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172-PT

PROFICIENCY TESTING 2014

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: 16 JUNE 2014

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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 'PT2014AUJgBSERNS1' and 'PT2014AUJgBSERNS2') or containing detectable ADV gB-specific antibodies (n = 4; coded 'PT2014AUJgBSERPS1', 'PT2014AUJgBSERPS2', 'PT2014AUJgBSERPS3' and 'PT2014AUJgBSERPS4'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2014AUJgBSERPS1, PT2014AUJgBSERPS2, PT2014AUJgBSERPS3, PT2014AUJgBSERPS4 and 4 aliquots of the reference serum samples PT2014AUJgBSERNS1 and PT2014AUJgBSERNS2. 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 and a seroneutralisation assay (SN). The reference serum samples PT2014AUJgBSERNS1 and PT2014AUJgBSERNS2 were obtained from both uninfected and non-vaccinated animals. The reference serum samples PT2014AUJgBSERPS1 and PT2014AUJgBSERPS2 were obtained from two 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 PT2014AUJgBSERPS2 was a 1/16 dilution of the original serum. The reference serum samples PT2014AUJgBSERPS3 and PT2014AUJgBSERPS4 were a 1/4 and 1/32 dilution respectively of a serum obtained from a naturally ADV-infected animal.

For each reference serum sample, the same qualitative result was obtained with both test methods used. Taken together, the reference serum samples PT2014AUJgBSERNS1 and PT2014AUJgBSERNS2 were considered as negative sera, and the reference serum samples PT2014AUJgBSERPS1, PT2014AUJgBSERPS2, PT2014AUJgBSERPS3 and PT2014AUJgBSERPS4 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, 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.

Remark: For the PT ADV gB, an additional panel consisting of 9 aliquots from 3 different serum samples (3 aliquots per sample) with variable qualitative results in ELISA but negative in SN, the reference test (golden standard) for the detection of ADV gB-specific antibodies in serum, was sent to the 6 participating laboratories along with the regular ADV gB PT samples. Since this was out of scope of this PT, a description of these additional serum samples as well as the qualitative and quantitative data analysis is summarized in Annex 3.

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 'PT2014AUJgESERNS1', 'PT2014AUJgESERNS2' and 'PT2014AUJgESERNS3') or containing detectable ADV gE-specific antibodies ($n = 3$; coded 'PT2014AUJgESERPS1', 'PT2014AUJgESERPS2' and 'PT2014AUJgESERPS3'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2014AUJgESERNS1, PT2014AUJgESERNS2, PT2014AUJgESERNS3, PT2014AUJgESERPS1 and PT2014AUJgESERPS2 and 4 aliquots of the reference serum samples PT2014AUJgESERPS3 and PT2014AUJgESERPS4. 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 gpl antibody ELISA test from IDEXX. The reference serum samples free from detectable ADV gE-specific antibodies are the same as the ADVgB reference samples and described in 2.1.: PT2014AUJgESERNS1 (=PT2014AUJgBSERNS1), PT2014AUJgESERNS2 (=PT2014AUJgBSERNS2) and PT2014AUJgESERNS3 (=PT2014AUJgBSERNS3). The reference serum sample PT2014AUJgESERPS1 is the same sample as PT2014AUJgBSERPS4, a 1/32 dilution of a serum obtained from a naturally ADV-infected animal. The reference serum samples PT2014AUJgESERPS2 and PT2014AUJgESERPS3 were a 1/2 and a 1/16 dilution of a serum obtained from an experimentally ADV-infected animal (with an ADV strain isolated from a wolf in 2011 in Belgium). Taken together, the reference serum samples PT2014AUJgESERNS1, PT2014AUJgESERNS2 and PT2014AUJgESERNS3 were considered as negative sera, whereas the reference serum samples PT2014AUJgESERPS1, PT2014AUJgESERPS2 and PT2014AUJgESERPS3 as variably 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 gpl 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 gpl 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 operational unit CVD-ERA of CODA-CERVA.

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

LAB1 until LAB5 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, LAB7 only participated in the PT ADV gE while LAB 6 only participated in the PT ADV gB. Those labs 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 16th of June 2014 (240 aliquots in total). LAB2, LAB3, LAB4 and LAB7 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 17th (LAB1 and LAB5) or 18th (LAB6) of June 2014. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 17th and 28th of June 2014 (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 18th of June and 7th of July 2014. Only one laboratory (LAB5) did not respect the deadline of 4th of July 2014 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.

Participating laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	17/06/2014	23/06/2014	23/06/2014	02/07/2014
LAB2	16/06/2014	25/06/2014	24/06/2014	26/06/2014
LAB3	16/06/2014	23/06/2014	23/06/2014	30/06/2014
LAB4	16/06/2014	17/06/2014	17/06/2014	28/06/2014
LAB5	17/06/2014	28/06/2014	28/06/2014	07/07/2014
LAB6	18/06/2014	01/07/2014	NA	03/07/2014
LAB7	16/06/2014	NA	18/06/2014	18/06/2014

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**, 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 2).



- (ii) For the detection of **ADV gE-specific antibodies**, all participating laboratories, except LAB5, provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence obtained 100% of agreement. LAB5 misclassified 7 samples and hence obtained 65% 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 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
failure	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)

Table 3. Agreement between results obtained by the participating laboratories (LABNR) and the status of the **ADV gE** reference serum samples assigned by 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	7
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (35.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	13 (65.0)	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 antibodies in reference serum samples, no variability between qualitative laboratory results could be observed.

For the detection of ADVgE-specific antibodies, LAB5 misclassified 7 aliquots in total: 2 aliquots of the reference serum samples PT2014AUJgESERNS1 and PT2014AUJgESERNS2 and 3 aliquots of the reference serum sample PT2014AUJgESERNS3. All these aliquots were reported as positive instead of negative.

