT2012AUJgBSERPS2	POS	POS	1
100	5	20	PT2012AUJgBSERPS3	POS	POS	1
101	6	1	PT2012AUJgBSERPS1	POS	POS	1
102	6	2	PT2012AUJgBSERPS2	POS	POS	1
103	6	3	PT2012AUJgBSERPS3	POS	POS	1
104	6	4	PT2012AUJgBSERNS1	NEG	NEG	1
105	6	5	PT2012AUJgBSERPS4	POS	POS	1
106	6	6	PT2012AUJgBSERNS1	NEG	NEG	1
107	6	7	PT2012AUJgBSERNS2	NEG	NEG	1
108	6	8	PT2012AUJgBSERNS1	NEG	NEG	1
109	6	9	PT2012AUJgBSERPS2	POS	POS	1
110	6	10	PT2012AUJgBSERPS1	POS	POS	1
111	6	11	PT2012AUJgBSERPS3	POS	POS	1
112	6	12	PT2012AUJgBSERPS2	POS	POS	1
113	6	13	PT2012AUJgBSERNS2	NEG	NEG	1
114	6	14	PT2012AUJgBSERPS3	POS	POS	1
115	6	15	PT2012AUJgBSERPS4	POS	POS	1
116	6	16	PT2012AUJgBSERNS2	NEG	NEG	1
117	6	17	PT2012AUJgBSERPS3	POS	POS	1
118	6	18	PT2012AUJgBSERPS1	POS	POS	1
119	6	19	PT2012AUJgBSERPS4	POS	POS	1
120	6	20	PT2012AUJgBSERPS4	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2012AUJgBSERPS1	POS	POS	1
122	7	2	PT2012AUJgBSERPS4	POS	POS	1
123	7	3	PT2012AUJgBSERPS4	POS	POS	1
124	7	4	PT2012AUJgBSERPS1	POS	POS	1
125	7	5	PT2012AUJgBSERPS2	POS	POS	1
126	7	6	PT2012AUJgBSERPS3	POS	POS	1
127	7	7	PT2012AUJgBSERNS1	NEG	NEG	1
128	7	8	PT2012AUJgBSERPS4	POS	POS	1
129	7	9	PT2012AUJgBSERNS1	NEG	NEG	1
130	7	10	PT2012AUJgBSERNS2	NEG	NEG	1
131	7	11	PT2012AUJgBSERNS1	NEG	NEG	1
132	7	12	PT2012AUJgBSERPS2	POS	POS	1
133	7	13	PT2012AUJgBSERPS1	POS	POS	1
134	7	14	PT2012AUJgBSERPS3	POS	POS	1
135	7	15	PT2012AUJgBSERPS2	POS	POS	1
136	7	16	PT2012AUJgBSERNS2	NEG	NEG	1
137	7	17	PT2012AUJgBSERPS3	POS	POS	1
138	7	18	PT2012AUJgBSERPS4	POS	POS	1
139	7	19	PT2012AUJgBSERNS2	NEG	NEG	1
140	7	20	PT2012AUJgBSERPS3	POS	POS	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 ADV reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012AUJgESERNS2	NEG	NEG	1
2	1	2	PT2012AUJgESERPS1	POS	POS	1
3	1	3	PT2012AUJgESERPS3	POS	POS	1
4	1	4	PT2012AUJgESERNS1	NEG	NEG	1
5	1	5	PT2012AUJgESERPS1	POS	POS	1
6	1	6	PT2012AUJgESERNS3	NEG	NEG	1
7	1	7	PT2012AUJgESERPS2	POS	POS	1
8	1	8	PT2012AUJgESERPS1	POS	POS	1
9	1	9	PT2012AUJgESERPS3	POS	POS	1
10	1	10	PT2012AUJgESERNS1	NEG	NEG	1
11	1	11	PT2012AUJgESERPS3	POS	POS	1
12	1	12	PT2012AUJgESERNS3	NEG	NEG	1
13	1	13	PT2012AUJgESERPS2	POS	POS	1
14	1	14	PT2012AUJgESERNS2	NEG	NEG	1
15	1	15	PT2012AUJgESERPS1	POS	POS	1
16	1	16	PT2012AUJgESERNS3	NEG	NEG	1
17	1	17	PT2012AUJgESERPS2	POS	POS	1
18	1	18	PT2012AUJgESERNS1	NEG	NEG	1
19	1	19	PT2012AUJgESERPS2	POS	POS	1
20	1	20	PT2012AUJgESERNS2	NEG	NEG	1
21	2	1	PT2012AUJgESERNS1	NEG	NEG	1
22	2	2	PT2012AUJgESERPS2	POS	POS	1
23	2	3	PT2012AUJgESERNS2	NEG	NEG	1
24	2	4	PT2012AUJgESERNS2	NEG	NEG	1
25	2	5	PT2012AUJgESERPS1	POS	POS	1
26	2	6	PT2012AUJgESERPS3	POS	POS	1
27	2	7	PT2012AUJgESERNS1	NEG	NEG	1
28	2	8	PT2012AUJgESERPS1	POS	POS	1
29	2	9	PT2012AUJgESERNS3	NEG	NEG	1
30	2	10	PT2012AUJgESERPS2	POS	POS	1
31	2	11	PT2012AUJgESERPS1	POS	POS	1
32	2	12	PT2012AUJgESERPS3	POS	POS	1
33	2	13	PT2012AUJgESERNS1	NEG	NEG	1
34	2	14	PT2012AUJgESERPS3	POS	POS	1
35	2	15	PT2012AUJgESERNS3	NEG	NEG	1
36	2	16	PT2012AUJgESERPS2	POS	POS	1
37	2	17	PT2012AUJgESERNS2	NEG	NEG	1
38	2	18	PT2012AUJgESERPS1	POS	POS	1
39	2	19	PT2012AUJgESERNS3	NEG	NEG	1
40	2	20	PT2012AUJgESERPS2	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012AUJgESERPS1	POS	POS	1
42	3	2	PT2012AUJgESERNS3	NEG	NEG	1
43	3	3	PT2012AUJgESERPS2	POS	POS	1
44	3	4	PT2012AUJgESERNS1	NEG	NEG	1
45	3	5	PT2012AUJgESERPS2	POS	POS	1
46	3	6	PT2012AUJgESERNS2	NEG	NEG	1
47	3	7	PT2012AUJgESERNS2	NEG	NEG	1
48	3	8	PT2012AUJgESERPS1	POS	POS	1
49	3	9	PT2012AUJgESERPS3	POS	POS	1
50	3	10	PT2012AUJgESERNS1	NEG	NEG	1
51	3	11	PT2012AUJgESERPS1	POS	POS	1
52	3	12	PT2012AUJgESERNS3	NEG	NEG	1
53	3	13	PT2012AUJgESERPS2	POS	POS	1
54	3	14	PT2012AUJgESERPS1	POS	POS	1
55	3	15	PT2012AUJgESERPS3	POS	POS	1
56	3	16	PT2012AUJgESERNS1	NEG	NEG	1
57	3	17	PT2012AUJgESERPS3	POS	POS	1
58	3	18	PT2012AUJgESERNS3	NEG	NEG	1
59	3	19	PT2012AUJgESERPS2	POS	POS	1
60	3	20	PT2012AUJgESERNS2	NEG	NEG	1
