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PROFICIENCY TESTING 2013

INFECTIOUS BOVINE RHINOTRACHEITIS (IBR)

Detection of IBRgB- and IBRgE-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: 10 JUNE 2013

DATE REPORT: 27 JANUARY 2014

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 IBRgB- and/or IBRgE-specific antibodies in bovine 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. IBRgB reference samples

Replicates of 6 reference serum samples of bovine origin, either free from detectable IBRgB-specific antibodies (n = 2; coded 'PT2013IBRgBSERNS1' and 'PT2013IBRgBSERNS2') or containing detectable IBRgB-specific antibodies (n = 4; coded 'PT2013IBRgBSERPS1', 'PT2013IBRgBSERPS2', 'PT2013IBRgBSERPS3' and 'PT2013IBRgBSERPS4'), were used. In total, 180 aliquots were distributed to 9 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2013IBRgBSERPS1, PT2013IBRgBSERPS2, PT2013IBRgBSERPS3 and PT2013IBRgBSERPS4, and 4 aliquots of the reference serum samples PT2013IBRgBSERNS1 and PT2013IBRgBSERNS2. 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 HerdChek IBRgB antibody ELISA test from IDEXX, the indirect ELISA test from LSI (LSIVET serum IBR screening) and a seroneutralisation assay (SN). The reference serum samples PT2013IBRgBSERNS1 and PT2013IBRgBSERNS2 were obtained from 2 animals from a Belgian I4-certified farm (IBR-free without vaccination). The reference serum samples PT2013IBRgBSERPS1 and PT2013IBRgBSERPS2 were obtained from 2 different vaccinated but uninfected animals. Hereby, the reference serum sample PT2013IBRgBSERPS1 was a 1/128 dilution of the original serum. The reference serum samples PT2013IBRgBSERPS3 and PT2013IBRgBSERPS4 were a 1/4 and a 1/32 dilution, respectively, of 2 different sera from experimentally infected but non-vaccinated animals. For each reference serum sample, the same qualitative result was obtained with all test methods used. Taken together, the reference serum samples PT2013IBRgBSERNS1 and PT2013IBRgBSERNS2 were considered as negative sera, and the reference serum samples PT2013IBRgBSERPS1, PT2013IBRgBSERPS2, PT2013IBRgBSERPS3 and PT2013IBRgBSERPS4 as strong positive sera in IBRgB ELISA (but rather weak positive in SN).

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek IBRgB 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 IBRgB-specific antibodies in bovine 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 IBRgB antibody ELISA test from IDEXX.

Remark: For the PT IBRgB, an additional panel consisting of 12 serum samples with variable qualitative results in ELISA but negative in SN, the reference test (golden standard) for the detection of IBRgB-specific antibodies in serum, was sent

to the 9 participating laboratories along with the regular IBRgB PT samples. Since this was out of scope of this PT according to ISO 17043, a description of these additional serum samples as well as the qualitative and quantitative data analysis is summarized in Annex 3.