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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014AUJgBSERPS1	POS	POS	1
2	1	2	PT2014AUJgBSERNS1	NEG	NEG	1
3	1	3	PT2014AUJgBSERPS2	POS	POS	1
4	1	4	PT2014AUJgBSERNS2	NEG	NEG	1
5	1	5	PT2014AUJgBSERNS2	NEG	NEG	1
6	1	6	PT2014AUJgBSERPS2	POS	POS	1
7	1	7	PT2014AUJgBSERPS1	POS	POS	1
8	1	8	PT2014AUJgBSERPS3	POS	POS	1
9	1	9	PT2014AUJgBSERNS1	NEG	NEG	1
10	1	10	PT2014AUJgBSERPS4	POS	POS	1
11	1	11	PT2014AUJgBSERPS3	POS	POS	1
12	1	12	PT2014AUJgBSERPS4	POS	POS	1
13	1	13	PT2014AUJgBSERNS1	NEG	NEG	1
14	1	14	PT2014AUJgBSERNS2	NEG	NEG	1
15	1	15	PT2014AUJgBSERPS3	POS	POS	1
16	1	16	PT2014AUJgBSERNS1	NEG	NEG	1
17	1	17	PT2014AUJgBSERPS1	POS	POS	1
18	1	18	PT2014AUJgBSERPS2	POS	POS	1
19	1	19	PT2014AUJgBSERNS2	NEG	NEG	1
20	1	20	PT2014AUJgBSERPS4	POS	POS	1
21	2	1	PT2014AUJgBSERNS2	NEG	NEG	1
22	2	2	PT2014AUJgBSERNS2	NEG	NEG	1
23	2	3	PT2014AUJgBSERPS2	POS	POS	1
24	2	4	PT2014AUJgBSERPS1	POS	POS	1
25	2	5	PT2014AUJgBSERPS3	POS	POS	1
26	2	6	PT2014AUJgBSERNS1	NEG	NEG	1
27	2	7	PT2014AUJgBSERPS4	POS	POS	1
28	2	8	PT2014AUJgBSERPS3	POS	POS	1
29	2	9	PT2014AUJgBSERPS4	POS	POS	1
30	2	10	PT2014AUJgBSERNS1	NEG	NEG	1
31	2	11	PT2014AUJgBSERNS2	NEG	NEG	1
32	2	12	PT2014AUJgBSERPS3	POS	POS	1
33	2	13	PT2014AUJgBSERNS1	NEG	NEG	1
34	2	14	PT2014AUJgBSERPS1	POS	POS	1
35	2	15	PT2014AUJgBSERPS2	POS	POS	1
36	2	16	PT2014AUJgBSERNS2	NEG	NEG	1
37	2	17	PT2014AUJgBSERPS4	POS	POS	1
38	2	18	PT2014AUJgBSERPS1	POS	POS	1
39	2	19	PT2014AUJgBSERNS1	NEG	NEG	1
40	2	20	PT2014AUJgBSERPS2	POS	POS	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2014AUJgBSERPS1	POS	POS	1
42	3	2	PT2014AUJgBSERNS1	NEG	NEG	1
43	3	3	PT2014AUJgBSERPS2	POS	POS	1
44	3	4	PT2014AUJgBSERNS2	NEG	NEG	1
45	3	5	PT2014AUJgBSERNS2	NEG	NEG	1
46	3	6	PT2014AUJgBSERPS2	POS	POS	1
47	3	7	PT2014AUJgBSERPS1	POS	POS	1
48	3	8	PT2014AUJgBSERPS3	POS	POS	1
49	3	9	PT2014AUJgBSERNS1	NEG	NEG	1
50	3	10	PT2014AUJgBSERPS4	POS	POS	1
51	3	11	PT2014AUJgBSERPS3	POS	POS	1
52	3	12	PT2014AUJgBSERPS4	POS	POS	1
53	3	13	PT2014AUJgBSERNS1	NEG	NEG	1
54	3	14	PT2014AUJgBSERNS2	NEG	NEG	1
55	3	15	PT2014AUJgBSERPS3	POS	POS	1
56	3	16	PT2014AUJgBSERNS1	NEG	NEG	1
57	3	17	PT2014AUJgBSERPS1	POS	POS	1
58	3	18	PT2014AUJgBSERPS2	POS	POS	1
59	3	19	PT2014AUJgBSERNS2	NEG	NEG	1
60	3	20	PT2014AUJgBSERPS4	POS	POS	1
61	4	1	PT2014AUJgBSERNS2	NEG	NEG	1
62	4	2	PT2014AUJgBSERNS2	NEG	NEG	1
63	4	3	PT2014AUJgBSERPS2	POS	POS	1
64	4	4	PT2014AUJgBSERPS1	POS	POS	1
65	4	5	PT2014AUJgBSERPS3	POS	POS	1
66	4	6	PT2014AUJgBSERNS1	NEG	NEG	1
67	4	7	PT2014AUJgBSERPS4	POS	POS	1
68	4	8	PT2014AUJgBSERPS3	POS	POS	1
69	4	9	PT2014AUJgBSERPS4	POS	POS	1
70	4	10	PT2014AUJgBSERNS1	NEG	NEG	1
71	4	11	PT2014AUJgBSERNS2	NEG	NEG	1
72	4	12	PT2014AUJgBSERPS3	POS	POS	1
73	4	13	PT2014AUJgBSERNS1	NEG	NEG	1
74	4	14	PT2014AUJgBSERPS1	POS	POS	1
75	4	15	PT2014AUJgBSERPS2	POS	POS	1
76	4	16	PT2014AUJgBSERNS2	NEG	NEG	1
77	4	17	PT2014AUJgBSERPS4	POS	POS	1
78	4	18	PT2014AUJgBSERPS1	POS	POS	1
79	4	19	PT2014AUJgBSERNS1	NEG	NEG	1
80	4	20	PT2014AUJgBSERPS2	POS	POS	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2014AUJgBSERPS1	POS	POS	1
82	5	2	PT2014AUJgBSERNS1	NEG	NEG	1
83	5	3	PT2014AUJgBSERPS2	POS	POS	1
84	5	4	PT2014AUJgBSERNS2	NEG	NEG	1
85	5	5	PT2014AUJgBSERNS2	NEG	NEG	1
86	5	6	PT2014AUJgBSERPS2	POS	POS	1
87	5	7	PT2014AUJgBSERPS1	POS	POS	1
88	5	8	PT2014AUJgBSERPS3	POS	POS	1
89	5	9	PT2014AUJgBSERNS1	NEG	NEG	1
90	5	10	PT2014AUJgBSERPS4	POS	POS	1
91	5	11	PT2014AUJgBSERPS3	POS	POS	1
92	5	12	PT2014AUJgBSERPS4	POS	POS	1
93	5	13	PT2014AUJgBSERNS1	NEG	NEG	1
94	5	14	PT2014AUJgBSERNS2	NEG	NEG	1
95	5	15	PT2014AUJgBSERPS3	POS	POS	1
96	5	16	PT2014AUJgBSERNS1	NEG	NEG	1
97	5	17	PT2014AUJgBSERPS1	POS	POS	1
98	5	18	PT2014AUJgBSERPS2	POS	POS	1
99	5	19	PT2014AUJgBSERNS2	NEG	NEG	1
100	5	20	PT2014AUJgBSERPS4	POS	POS	1
101	6	1	PT2014AUJgBSERNS2	NEG	NEG	1
102	6	2	PT2014AUJgBSERNS2	NEG	NEG	1
103	6	3	PT2014AUJgBSERPS2	POS	POS	1
104	6	4	PT2014AUJgBSERPS1	POS	POS	1
105	6	5	PT2014AUJgBSERPS3	POS	POS	1
106	6	6	PT2014AUJgBSERNS1	NEG	NEG	1
107	6	7	PT2014AUJgBSERPS4	POS	POS	1
108	6	8	PT2014AUJgBSERPS3	POS	POS	1
109	6	9	PT2014AUJgBSERPS4	POS	POS	1
110	6	10	PT2014AUJgBSERNS1	NEG	NEG	1
111	6	11	PT2014AUJgBSERNS2	NEG	NEG	1
112	6	12	PT2014AUJgBSERPS3	POS	POS	1
113	6	13	PT2014AUJgBSERNS1	NEG	NEG	1
114	6	14	PT2014AUJgBSERPS1	POS	POS	1
115	6	15	PT2014AUJgBSERPS2	POS	POS	1
116	6	16	PT2014AUJgBSERNS2	NEG	NEG	1
117	6	17	PT2014AUJgBSERPS4	POS	POS	1
118	6	18	PT2014AUJgBSERPS1	POS	POS	1
119	6	19	PT2014AUJgBSERNS1	NEG	NEG	1