61	4	1	PT2012AUJgESERNS3	NEG	NEG	1
62	4	2	PT2012AUJgESERPS2	POS	POS	1
63	4	3	PT2012AUJgESERNS2	NEG	NEG	1
64	4	4	PT2012AUJgESERPS1	POS	POS	1
65	4	5	PT2012AUJgESERNS3	NEG	NEG	1
66	4	6	PT2012AUJgESERPS2	POS	POS	1
67	4	7	PT2012AUJgESERNS1	NEG	NEG	1
68	4	8	PT2012AUJgESERPS2	POS	POS	1
69	4	9	PT2012AUJgESERNS2	NEG	NEG	1
70	4	10	PT2012AUJgESERNS2	NEG	NEG	1
71	4	11	PT2012AUJgESERPS1	POS	POS	1
72	4	12	PT2012AUJgESERPS3	POS	POS	1
73	4	13	PT2012AUJgESERNS1	NEG	NEG	1
74	4	14	PT2012AUJgESERPS1	POS	POS	1
75	4	15	PT2012AUJgESERNS3	NEG	NEG	1
76	4	16	PT2012AUJgESERPS2	POS	POS	1
77	4	17	PT2012AUJgESERPS1	POS	POS	1
78	4	18	PT2012AUJgESERPS3	POS	POS	1
79	4	19	PT2012AUJgESERNS1	NEG	NEG	1
80	4	20	PT2012AUJgESERPS3	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2012AUJgESERPS3	POS	POS	1
82	5	2	PT2012AUJgESERNS1	NEG	NEG	1
83	5	3	PT2012AUJgESERPS3	POS	POS	1
84	5	4	PT2012AUJgESERNS3	NEG	NEG	1
85	5	5	PT2012AUJgESERPS2	POS	POS	1
86	5	6	PT2012AUJgESERNS2	NEG	NEG	1
87	5	7	PT2012AUJgESERPS1	POS	POS	1
88	5	8	PT2012AUJgESERNS3	NEG	NEG	1
89	5	9	PT2012AUJgESERPS2	POS	POS	1
90	5	10	PT2012AUJgESERNS1	NEG	NEG	1
91	5	11	PT2012AUJgESERPS2	POS	POS	1
92	5	12	PT2012AUJgESERNS2	NEG	NEG	1
93	5	13	PT2012AUJgESERNS2	NEG	NEG	1
94	5	14	PT2012AUJgESERPS1	POS	POS	1
95	5	15	PT2012AUJgESERPS3	POS	POS	1
96	5	16	PT2012AUJgESERNS1	NEG	NEG	1
97	5	17	PT2012AUJgESERPS1	POS	POS	1
98	5	18	PT2012AUJgESERNS3	NEG	NEG	1
99	5	19	PT2012AUJgESERPS2	POS	POS	1
100	5	20	PT2012AUJgESERPS1	POS	POS	1
101	6	1	PT2012AUJgESERNS3	NEG	NEG	1
102	6	2	PT2012AUJgESERPS2	POS	POS	1
103	6	3	PT2012AUJgESERPS1	POS	POS	1
104	6	4	PT2012AUJgESERPS3	POS	POS	1
105	6	5	PT2012AUJgESERNS1	NEG	NEG	1
106	6	6	PT2012AUJgESERPS3	POS	POS	1
107	6	7	PT2012AUJgESERNS3	NEG	NEG	1
108	6	8	PT2012AUJgESERPS2	POS	POS	1
109	6	9	PT2012AUJgESERNS2	NEG	NEG	1
110	6	10	PT2012AUJgESERPS1	POS	POS	1
111	6	11	PT2012AUJgESERNS3	NEG	NEG	1
112	6	12	PT2012AUJgESERPS2	POS	POS	1
113	6	13	PT2012AUJgESERNS1	NEG	NEG	1
114	6	14	PT2012AUJgESERPS2	POS	POS	1
115	6	15	PT2012AUJgESERNS2	NEG	NEG	1
116	6	16	PT2012AUJgESERNS2	NEG	NEG	1
117	6	17	PT2012AUJgESERPS1	POS	POS	1
118	6	18	PT2012AUJgESERPS3	POS	POS	1
119	6	19	PT2012AUJgESERNS1	NEG	NEG	1
120	6	20	PT2012AUJgESERPS1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	8	1	PT2012AUJgESERPS3	POS	POS	1
122	8	2	PT2012AUJgESERNS1	NEG	NEG	1
123	8	3	PT2012AUJgESERPS1	POS	POS	1
124	8	4	PT2012AUJgESERNS3	NEG	NEG	1
125	8	5	PT2012AUJgESERPS2	POS	POS	1
126	8	6	PT2012AUJgESERPS1	POS	POS	1
127	8	7	PT2012AUJgESERPS3	POS	POS	1
128	8	8	PT2012AUJgESERNS1	NEG	NEG	1
129	8	9	PT2012AUJgESERPS3	POS	POS	1
130	8	10	PT2012AUJgESERNS3	NEG	NEG	1
131	8	11	PT2012AUJgESERPS2	POS	POS	1
132	8	12	PT2012AUJgESERNS2	NEG	NEG	1
133	8	13	PT2012AUJgESERPS1	POS	POS	1
134	8	14	PT2012AUJgESERNS3	NEG	NEG	1
135	8	15	PT2012AUJgESERPS2	POS	POS	1
136	8	16	PT2012AUJgESERNS1	NEG	NEG	1
137	8	17	PT2012AUJgESERPS2	POS	POS	1
138	8	18	PT2012AUJgESERNS2	NEG	NEG	1
139	8	19	PT2012AUJgESERNS2	NEG	NEG	1
140	8	20	PT2012AUJgESERPS1	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, all 7 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (Table 2 and Table 4).
The ADV gB participating laboratories used ELISA kits from 4 different producers as well as different batches from the same ELISA kit: IDEXX (1 batch: FH942), Prionics (3 batches: Z110701L, Z110301L, Z110901L), LSI (1 batch: 2-PRVGB-004) and BioChek (1 batch: FS5437). LAB1 and LAB7 on the one hand, and LAB4, LAB5 and LAB6 on the other hand, used an ADV gB ELISA kit from the same producer. Hereby, LAB1 and LAB7 used the same batch and both performed the long incubation protocol. In contrast, LAB4, LAB5 and LAB6 used different batches. LAB4 and LAB6 both performed the short incubation protocol of the used ELISA kit, whereas LAB5 did not provide information about the used incubation protocol.