III.2.2. IBRgE reference samples

Replicates of 6 reference serum samples of bovine origin, either free from detectable IBRgE-specific antibodies (n = 3; coded 'PT2013IBRgESERNS1', 'PT2013IBRgESERNS2' and 'PT2013IBRgESERNS3') or containing detectable IBRgE-specific antibodies (n=3, coded 'PT2013IBRgESERPS1', 'PT2013IBRgESERPS2' and 'PT2013IBRgESERPS3'), were used. In total, 180 aliquots were distributed to 9 different participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2013IBRgESERNS1, PT2013IBRgESERNS2, PT2013IBRgESERNS3 and PT2013IBRgESERPS3, and 4 aliquots of the reference serum samples PT2013IBRgESERPS1 and PT2013IBRgESERPS2. 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 IBRgE antibody ELISA test from IDEXX. The reference serum sample PT2013IBRgESERNS1 (=PT2013IBRgBSERNS1) was from an animal from a Belgian I4-certified farm (IBR-free without vaccination), whereas the reference serum samples PT2013IBRgESERNS2 and PT2013IBRgESERNS3 (=PT2013IBRgBSERPS2) were from vaccinated but uninfected animals. The reference serum samples PT2013IBRgESERPS1 (=PT2013IBRgBSERPS3), PT2013IBRgESERPS2 and PT2013IBRgESERPS3 (=PT2013IBRgBSERPS4) were derived from 2 different experimentally infected but non-vaccinated animals. Hereby, the reference serum sample PT2013IBRgESERPS1 was a 1/4 dilution of a serum collected from one animal, whereas the reference serum samples PT2013IBRgESERPS2 and PT2013IBRgESERPS3 were a 1/16 and a 1/32 dilution, respectively, of a serum obtained from another animal. Taken together, the reference serum samples PT2013IBRgESERNS1, PT2013IBRgESERNS2 and PT2013IBRgESERNS3 were considered as negative sera, the reference serum sample PT2013IBRgESERPS1 as a strong positive serum, and the reference serum samples PT2013IBRgESERPS2 and PT2013IBRgESERPS3 as (weak) positive sera in IBRgE ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek IBRgE 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 IBRgE-specific antibodies in bovine 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 IBRgE 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 IBRgB or the PT IBRgE.

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 IBRgB and 90% for the PT IBRgE.

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 LAB8 participated in both the PT IBRgB and the PT IBRgE and hence received 40 aliquots of reference serum samples (20 for the PT IBRgB and 20 for the PT IBRgE). LAB9 only participated in the PT IBRgB, whereas LAB10 only participated in the PT IBRgE. LAB9 and LAB10 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 10th of June 2013. LAB1, LAB2, LAB3 and LAB4 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 11th (LAB6, LAB8 and LAB9) or 12th (LAB5, LAB7 and LAB10) of June 2013. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 11th and 28th of June 2013 (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 and 28th of June 2013 (Table 1). All participants hereby respected the deadline of 28th of June 2013.

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	10/06/2013	11/06/2013	14/06/2013	20/06/2013
LAB2	10/06/2013	12/06/2013	12/06/2013	25/06/2013
LAB3	10/06/2013	14 & 18/06/2013 (#)	12/06/2013	21/06/2013
LAB4	10/06/2013	10 & 13/06/2013 (#)	12/06/2013	18/06/2013
LAB5	12/06/2013	14 & 21/06/2013 (#)	14/06/2013 (°)	25/06/2013
LAB6	11/06/2013	25/06/2013	24/06/2013	28/06/2013
LAB7	12/06/2013	28/06/2013	19 & 26/06/2013 (°)	28/06/2013
LAB8	11/06/2013	14/06/2013	19/06/2013	20/06/2013
LAB9	11/06/2013	20/06/2013	NA	25/06/2013
LAB10	12/06/2013	NA	25/06/2013	28/06/2013

Legend: NA = not applicable; (#) = this laboratory tested ELISA kits from 2 different producers; (°) = this laboratory tested different batches of the same ELISA kit

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

LAB3, LAB4, LAB5 and LAB7 submitted 2 sets of results for the PT IBRgB and/or the PT IBRgE since they analysed the 20 aliquots of reference serum samples using ELISA kits from different producers and/or different batches of the same ELISA kit. In order to analyse the provided data, these 4 laboratories have been divided into different sublaboratories, namely: LAB3 into LAB3.1 (kit 1 for PT IBRgB) and LAB3.2 (kit 2 for PT IBRgB), LAB4 into LAB4.1 (kit 1 for PT IBRgB) and LAB4.2 (kit 2 for PT IBRgB), LAB5 into LAB5.1 (kit 1 for PT IBRgB and batch1 for PT IBRgE) and LAB5.2 (kit 2 for PT IBRgB and batch 2 for PT IBRgE) and LAB7 into LAB7.1 (batch 1 for PT IBRgE) and LAB7.2 (batch 2 for PT IBRgE).