120	6	20	PT2014AUJgBSERPS2	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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014AUJgESERPS2	POS	POS	1
2	1	2	PT2014AUJgESERNS2	NEG	NEG	1
3	1	3	PT2014AUJgESERNS1	NEG	NEG	1
4	1	4	PT2014AUJgESERPS2	POS	POS	1
5	1	5	PT2014AUJgESERNS3	NEG	NEG	1
6	1	6	PT2014AUJgESERPS3	POS	POS	1
7	1	7	PT2014AUJgESERNS3	NEG	NEG	1
8	1	8	PT2014AUJgESERPS2	POS	POS	1
9	1	9	PT2014AUJgESERNS2	NEG	NEG	1
10	1	10	PT2014AUJgESERPS1	POS	POS	1
11	1	11	PT2014AUJgESERNS1	NEG	NEG	1
12	1	12	PT2014AUJgESERPS3	POS	POS	1
13	1	13	PT2014AUJgESERNS3	NEG	NEG	1
14	1	14	PT2014AUJgESERPS3	POS	POS	1
15	1	15	PT2014AUJgESERNS2	NEG	NEG	1
16	1	16	PT2014AUJgESERPS1	POS	POS	1
17	1	17	PT2014AUJgESERPS2	POS	POS	1
18	1	18	PT2014AUJgESERNS1	NEG	NEG	1
19	1	19	PT2014AUJgESERPS1	POS	POS	1
20	1	20	PT2014AUJgESERPS3	POS	POS	1
21	2	1	PT2014AUJgESERPS2	POS	POS	1
22	2	2	PT2014AUJgESERNS3	NEG	NEG	1
23	2	3	PT2014AUJgESERPS3	POS	POS	1
24	2	4	PT2014AUJgESERNS3	NEG	NEG	1
25	2	5	PT2014AUJgESERPS2	POS	POS	1
26	2	6	PT2014AUJgESERNS2	NEG	NEG	1
27	2	7	PT2014AUJgESERPS1	POS	POS	1
28	2	8	PT2014AUJgESERNS1	NEG	NEG	1
29	2	9	PT2014AUJgESERPS3	POS	POS	1
30	2	10	PT2014AUJgESERNS3	NEG	NEG	1
31	2	11	PT2014AUJgESERPS3	POS	POS	1
32	2	12	PT2014AUJgESERNS2	NEG	NEG	1
33	2	13	PT2014AUJgESERPS1	POS	POS	1
34	2	14	PT2014AUJgESERPS2	POS	POS	1
35	2	15	PT2014AUJgESERNS1	NEG	NEG	1
36	2	16	PT2014AUJgESERPS1	POS	POS	1
37	2	17	PT2014AUJgESERPS3	POS	POS	1
38	2	18	PT2014AUJgESERPS2	POS	POS	1
39	2	19	PT2014AUJgESERNS2	NEG	NEG	1
40	2	20	PT2014AUJgESERNS1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2014AUJgESERPS2	POS	POS	1
42	3	2	PT2014AUJgESERNS2	NEG	NEG	1
43	3	3	PT2014AUJgESERNS1	NEG	NEG	1
44	3	4	PT2014AUJgESERPS2	POS	POS	1
45	3	5	PT2014AUJgESERNS3	NEG	NEG	1
46	3	6	PT2014AUJgESERPS3	POS	POS	1
47	3	7	PT2014AUJgESERNS3	NEG	NEG	1
48	3	8	PT2014AUJgESERPS2	POS	POS	1
49	3	9	PT2014AUJgESERNS2	NEG	NEG	1
50	3	10	PT2014AUJgESERPS1	POS	POS	1
51	3	11	PT2014AUJgESERNS1	NEG	NEG	1
52	3	12	PT2014AUJgESERPS3	POS	POS	1
53	3	13	PT2014AUJgESERNS3	NEG	NEG	1
54	3	14	PT2014AUJgESERPS3	POS	POS	1
55	3	15	PT2014AUJgESERNS2	NEG	NEG	1
56	3	16	PT2014AUJgESERPS1	POS	POS	1
57	3	17	PT2014AUJgESERPS2	POS	POS	1
58	3	18	PT2014AUJgESERNS1	NEG	NEG	1
59	3	19	PT2014AUJgESERPS1	POS	POS	1
60	3	20	PT2014AUJgESERPS3	POS	POS	1
61	4	1	PT2014AUJgESERPS2	POS	POS	1
62	4	2	PT2014AUJgESERNS3	NEG	NEG	1
63	4	3	PT2014AUJgESERPS3	POS	POS	1
64	4	4	PT2014AUJgESERNS3	NEG	NEG	1
65	4	5	PT2014AUJgESERPS2	POS	POS	1
66	4	6	PT2014AUJgESERNS2	NEG	NEG	1
67	4	7	PT2014AUJgESERPS1	POS	POS	1
68	4	8	PT2014AUJgESERNS1	NEG	NEG	1
69	4	9	PT2014AUJgESERPS3	POS	POS	1
70	4	10	PT2014AUJgESERNS3	NEG	NEG	1
71	4	11	PT2014AUJgESERPS3	POS	POS	1
72	4	12	PT2014AUJgESERNS2	NEG	NEG	1
73	4	13	PT2014AUJgESERPS1	POS	POS	1
74	4	14	PT2014AUJgESERPS2	POS	POS	1
75	4	15	PT2014AUJgESERNS1	NEG	NEG	1
76	4	16	PT2014AUJgESERPS1	POS	POS	1
77	4	17	PT2014AUJgESERPS3	POS	POS	1
78	4	18	PT2014AUJgESERPS2	POS	POS	1
79	4	19	PT2014AUJgESERNS2	NEG	NEG	1
80	4	20	PT2014AUJgESERNS1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2014AUJgESERPS2	POS	POS	1
82	5	2	PT2014AUJgESERNS2	NEG	POS	0
83	5	3	PT2014AUJgESERNS1	NEG	NEG	1
84	5	4	PT2014AUJgESERPS2	POS	POS	1
85	5	5	PT2014AUJgESERNS3	NEG	POS	0
86	5	6	PT2014AUJgESERPS3	POS	POS	1
87	5	7	PT2014AUJgESERNS3	NEG	POS	0
88	5	8	PT2014AUJgESERPS2	POS	POS	1
89	5	9	PT2014AUJgESERNS2	NEG	POS	0
90	5	10	PT2014AUJgESERPS1	POS	POS	1
91	5	11	PT2014AUJgESERNS1	NEG	POS	0
92	5	12	PT2014AUJgESERPS3	POS	POS	1
93	5	13	PT2014AUJgESERNS3	NEG	POS	0
94	5	14	PT2014AUJgESERPS3	POS	POS	1
95	5	15	PT2014AUJgESERNS2	NEG	NEG	1
96	5	16	PT2014AUJgESERPS1	POS	POS	1
97	5	17	PT2014AUJgESERPS2	POS	POS	1
98	5	18	PT2014AUJgESERNS1	NEG	POS	0
99	5	19	PT2014AUJgESERPS1	POS	POS	1
100	5	20	PT2014AUJgESERPS3	POS	POS	1
101	7	1	PT2014AUJgESERPS2	POS	POS	1
102	7	2	PT2014AUJgESERNS3	NEG	NEG	1
103	7	3	PT2014AUJgESERPS3	POS	POS	1
104	7	4	PT2014AUJgESERNS3	NEG	NEG	1
105	7	5	PT2014AUJgESERPS2	POS	POS	1
106	7	6	PT2014AUJgESERNS2	NEG	NEG	1
107	7	7	PT2014AUJgESERPS1	POS	POS	1
108	7	8	PT2014AUJgESERNS1	NEG	NEG	1
109	7	9	PT2014AUJgESERPS3	POS	POS	1
110	7	10	PT2014AUJgESERNS3	NEG	NEG	1
111	7	11	PT2014AUJgESERPS3	POS	POS	1
112	7	12	PT2014AUJgESERNS2	NEG	NEG	1
113	7	13	PT2014AUJgESERPS1	POS	POS	1
114	7	14	PT2014AUJgESERPS2	POS	POS	1
115	7	15	PT2014AUJgESERNS1	NEG	NEG	1
116	7	16	PT2014AUJgESERPS1	POS	POS	1
117	7	17	PT2014AUJgESERPS3	POS	POS	1
118	7	18	PT2014AUJgESERPS2	POS	POS	1
119	7	19	PT2014AUJgESERNS2	NEG	NEG	1
120	7	20	PT2014AUJgESERNS1	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, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement).