For the detection of ADV gE-specific antibodies in reference serum samples, all 7 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (Table 3 and Table 5).
The ADV gE participating laboratories used ELISA kits from 3 different producers as well as different batches from the same ELISA kit: IDEXX (4 batches: HG592, BH557, JG917, MG264), LSI (1 batch: 2-PRVGE-003) and BioChek (1 batch: FS5218). LAB1, LAB4, LAB5, LAB6 and LAB8 used an ADV gE ELISA kit from the same producer. Hereby, LAB5 and LAB6 used the same batch. Furthermore, LAB1 performed the long incubation protocol, whereas LAB4 and LAB6 performed the short incubation protocol (LAB5 and LAB8 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 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the ADV reference laboratory of CODA-CERVA (see III.3.3.). Consequently, all participants to the ADV gB PT achieved a satisfactory performance for the detection of ADV gB-specific antibodies in reference serum samples and all participants to the ADV gE PT achieved a satisfactory performance for the detection of ADV gE-specific antibodies in reference serum samples.

Head CVD-ERA
Yves Van der Stede

Appendix

Names of the participating laboratories

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

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

BioChek BV (Reeuwijk, The Netherlands)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

IDEXX Switzerland AG (Bern-Liebefeld, Switzerland)

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

Laboratoire Service International (LSI) (Lissieu, France)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, 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 programs SAS 9.2. (summary statistics) and R (box plots). All quantitative data analyses were performed on the normalized data, namely the percentages blocking calculated according to the instructions for this PT: [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.