All participating laboratories used ADV gB antibody ELISA kits from 3 different commercial kit producers. Hereby, different batches from the same ELISA kit were used: Prionics (2 batches: Z120701L, Z131201L). LAB2, LAB4 and LAB6 used the same batch. One batch (KJ257) of IDEXX and 1 batch (FS5895) of BioChek was used by LAB1 and LAB5 respectively. In addition, LAB1 performed the long incubation protocol, whereas all other laboratories performed the short incubation protocol of the same ELISA kit (different batches).

For the detection of ADV gE-specific antibodies in reference serum samples, 5 out of 6 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB5 misclassified 7 aliquots: 2 aliquots (out of 3) of the reference serum samples PT2014AUJgESERNS1 and PT2014AUJgESERNS2 and all (=3) aliquots of the reference serum sample PT2014AUJgESERNS3. All these aliquots were reported as positive instead of negative (Table 3 and Table 5). Hereby, LAB5 only obtained 65% of agreement with the assigned status of the reference serum samples.

All participants, except LAB5, used the ADV gE antibody ELISA kit from the same commercial kit producer. Hereby, different batches from the same ELISA kit were used: IDEXX (3 batches: LJ351, AK627, GJ838). LAB2, LAB3 and LAB4 used the same batch while LAB1 and LAB7 used another batch. LAB5 used the ADV gE antibody ELISA from Biochek (batch FS5841). Furthermore, LAB1 performed the long incubation protocol, whereas LAB2, LAB3, LAB4 and LAB7 used the short incubation protocol (LAB5 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 CODA-CERVA (see III.3.3.). Consequently, all participants to the PT ADV gB achieved a satisfactory performance for the detection of ADV gB-specific antibodies in porcine serum samples with all kits used.

For the PT ADVgE , 5 out of 6 laboratories achieved a satisfactory performance for the detection of ADV gE-specific antibodies in porcine serum samples. LAB 5 did not achieve a satisfactory performance for the detection of ADV gE-specific antibodies in 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)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

BioChek (Reeuwijk, The Netherlands)

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

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

Prionics Lelystad (Lelystad, The Netherlands)



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 R (box plots) and SAS 9.2. (summary statistics). All quantitative data analyses were performed on the normalized data, namely the percentages blocking calculated according to the instructions for this PT: $[1 - (\text{OD}_{\text{Sample}} / \text{mean OD}_{\text{Negative Kit Controls}})] \times 100$.

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.

I. Box plots

Box plots of the percentages blocking per reference serum sample and per participating (sub)laboratory were made using the statistical software R. 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. Because LAB5 used a different method in order to calculate the percentages of blocking compared to the other participating laboratories, this lab was left out of the box plots in order to avoid scaling problems.

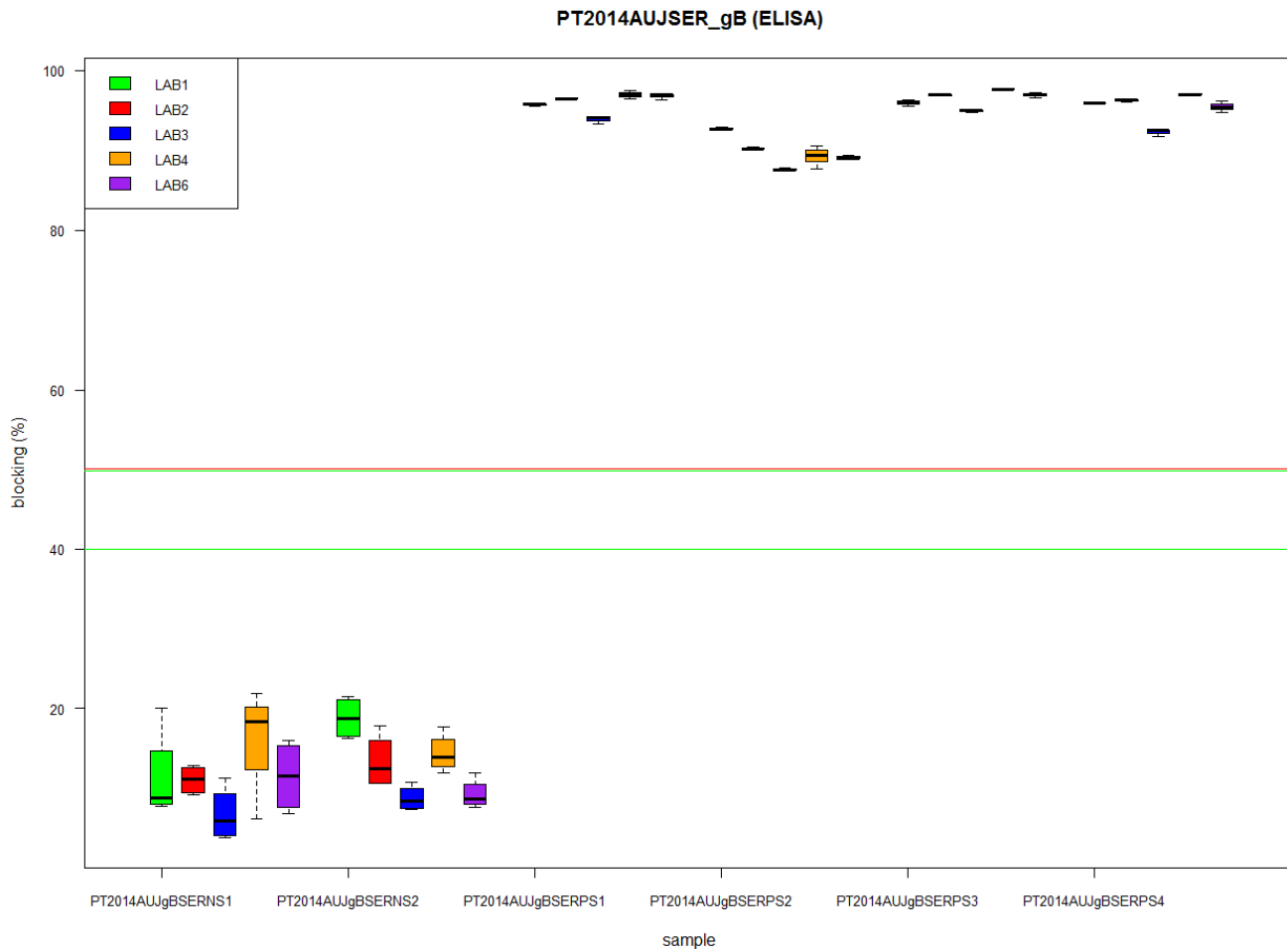


Figure 1. Box plots showing the percentage blocking per ADV gB reference serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are: 40-50% (green lines; LAB1) and 50% (LAB2, LAB3, LAB4 and LAB6). LAB2, LAB4 and LAB6 used the same batch of the same producer while LAB 3 used another batch of that producer. LAB1 used a different producer. LAB1 performed the long incubation protocol, whereas all other laboratories performed the short incubation protocol.

PT2014AUJSER_gE (ELISA)

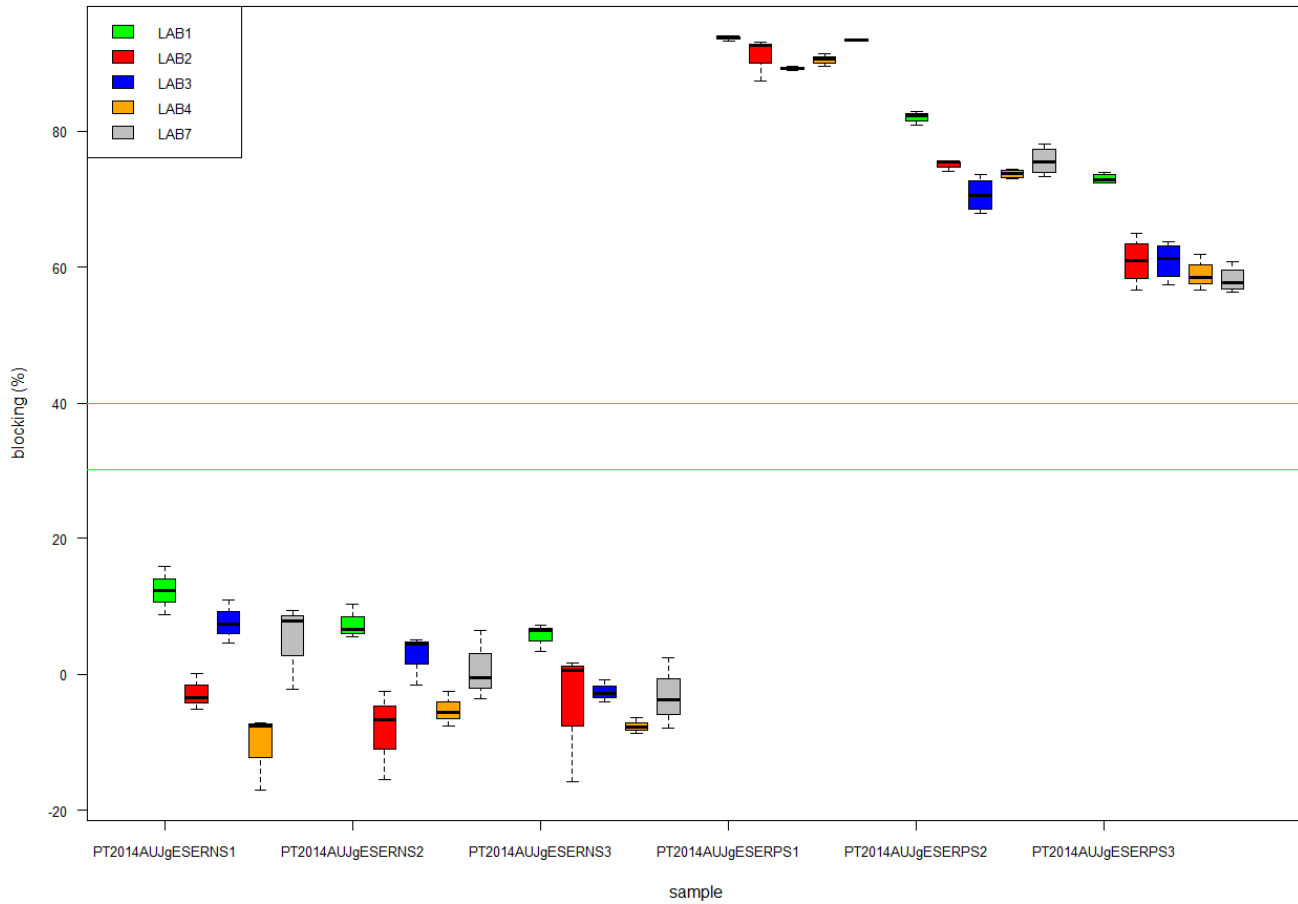


Figure 2. Box plots showing the percentage blocking per ADV gE reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by all the participating laboratories are shown in green (30-40%). All participants used the ADV gE antibody ELISA kit from the same commercial kit producer IDEXX. Hereby, different batches from the same ELISA kit were used: IDEXX (3 batches: LJ351, AK627, GJ838). LAB2, LAB3 and LAB4 used the same batch while LAB1 and LAB7 used another batch. Furthermore, LAB1 performed the long incubation protocol, whereas LAB2, LAB3, LAB4 and LAB7 used the short incubation protocol.

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 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
12	1,83	1,92	1,69	1,58	1,51	1,46	1,42	1,40	1,37	1,35
13	1,84	1,92	1,69	1,58	1,51	1,46	1,43	1,40	1,37	1,35
14	1,85	1,92	1,70	1,59	1,52	1,47	1,43	1,40	1,37	1,35
15	1,86	1,93	1,70	1,59	1,52	1,47	1,43	1,40	1,38	1,36

Based on Table 1, the maximum absolute value for Mandel's h-statistic is 1,57 for the PT ADV gB (p=5) and the PT ADV gE (p=5). For the PT ADV gB, the maximum value for Mandel's k-statistic is 1,53 for the reference serum samples PT2014AUJgBSERNS1 and PT2014AUJgBSERNS2 (p=5 and n=4), and 1,62 for the other samples. For the PT ADV gE, the maximum value for Mandel's k-statistic is 1,62 for the reference serum samples PT2014AUJgESERNS1, PT2014AUJgESERNS2, PT2014AUJgESERNS3 and PT2014AUJgESERPS1 (p=5 and n=3) and 1,53 for the reference serum samples PT2014AUJgESERPS2 and PT2014AUJgESERPS3 (p=5 and n=4).

For the detection of ADV gB-specific antibodies, 3 out of 5 participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB3, which showed an increased value for Mandel's h-statistic for the samples PT2014AujgBSERPS1 (h=-1.65) and PT2014AujgBSERPS4 (h=-1.70). Furthermore, 3 out of 5 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB4 and LAB6 which showed an increased value for Mandel's k-statistic for samples PT2014AujgBSERPS2 (k=2.16) and PT2014AujgBSERPS4 (k=1.76) respectively.

For the detection of ADV gE-specific antibodies, 4 out of 5 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB1, increased value for Mandel's h-statistic reference serum samples PT2014AujgESERNS3 (h=1.64), PT2014AujgESERPS2 (h=1.59) and PT2014AujgBSERPS3 (h=1.75). Furthermore, 3 out of 5 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB2 with increased values for the Mandel's k-statistic for serum reference samples PT2014AujgESERNS3 (k=1.91) and PT2014AujgESERPS1 (k= 2.12). LAB3 showed an increased value for Mandel's k-statistic for reference serum sample PT2014AujgESERPS2 (k=1.62).