Remark: Since LAB2 was the only participant using an indirect ADV gB antibody ELISA, data from this participant could not be included into the comparative quantitative data analysis for the ADV gB PT.

I. Box plots

Box plots of the percentages blocking per reference serum sample and per participating laboratory were made using the statistical software R. Box plots for the laboratories participating in the ADV gB PT and the ADV gE PT are shown in Figure 1 and Figure 2, respectively.

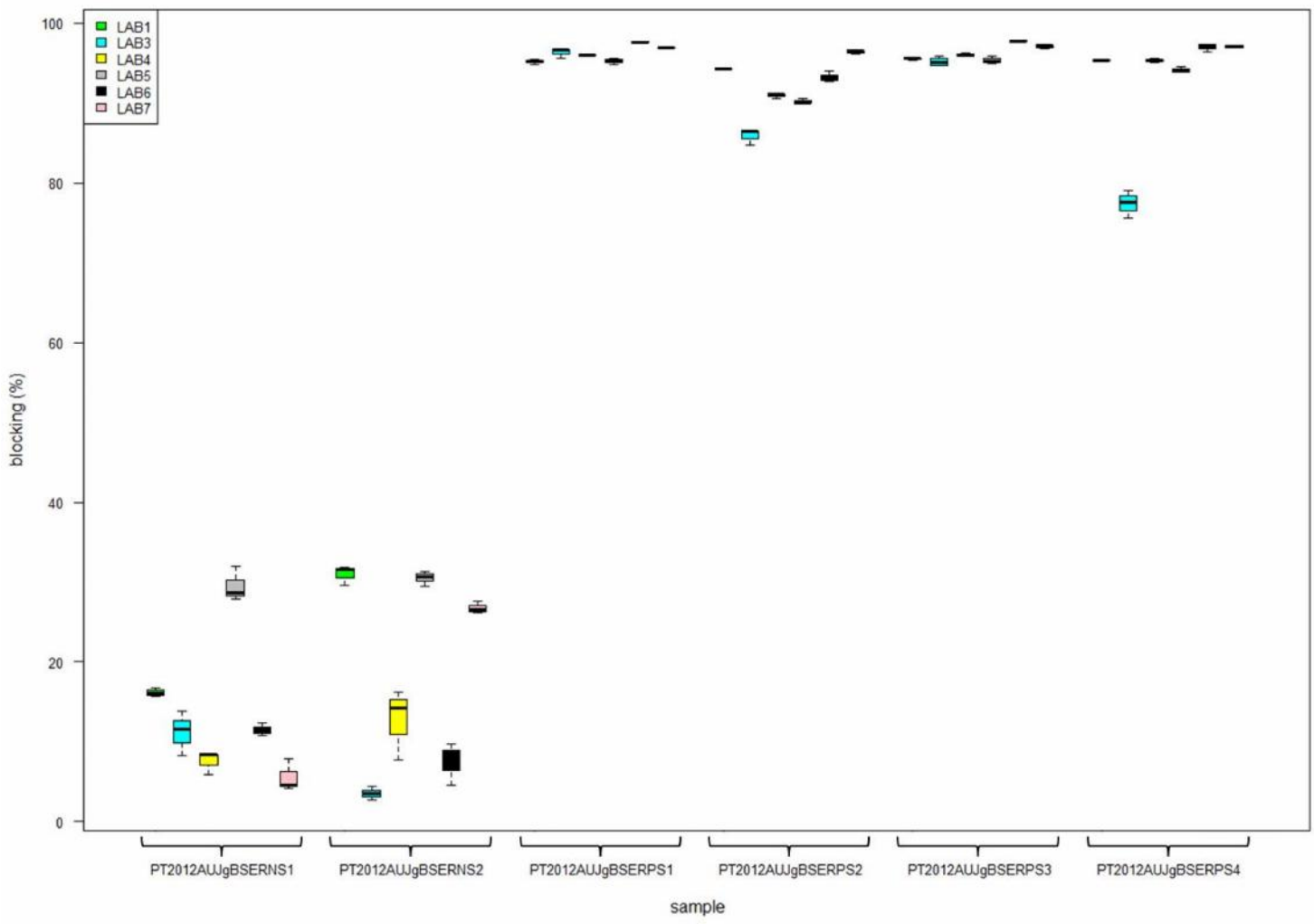

Figure 1. Box plots showing the percentage blocking per ADV gB reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values are not shown since ADV gB ELISA kits from different producers were used. LAB1 and LAB7 on the one hand, and LAB4, LAB5 and LAB6 on the other hand, used an ADV gB ELISA kit from the same producer.