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 as a dependent variable, it was investigated whether statistically significant differences exist ($\alpha=0,05$) 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 PT ADV gB, no statistically significant differences were observed between laboratories or between different kits at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were observed for the negative but not for the positive reference serum samples. For the negative reference serum samples, LAB4 and LAB1 reported percentages blocking that were significantly higher than those reported by LAB3..

III.2. ADV gE

For the PT ADV gE, 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 the negative but not for the positive reference serum samples. For the negative reference serum samples, LAB1 reported percentages blocking that were significantly higher than those reported by LAB4.



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

A. ADV qB

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
PT2014AUJgBSERNS1	1	4	33,84	11,36	11,37	0,06	4,82	4,96	1,17	0,00	1,21	51,20
PT2014AUJgBSERNS1	2	4	3,49	11,06	11,37	0,06	4,82	4,96	1,17	-0,09	0,39	16,88
PT2014AUJgBSERNS1	3	4	11,56	6,72	11,37	0,06	4,82	4,96	1,17	-1,38	0,71	50,62
PT2014AUJgBSERNS1	4	4	46,70	16,21	11,37	0,06	4,82	4,96	1,17	1,44	1,42	42,14
PT2014AUJgBSERNS1	6	4	20,68	11,49	11,37	0,06	4,82	4,96	1,17	0,04	0,94	39,57
PT2014AUJgBSERNS2	1	4	7,41	18,82	12,91	0,38	2,50	3,18	1,96	1,43	1,09	14,47
PT2014AUJgBSERNS2	2	4	11,63	13,35	12,91	0,38	2,50	3,18	1,96	0,11	1,37	25,55
PT2014AUJgBSERNS2	3	4	2,44	8,75	12,91	0,38	2,50	3,18	1,96	-1,01	0,63	17,86
PT2014AUJgBSERNS2	4	4	6,02	14,40	12,91	0,38	2,50	3,18	1,96	0,36	0,98	17,04
PT2014AUJgBSERNS2	6	4	3,68	9,25	12,91	0,38	2,50	3,18	1,96	-0,89	0,77	20,74
PT2014AUJgBSERPS1	1	3	0,01	95,69	95,98	0,76	0,35	0,72	0,62	-0,23	0,28	0,10
PT2014AUJgBSERPS1	2	3	0,01	96,51	95,98	0,76	0,35	0,72	0,62	0,43	0,23	0,08
PT2014AUJgBSERPS1	3	3	0,21	93,90	95,98	0,76	0,35	0,72	0,62	-1,65	1,32	0,49
PT2014AUJgBSERPS1	4	3	0,27	97,00	95,98	0,76	0,35	0,72	0,62	0,81	1,47	0,53
PT2014AUJgBSERPS1	6	3	0,12	96,78	95,98	0,76	0,35	0,72	0,62	0,63	0,98	0,36



Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
PT2014AUJgBSERPS2	1	3	0,02	92,74	89,79	0,66	0,67	1,15	0,93	1,55	0,20	0,14
PT2014AUJgBSERPS2	2	3	0,05	90,24	89,79	0,66	0,67	1,15	0,93	0,23	0,32	0,24
PT2014AUJgBSERPS2	3	3	0,04	87,60	89,79	0,66	0,67	1,15	0,93	-1,15	0,31	0,24
PT2014AUJgBSERPS2	4	3	2,10	89,26	89,79	0,66	0,67	1,15	0,93	-0,28	2,16	1,62
PT2014AUJgBSERPS2	6	3	0,05	89,12	89,79	0,66	0,67	1,15	0,93	-0,35	0,34	0,25
PT2014AUJgBSERPS3	1	3	0,12	95,95	96,48	0,85	0,22	0,57	0,52	-0,50	1,61	0,37
PT2014AUJgBSERPS3	2	3	0,01	96,93	96,48	0,85	0,22	0,57	0,52	0,43	0,37	0,08
PT2014AUJgBSERPS3	3	3	0,02	94,93	96,48	0,85	0,22	0,57	0,52	-1,46	0,59	0,14
PT2014AUJgBSERPS3	4	3	0,01	97,66	96,48	0,85	0,22	0,57	0,52	1,12	0,41	0,09
PT2014AUJgBSERPS3	6	3	0,08	96,92	96,48	0,85	0,22	0,57	0,52	0,42	1,32	0,30
PT2014AUJgBSERPS4	1	3	0,00	95,92	95,37	0,84	0,39	0,99	0,91	0,30	0,00	0,00
PT2014AUJgBSERPS4	2	3	0,02	96,20	95,37	0,84	0,39	0,99	0,91	0,45	0,38	0,16
PT2014AUJgBSERPS4	3	3	0,26	92,28	95,37	0,84	0,39	0,99	0,91	-1,70	1,30	0,55
PT2014AUJgBSERPS4	4	3	0,01	97,03	95,37	0,84	0,39	0,99	0,91	0,91	0,29	0,12
PT2014AUJgBSERPS4	6	3	0,48	95,44	95,37	0,84	0,39	0,99	0,91	0,04	1,76	0,72

Legend: Labnr = number attributed to a laboratory during the PT; n_i = number of replicates; v_i = total variability (variance) in the normalized data (% blocking); x_i_m = mean of normalized data (% blocking); x_g_m = mean of normalized data (% blocking) obtained by all laboratories; between_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 qE

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
PT2014AUJgESERNS1	1	3	12,36	12,31	2,31	0,49	4,47	6,24	4,34	1,10	0,79	28,55
PT2014AUJgESERNS1	2	3	7,15	-2,81	2,31	0,49	4,47	6,24	4,34	-0,57	0,60	-95,00
PT2014AUJgESERNS1	3	3	10,18	7,66	2,31	0,49	4,47	6,24	4,34	0,59	0,71	41,62
PT2014AUJgESERNS1	4	3	31,06	-10,59	2,31	0,49	4,47	6,24	4,34	-1,42	1,25	-52,61
PT2014AUJgESERNS1	7	3	39,33	4,96	2,31	0,49	4,47	6,24	4,34	0,29	1,40	126,33
PT2014AUJgESERNS2	1	3	6,31	7,44	-0,51	0,30	4,39	5,24	2,86	1,27	0,57	33,75
PT2014AUJgESERNS2	2	3	43,22	-8,24	-0,51	0,30	4,39	5,24	2,86	-1,23	1,50	-79,79
PT2014AUJgESERNS2	3	3	13,60	2,68	-0,51	0,30	4,39	5,24	2,86	0,51	0,84	137,72
PT2014AUJgESERNS2	4	3	6,45	-5,21	-0,51	0,30	4,39	5,24	2,86	-0,75	0,58	-48,71
PT2014AUJgESERNS2	7	3	26,74	0,77	-0,51	0,30	4,39	5,24	2,86	0,20	1,18	670,75
<u>PT2014AUJgESERNS3</u>	<u>1</u>	3	4,10	5,65	-2,44	0,13	5,14	5,50	1,97	<u>1,64</u>	0,39	35,82
<u>PT2014AUJgESERNS3</u>	<u>2</u>	3	96,52	-4,53	-2,44	0,13	5,14	5,50	1,97	-0,42	<u>1,91</u>	-216,80
PT2014AUJgESERNS3	3	3	2,82	-2,54	-2,44	0,13	5,14	5,50	1,97	-0,02	0,33	-66,05
PT2014AUJgESERNS3	4	3	1,31	-7,65	-2,44	0,13	5,14	5,50	1,97	-1,06	0,22	-14,96
PT2014AUJgESERNS3	7	3	27,18	-3,11	-2,44	0,13	5,14	5,50	1,97	-0,14	1,01	-167,40
PT2014AUJgESERPS1	1	3	0,10	93,68	91,57	0,24	1,50	1,73	0,85	1,10	0,21	0,34
<u>PT2014AUJgESERPS1</u>	<u>2</u>	3	10,19	91,01	91,57	0,24	1,50	1,73	0,85	-0,29	<u>2,12</u>	3,51
PT2014AUJgESERPS1	3	3	0,10	89,25	91,57	0,24	1,50	1,73	0,85	-1,21	0,21	0,35
PT2014AUJgESERPS1	4	3	0,90	90,50	91,57	0,24	1,50	1,73	0,85	-0,56	0,63	1,05
PT2014AUJgESERPS1	7	3	0,04	93,40	91,57	0,24	1,50	1,73	0,85	0,96	0,13	0,21



Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
<u>PT2014AUJgESERPS2</u>	<u>1</u>	4	0,82	82,09	75,43	0,62	1,62	2,62	2,06	<u>1,59</u>	0,56	1,10
PT2014AUJgESERPS2	2	4	0,46	75,15	75,43	0,62	1,62	2,62	2,06	-0,07	0,42	0,90
<u>PT2014AUJgESERPS2</u>	<u>3</u>	4	6,89	70,62	75,43	0,62	1,62	2,62	2,06	-1,15	<u>1,62</u>	3,72
PT2014AUJgESERPS2	4	4	0,45	73,71	75,43	0,62	1,62	2,62	2,06	-0,41	0,41	0,91
PT2014AUJgESERPS2	7	4	4,49	75,58	75,43	0,62	1,62	2,62	2,06	0,04	1,31	2,80
<u>PT2014AUJgESERPS3</u>	<u>1</u>	4	0,61	72,99	62,36	0,60	2,42	3,83	2,97	<u>1,75</u>	0,32	1,07
PT2014AUJgESERPS3	2	4	12,11	60,87	62,36	0,60	2,42	3,83	2,97	-0,25	1,44	5,72
PT2014AUJgESERPS3	3	4	8,10	60,91	62,36	0,60	2,42	3,83	2,97	-0,24	1,17	4,67
PT2014AUJgESERPS3	4	4	4,88	58,89	62,36	0,60	2,42	3,83	2,97	-0,57	0,91	3,75
PT2014AUJgESERPS3	7	4	3,66	58,16	62,36	0,60	2,42	3,83	2,97	-0,69	0,79	3,29

Legend: Labnr = number attributed to a laboratory during the PT; n_i = number of replicates; v_i = total variability (variance) in the normalized data (% blocking); x_i_m = mean of normalized data (% blocking); x_g_m = mean of normalized data (% blocking) obtained by all laboratories; between_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).



Annex 3: Qualitative and quantitative data analysis of the additional panel ADV gB samples

For the PT ADV gB, an additional panel consisting of serum samples with variable qualitative results in ELISA but all negative in SN, the reference test (golden standard) for the detection of ADV gB-specific antibodies in serum, was sent to the participating laboratories along with the regular ADV gB PT samples. The aim was to include serum samples that score weak positive and cut-off in ELISA in order to achieve more information about the analytical sensitivity of the used ADV gB ELISA kits by the participating laboratories. This part was out of scope of this PT and should only be considered as educational information for the participants. The data analysis of these additional serum samples was thus not used to evaluate the participants in this PT.

I. Information about the additional ADVgB serum samples

In total, 54 aliquots of these additional serum samples were distributed to the 6 laboratories participating in the PT ADV gB. All participants received 3 aliquots of 3 different serum samples of porcine origin (PT2014AUJgBSER-S1A, PT2014AUJgBSER-S1B, PT2014AUJgBSER-S1C), i.e. 9 aliquots in total. The positions of the serum samples in the sent blocks were randomized for each participant. Only one laboratory did not sent back the results for the additional samples.

The serum samples PT2014AUJgBSER-S1A, PT2014AUJgBSER-S1B and PT2014AUJgBSER-S1C were a 1/1024, a 1/2048 and a 1/4096 dilution, respectively, of a serum obtained from an animal that was experimentally infected with an ADV strain isolated from a wolf in 2011 in Belgium (different animal than the one reference serum sample PT2014AUJgBSERPS4 was derived from (see III.2.1)). During pre-verification, all 3 additional serum samples were negative using SN, whereas variable results were obtained using the PrioCheck PRV gB antibody ELISA test from Prionics (POS - POS/NEG - NEG, respectively).

After aliquoting the different serum samples, a homogeneity check was performed on 10 aliquots of each serum sample using the PrioCheck PRV gB antibody ELISA test from Prionics. In addition, 3 aliquots of each 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.

II. Qualitative data analysis

The qualitative results reported by the participating laboratories are summarized in Table 1.

Table 1. Overview of the qualitative results reported by the participating laboratories for the 9 aliquots of additional ADVgB serum samples. Lab 5 did not report the results. (NA: not applicable- NI could not be reported by the ELISA's used by these laboratories)

	LAB1	LAB2	LAB3	LAB4	LAB6	TOTAL
PT2014AUJgBSER-S1A						
POS	3	3	3	3	3	15
NI	0	NA	NA	NA	NA	NA
NEG	0	0	0	0	0	0
PT2014AUJgBSER-S1B						
POS	3	1	3	3	0	10
NI	0	NA	NA	NA	NA	NA
NEG	0	2	0	0	3	5
PT2014AUJgBSER-S1C						
POS	3	0	0	0	0	3
NI	0	NA	NA	NA	NA	NA
NEG	0	3	3	3	3	12

III. Quantitative data analysis

The quantitative data analysis was performed using the statistical software programs R (box plots) and SAS 9.2. (summary statistics), all as described in Annex 1.

III.1 Box plots

Box plots of the percentages blocking per additional ADV gB serum sample and per participating (sub)laboratory were made using the statistical software R and are shown in Figure 1.