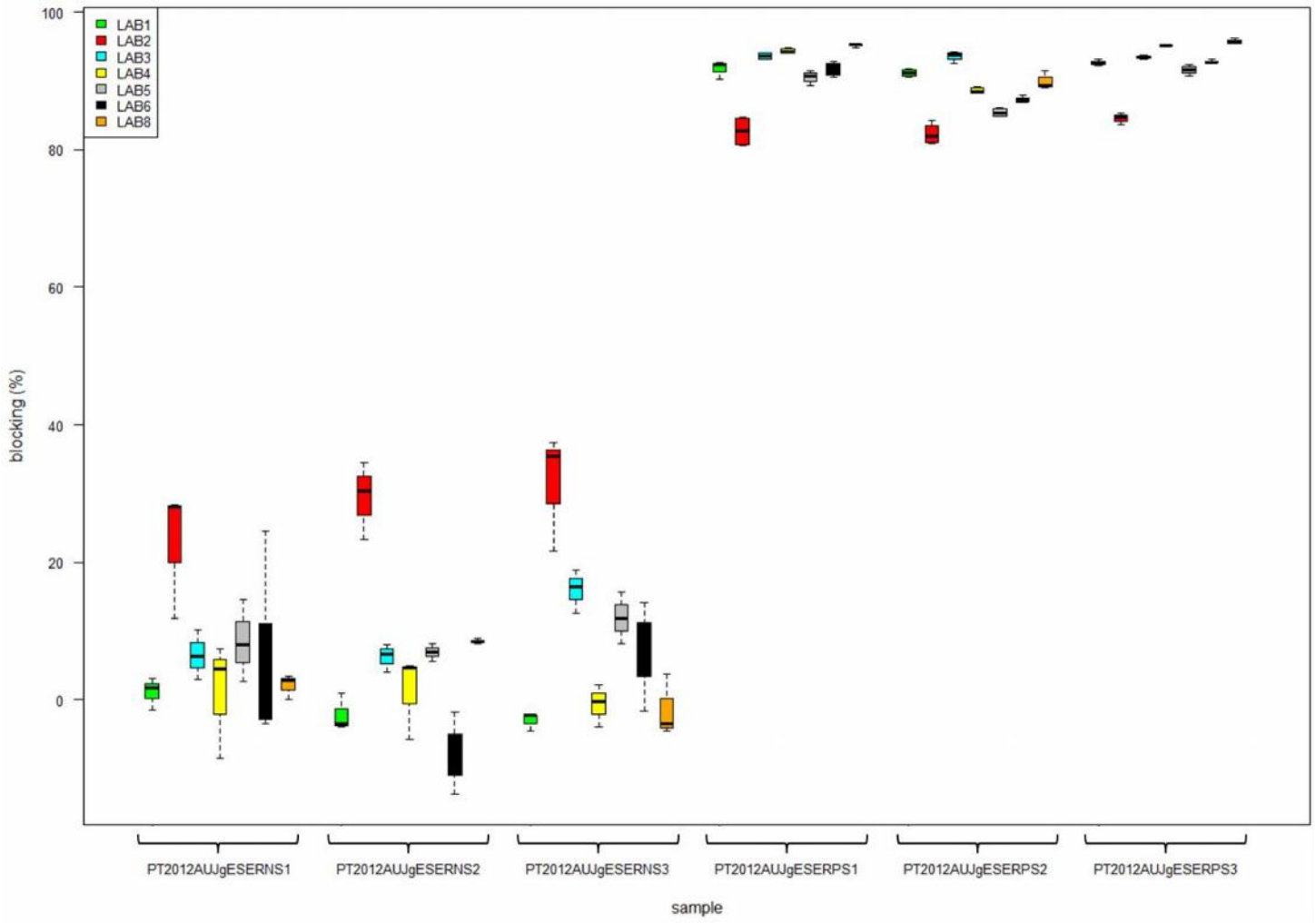

Figure 2. Box plots showing the percentage blocking per ADV gE reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values are not shown since ADV gE ELISA kits from different producers were used. LAB1, LAB4, LAB5, LAB6 and LAB8 used an ADV gE ELISA kit from the same producer.

II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-lab variability (reproducibility) and within-lab variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated based on the percentages blocking per reference serum sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,40	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35
11	1,82	1,91	1,69	1,58	1,51	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic is 1,66 for the ADV gB PT (p=6) and 1,71 for the ADV gE PT (p=7). For the ADV gB PT, the maximum value for Mandel's k-statistic are 1,64 for the reference serum samples PT2012AUJgBSERNS1, PT2012AUJgBSERNS2, PT2012AUJgBSERPS1 and PT2012AUJgBSERPS2 (p=6 and n=3), and 1,54 for the reference serum samples PT2012AUJgBSERPS3 and PT2012AUJgBSERPS4 (p=6 and n=4). For the ADV gE PT, the maximum value for Mandel's k-statistic are 1,66 for the reference serum samples PT2012AUJgESERNS1, PT2012AUJgESERNS2, PT2012AUJgESERNS3 and PT2012AUJgESERPS3 (p=7 and n=3) and 1,55 for the reference serum samples PT2012AUJgESERPS1 and PT2012AUJgESERPS2 (p=7 and n=4).

For the detection of ADV gB-specific antibodies, 4 out of 6 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples: LAB1, LAB4, LAB6 and LAB7. The other participants showed an increased value for Mandel's h-statistic for 1 reference serum sample: LAB3 for the positive reference serum sample PT2012AUJgBSERPS4 (h=-2,02) and LAB5 for the negative reference serum sample PT2012AUJgBSERNS1 (h=1,85). LAB1 and LAB7 on the one hand, and LAB4, LAB5 and LAB6 on the other hand, used an ADV gB ELISA kit from the same producer.
Furthermore, 4 out of 6 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples: LAB1, LAB5, LAB6 and LAB7. The other participants showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB3 for the positive reference serum samples PT2012AUJgBSERPS1 (k=1,80), PT2012AUJgBSERPS2 (k=1,87), PT2012AUJgBSERPS3 (k=1,76) and PT2012AUJgBSERPS4 (k=2,27), and LAB4 for the negative reference serum sample PT2012AUJgBSERNS2 (k=1,98).

For the detection of ADV gE-specific antibodies, 6 out of 7 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples: LAB1, LAB3, LAB4, LAB5, LAB6 and LAB8. In contrast, LAB2 showed an increased value for Mandel's h-statistic for 5 out of 6 reference serum samples: PT2012AUJgESERNS1 (h=2,09), PT2012AUJgESERNS2 (h=1,98), PT2012AUJgESERNS3 (h=1,84), PT2012AUJgESERPS1 (h=-2,09) and PT2012AUJgESERPS3 (h=-2,09). LAB1, LAB4, LAB5, LAB6 and LAB8 used an ADV gE ELISA kit from the same producer.
Furthermore, 5 out of 7 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples: LAB1, LAB3, LAB4, LAB5 and LAB8. The other participants showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB2 for the positive reference serum samples PT2012AUJgESERPS1 (k=2,00) and PT2012AUJgESERPS2 (k=1,73), and LAB6 for the negative reference serum sample PT2012AUJgESERNS1 (k=1,93).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values (in this case the percentages blocking) as a dependent variable, it was investigated whether statistically significant differences exist (α=0,05) between participating laboratories. Comparisons were made at the global level (all reference serum samples were analysed together), status level (all reference serum samples with the same status were analysed together) and sample level (all reference serum samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

III.1. ADV gB

For the ADV gB PT, no statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.
At the status level, significant differences were observed for both the negative and positive reference serum samples. The percentages blocking for the negative reference serum samples reported by LAB5 were significantly higher than those reported by the other participants, except LAB1, whereas the percentages blocking for the positive reference serum samples reported by LAB3 were significantly lower than those reported by the other participants.

III.2. ADV gE

For the ADV gE PT, no statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.
At the status level, significant differences were observed for both the negative and positive reference serum samples. LAB2 reported percentages blocking that were significantly higher than those reported by the other participants for the negative reference serum samples, whereas the same participant reported percentages blocking were significantly lower than those reported by the other participants for the positive reference serum samples.

Annex 2: Calculations of Mandel's h- and k-statistics (based on % blocking)

A. ADV gB

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012AUJgBSERNS1	1	3	0,32	16,17	13,58	0,81	1,82	4,23	3,82	0,30	0,31	3,49
PT2012AUJgBSERNS1	3	3	7,71	11,21	13,58	0,81	1,82	4,23	3,82	-0,28	1,53	24,77
PT2012AUJgBSERNS1	4	3	2,30	7,58	13,58	0,81	1,82	4,23	3,82	-0,70	0,83	19,98
PT2012AUJgBSERNS1	5	3	4,80	29,47	13,58	0,81	1,82	4,23	3,82	1,85	1,20	7,43
PT2012AUJgBSERNS1	6	3	0,64	11,49	13,58	0,81	1,82	4,23	3,82	-0,24	0,44	6,97
PT2012AUJgBSERNS1	7	3	4,09	5,54	13,58	0,81	1,82	4,23	3,82	-0,93	1,11	36,51
PT2012AUJgBSERNS2	1	3	1,54	31,03	18,67	0,85	2,27	5,89	5,44	1,01	0,55	4,00
PT2012AUJgBSERNS2	3	3	0,70	3,51	18,67	0,85	2,27	5,89	5,44	-1,24	0,37	23,83
PT2012AUJgBSERNS2	4	3	20,14	12,72	18,67	0,85	2,27	5,89	5,44	-0,49	1,98	35,29
PT2012AUJgBSERNS2	5	3	0,90	30,51	18,67	0,85	2,27	5,89	5,44	0,97	0,42	3,11
PT2012AUJgBSERNS2	6	3	7,07	7,52	18,67	0,85	2,27	5,89	5,44	-0,91	1,17	35,37
PT2012AUJgBSERNS2	7	3	0,60	26,77	18,67	0,85	2,27	5,89	5,44	0,66	0,34	2,88
PT2012AUJgBSERPS1	1	3	0,11	95,15	96,21	0,62	0,33	0,54	0,42	-1,10	1,01	0,35
PT2012AUJgBSERPS1	3	3	0,35	96,35	96,21	0,62	0,33	0,54	0,42	0,14	1,80	0,62
PT2012AUJgBSERPS1	4	3	0,01	96,00	96,21	0,62	0,33	0,54	0,42	-0,22	0,29	0,10
PT2012AUJgBSERPS1	5	3	0,16	95,23	96,21	0,62	0,33	0,54	0,42	-1,02	1,21	0,42
PT2012AUJgBSERPS1	6	3	0,02	97,61	96,21	0,62	0,33	0,54	0,42	1,45	0,39	0,13
PT2012AUJgBSERPS1	7	3	0,01	96,94	96,21	0,62	0,33	0,54	0,42	0,76	0,22	0,07
PT2012AUJgBSERPS2	1	3	0,01	94,27	91,82	0,90	0,55	1,73	1,64	0,66	0,20	0,12
PT2012AUJgBSERPS2	3	3	1,04	85,88	91,82	0,90	0,55	1,73	1,64	-1,61	1,87	1,19
PT2012AUJgBSERPS2	4	3	0,11	90,91	91,82	0,90	0,55	1,73	1,64	-0,25	0,61	0,37
PT2012AUJgBSERPS2	5	3	0,11	90,20	91,82	0,90	0,55	1,73	1,64	-0,44	0,62	0,38
PT2012AUJgBSERPS2	6	3	0,46	93,25	91,82	0,90	0,55	1,73	1,64	0,39	1,24	0,73
PT2012AUJgBSERPS2	7	3	0,05	96,42	91,82	0,90	0,55	1,73	1,64	1,25	0,40	0,23