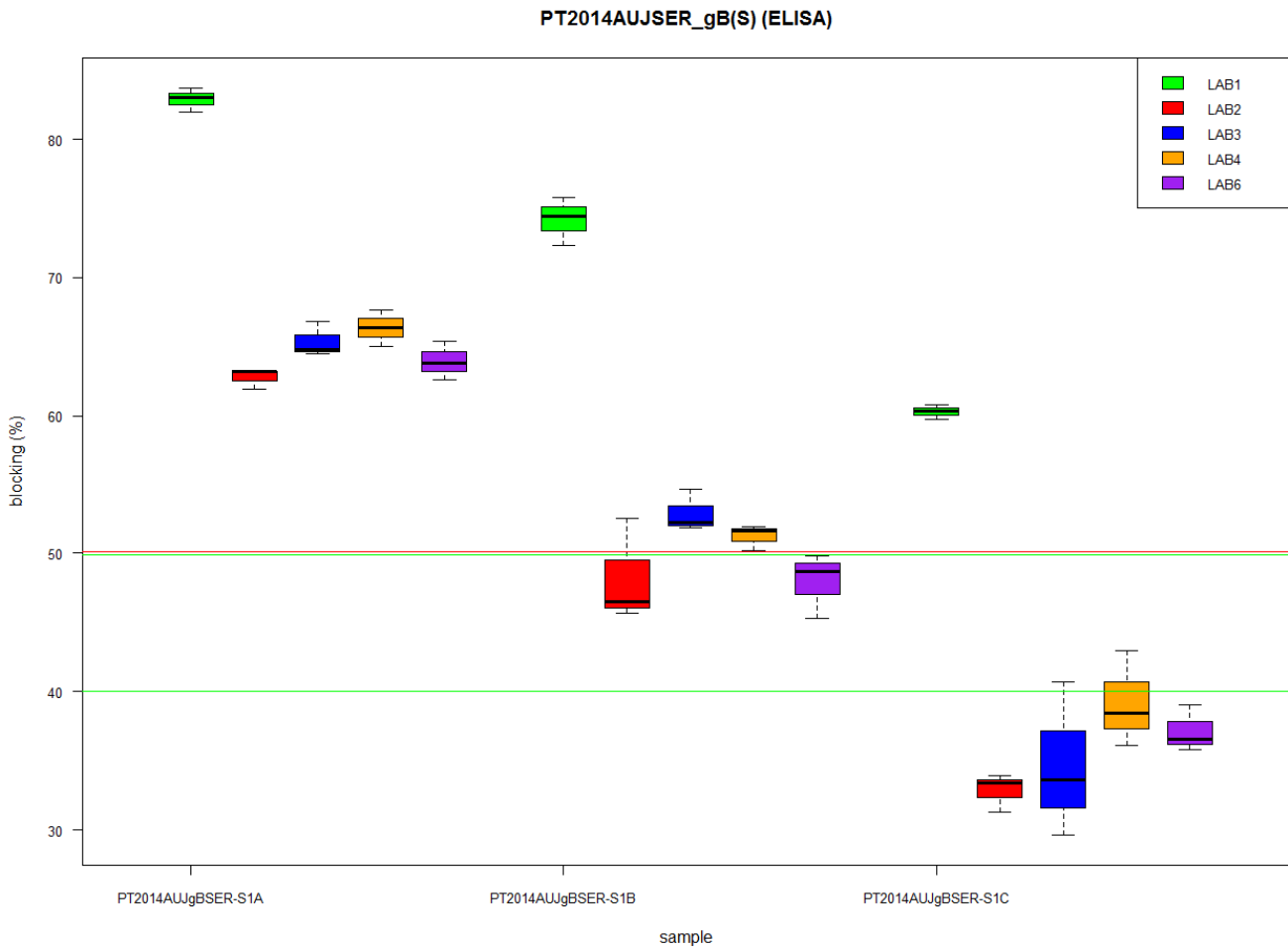


Figure 1. Box plots showing the percentage blocking per additional ADV gB reference serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are: 40-50% (green lines; LAB1) and 50% (LAB2, LAB3, LAB4 and LAB6). LAB2, LAB4 and LAB6 used the same batch of the same producer while LAB 3 used another batch of that producer. LAB1 used a different producer. LAB1 performed the long incubation protocol, whereas all other laboratories performed the short incubation protocol.

III.2 Mandel's h- and k-statistics

Based on Table 1 in Annex 1, the maximum absolute value for Mandel's h-statistic is 1,57 (p=5) and the maximum value for Mandel's k-statistic is 1,62 for all serum samples (p=5 and n=3).

For the detection of ADV gB-specific antibodies in the additional serum samples, 4 out of 5 participating laboratories obtained a satisfactory between-laboratory consistency for all serum samples. LAB1 reported increased values of the Mandel's h-statistic for all serum samples. Furthermore, 3 out of 5 participating laboratories obtained a satisfactory within-laboratory consistency for all serum samples. This was not the case for LAB2 and LAB3 which showed an increased value for Mandel's k-statistic for serum sample PT2014ADVgBSER-S1B (k=1,64) and PT2014ADVgBSER-S1C (k=1,80) respectively.

All data used for the calculations of Mandel's h- and k-statistics for the additional ADV gB serum samples can be found in Table 2 of this Annex.

III.3 ANOVA

For the additional ADV gB serum samples, there was a significant difference between the participating (sub)laboratories at a global level. LAB1 reported percentages blocking that were significantly higher than those reported by LAB2, LAB3, LAB4 and LAB6.



Table 2. Calculations of Mandel’s h- and k-statistics (based on % blocking) for the panel additional ADV gB serum samples.

Sample	Labnr	n _i	v _i	x _{i_m}	x _{g_m}	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
<u>PT2014AUJgBSER-S1A</u>	<u>1</u>	3	0,76	82,89	68,27	0,93	1,16	4,29	4,13	<u>1,76</u>	0,75	1,05
PT2014AUJgBSER-S1A	2	3	0,55	62,78	68,27	0,93	1,16	4,29	4,13	-0,66	0,64	1,18
PT2014AUJgBSER-S1A	3	3	1,62	65,38	68,27	0,93	1,16	4,29	4,13	-0,35	1,10	1,95
PT2014AUJgBSER-S1A	4	3	1,78	66,36	68,27	0,93	1,16	4,29	4,13	-0,23	1,15	2,01
PT2014AUJgBSER-S1A	6	3	1,98	63,95	68,27	0,93	1,16	4,29	4,13	-0,52	1,22	2,20
<u>PT2014AUJgBSER-S1B</u>	<u>1</u>	3	3,05	74,18	54,90	0,85	2,27	5,91	5,45	<u>1,76</u>	0,77	2,35
<u>PT2014AUJgBSER-S1B</u>	<u>2</u>	3	13,97	48,22	54,90	0,85	2,27	5,91	5,45	-0,61	<u>1,64</u>	7,75
PT2014AUJgBSER-S1B	3	3	2,38	52,90	54,90	0,85	2,27	5,91	5,45	-0,18	0,68	2,91
PT2014AUJgBSER-S1B	4	3	0,89	51,24	54,90	0,85	2,27	5,91	5,45	-0,33	0,42	1,84
PT2014AUJgBSER-S1B	6	3	5,54	47,94	54,90	0,85	2,27	5,91	5,45	-0,63	1,04	4,91
<u>PT2014AUJgBSER-S1C</u>	<u>1</u>	3	0,27	60,31	40,82	0,76	3,12	6,32	5,50	<u>1,75</u>	0,17	0,87
PT2014AUJgBSER-S1C	2	3	1,97	32,86	40,82	0,76	3,12	6,32	5,50	-0,71	0,45	4,27
<u>PT2014AUJgBSER-S1C</u>	<u>3</u>	3	31,56	34,65	40,82	0,76	3,12	6,32	5,50	-0,55	<u>1,80</u>	16,21
PT2014AUJgBSER-S1C	4	3	11,98	39,16	40,82	0,76	3,12	6,32	5,50	-0,15	1,11	8,84
PT2014AUJgBSER-S1C	6	3	2,78	37,15	40,82	0,76	3,12	6,32	5,50	-0,33	0,53	4,49

Legend: Labnr = number attributed to a laboratory during the PT; n_i = number of replicates; v_i = total variability (variance) in the normalized data (% blocking); x_{i_m} = mean of normalized data (% blocking); x_{g_m} = mean of normalized data (% blocking) obtained by all laboratories; between_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).