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012AUJgBSERPS3	1	4	0,02	95,57	96,15	0,69	0,31	0,56	0,46	-0,56	0,50	0,16
PT2012AUJgBSERPS3	**3**	4	0,29	95,16	96,15	0,69	0,31	0,56	0,46	-0,94	**1,76**	0,57
PT2012AUJgBSERPS3	4	4	0,04	95,99	96,15	0,69	0,31	0,56	0,46	-0,15	0,62	0,20
PT2012AUJgBSERPS3	5	4	0,15	95,33	96,15	0,69	0,31	0,56	0,46	-0,78	1,27	0,41
PT2012AUJgBSERPS3	6	4	0,01	97,75	96,15	0,69	0,31	0,56	0,46	1,52	0,31	0,10
PT2012AUJgBSERPS3	7	4	0,05	97,12	96,15	0,69	0,31	0,56	0,46	0,92	0,75	0,24
PT2012AUJgBSERPS4	1	4	0,00	95,38	92,73	0,97	0,61	3,44	3,38	0,35	0,04	0,03
PT2012AUJgBSERPS4	**3**	4	1,93	77,44	92,73	0,97	0,61	3,44	3,38	**-2,02**	**2,27**	1,79
PT2012AUJgBSERPS4	4	4	0,05	95,38	92,73	0,97	0,61	3,44	3,38	0,35	0,38	0,24
PT2012AUJgBSERPS4	5	4	0,09	94,13	92,73	0,97	0,61	3,44	3,38	0,18	0,49	0,32
PT2012AUJgBSERPS4	6	4	0,15	97,01	92,73	0,97	0,61	3,44	3,38	0,56	0,64	0,40
PT2012AUJgBSERPS4	7	4	0,01	97,06	92,73	0,97	0,61	3,44	3,38	0,57	0,18	0,11

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised data (% blocking); **x_i_m** = mean of normalized data (% blocking); **x_g_m** = mean of normalized data (% blocking) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).

B. ADV gE

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	Cv
PT2012AUJgESERNS1	1	3	5,25	1,10	6,89	0,08	8,20	8,55	2,42	-0,76	0,28	208,05
PT2012AUJgESERNS1	2	3	90,97	22,77	6,89	0,08	8,20	8,55	2,42	2,09	1,16	41,89
PT2012AUJgESERNS1	3	3	12,53	6,50	6,89	0,08	8,20	8,55	2,42	-0,05	0,43	54,49
PT2012AUJgESERNS1	4	3	71,70	1,12	6,89	0,08	8,20	8,55	2,42	-0,76	1,03	758,06
PT2012AUJgESERNS1	5	3	35,37	8,42	6,89	0,08	8,20	8,55	2,42	0,20	0,73	70,60
PT2012AUJgESERNS1	6	3	251,73	6,24	6,89	0,08	8,20	8,55	2,42	-0,09	1,93	254,38
PT2012AUJgESERNS1	8	3	3,36	2,08	6,89	0,08	8,20	8,55	2,42	-0,63	0,22	88,03
PT2012AUJgESERNS2	1	3	7,19	-2,20	6,02	0,57	4,12	6,27	4,73	-0,70	0,65	-121,76
PT2012AUJgESERNS2	2	3	31,81	29,41	6,02	0,57	4,12	6,27	4,73	1,98	1,37	19,17
PT2012AUJgESERNS2	3	3	4,26	6,19	6,02	0,57	4,12	6,27	4,73	0,01	0,50	33,35
PT2012AUJgESERNS2	4	3	38,03	1,28	6,02	0,57	4,12	6,27	4,73	-0,40	1,50	483,57
PT2012AUJgESERNS2	5	3	1,80	6,90	6,02	0,57	4,12	6,27	4,73	0,07	0,33	19,41
PT2012AUJgESERNS2	6	3	35,52	-7,94	6,02	0,57	4,12	6,27	4,73	-1,18	1,45	-75,08
PT2012AUJgESERNS2	8	3	0,18	8,50	6,02	0,57	4,12	6,27	4,73	0,21	0,10	4,97
PT2012AUJgESERNS3	1	3	1,60	-3,03	8,74	0,46	5,25	7,16	4,88	-0,95	0,24	-41,75
PT2012AUJgESERNS3	2	3	74,02	31,45	8,74	0,46	5,25	7,16	4,88	1,84	1,64	27,36
PT2012AUJgESERNS3	3	3	9,78	15,97	8,74	0,46	5,25	7,16	4,88	0,59	0,60	19,58
PT2012AUJgESERNS3	4	3	9,61	-0,69	8,74	0,46	5,25	7,16	4,88	-0,76	0,59	-451,19
PT2012AUJgESERNS3	5	3	14,07	11,90	8,74	0,46	5,25	7,16	4,88	0,26	0,72	31,51
PT2012AUJgESERNS3	6	3	62,93	6,97	8,74	0,46	5,25	7,16	4,88	-0,14	1,51	113,88
PT2012AUJgESERNS3	8	3	20,59	-1,43	8,74	0,46	5,25	7,16	4,88	-0,82	0,87	-318,34
PT2012AUJgESERPS1	1	4	1,21	91,88	91,43	0,71	1,08	2,01	1,70	0,11	1,02	1,20
PT2012AUJgESERPS1	2	4	4,64	82,66	91,43	0,71	1,08	2,01	1,70	-2,09	2,00	2,61
PT2012AUJgESERPS1	3	4	0,23	93,62	91,43	0,71	1,08	2,01	1,70	0,52	0,44	0,51
PT2012AUJgESERPS1	4	4	0,15	94,35	91,43	0,71	1,08	2,01	1,70	0,70	0,36	0,41

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	Cv
PT2012AUJgESERPS1	5	4	0,76	90,54	91,43	0,71	1,08	2,01	1,70	-0,21	0,81	0,96
PT2012AUJgESERPS1	6	4	1,09	91,74	91,43	0,71	1,08	2,01	1,70	0,07	0,97	1,14
PT2012AUJgESERPS1	8	4	0,05	95,23	91,43	0,71	1,08	2,01	1,70	0,90	0,21	0,23
PT2012AUJgESERPS2	1	4	0,33	91,14	88,30	0,75	0,87	1,75	1,51	0,76	0,66	0,63
PT2012AUJgESERPS2	**2**	4	2,27	82,29	88,30	0,75	0,87	1,75	1,51	-1,61	**1,73**	1,83
PT2012AUJgESERPS2	3	4	0,53	93,58	88,30	0,75	0,87	1,75	1,51	1,41	0,83	0,78
PT2012AUJgESERPS2	4	4	0,21	88,59	88,30	0,75	0,87	1,75	1,51	0,08	0,52	0,51
PT2012AUJgESERPS2	5	4	0,39	85,42	88,30	0,75	0,87	1,75	1,51	-0,77	0,72	0,73
PT2012AUJgESERPS2	6	4	0,22	87,27	88,30	0,75	0,87	1,75	1,51	-0,27	0,54	0,54
PT2012AUJgESERPS2	8	4	1,37	89,80	88,30	0,75	0,87	1,75	1,51	0,40	1,34	1,30
PT2012AUJgESERPS3	1	3	0,25	92,59	92,26	0,89	0,53	1,60	1,51	0,09	0,95	0,54
PT2012AUJgESERPS3	**2**	3	0,69	84,52	92,26	0,89	0,53	1,60	1,51	**-2,09**	1,56	0,98
PT2012AUJgESERPS3	3	3	0,07	93,45	92,26	0,89	0,53	1,60	1,51	0,32	0,50	0,28
PT2012AUJgESERPS3	4	3	0,01	95,13	92,26	0,89	0,53	1,60	1,51	0,77	0,21	0,12
PT2012AUJgESERPS3	5	3	0,72	91,58	92,26	0,89	0,53	1,60	1,51	-0,18	1,59	0,93
PT2012AUJgESERPS3	6	3	0,06	92,79	92,26	0,89	0,53	1,60	1,51	0,14	0,47	0,27
PT2012AUJgESERPS3	8	3	0,18	95,75	92,26	0,89	0,53	1,60	1,51	0,94	0,79	0,44

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised data (% blocking); **x_i_m** = mean of normalized data (% blocking); **x_g_m** = mean of normalized data (% blocking) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).