IV.4.1. Level of agreement

Qualitative data analysis showed that:

(i) For the detection of **IBRgB-specific antibodies**, 7 out of 9 participating laboratories (LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB8) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). Hereby, LAB3, LAB4 and LAB5 used each 2 ELISA kits from different producers. In contrast, LAB7 misclassified 3 aliquots (85% of agreement) and LAB9 misclassified 2 aliquots (90% of agreement) of reference serum samples (Table 2).

(ii) For the detection of **IBRgE-specific antibodies**, all 9 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). Hereby, LAB5 and LAB7 used 2 different batches of the same ELISA kit (Table 3).

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

	LABNR								
	1	**2**	**3.1**	**3.2**	**4.1**	**4.2**	**5.1**	**5.2**	**6**
Failure	0 (0.0)	0 (0.0)	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)	20 (100.0)	20 (100.0)

	LABNR		
	7	**8**	**9**
Failure	3 (15.0)	0 (0.0)	2 (10.0)
Success	**17 (85.0)**	20 (100.0)	**18 (90.0)**

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

	LABNR								
	1	**2**	**3**	**4**	**5.1**	**5.2**	**6**	**7.1**	**7.2**
Failure	0 (0.0)	0 (0.0)	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)	20 (100.0)	20 (100.0)

	LABNR	
	8	**10**
Failure	0 (0.0)	0 (0.0)
Success	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

Only a small variability between laboratories could be observed at the qualitative data level:

(i) For the detection of **IBRgB-specific antibodies**, no variability between LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB8 could be observed since these participants correctly identified all reference serum samples. Hereby, LAB3, LAB4 and LAB5 obtained identical qualitative results using 2 ELISA kits from different producers. In contrast, LAB7 misclassified all 3 aliquots of the reference serum sample PT2013IBRgBSERPS1 (3x NI instead

of POS), whereas LAB9 misclassified 2 out of 3 aliquots of the reference serum sample PT2013IBRgBSERPS2 (1x NI and 1x NEG instead of POS).

(ii) For the detection of **IBRgE-specific antibodies**, no variability between laboratories could be observed since all participants correctly identified all reference serum samples. Hereby, LAB5 and LAB7 analysed the reference serum samples using 2 different batches of the same ELISA kit.

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

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013IBRgBSERNS1	NEG	NEG	1
2	1	2	PT2013IBRgBSERPS1	POS	POS	1
3	1	3	PT2013IBRgBSERNS1	NEG	NEG	1
4	1	4	PT2013IBRgBSERPS2	POS	POS	1
5	1	5	PT2013IBRgBSERNS1	NEG	NEG	1
6	1	6	PT2013IBRgBSERPS2	POS	POS	1
7	1	7	PT2013IBRgBSERNS1	NEG	NEG	1
8	1	8	PT2013IBRgBSERPS2	POS	POS	1
9	1	9	PT2013IBRgBSERNS2	NEG	NEG	1
10	1	10	PT2013IBRgBSERPS3	POS	POS	1
11	1	11	PT2013IBRgBSERNS2	NEG	NEG	1
12	1	12	PT2013IBRgBSERPS3	POS	POS	1
13	1	13	PT2013IBRgBSERNS2	NEG	NEG	1
14	1	14	PT2013IBRgBSERPS3	POS	POS	1
15	1	15	PT2013IBRgBSERNS2	NEG	NEG	1
16	1	16	PT2013IBRgBSERPS4	POS	POS	1
17	1	17	PT2013IBRgBSERPS1	POS	POS	1
18	1	18	PT2013IBRgBSERPS4	POS	POS	1
19	1	19	PT2013IBRgBSERPS1	POS	POS	1
20	1	20	PT2013IBRgBSERPS4	POS	POS	1
21	2	1	PT2013IBRgBSERPS4	POS	POS	1
22	2	2	PT2013IBRgBSERNS1	NEG	NEG	1
23	2	3	PT2013IBRgBSERPS1	POS	POS	1
24	2	4	PT2013IBRgBSERNS1	NEG	NEG	1
25	2	5	PT2013IBRgBSERPS2	POS	POS	1
26	2	6	PT2013IBRgBSERNS1	NEG	NEG	1
27	2	7	PT2013IBRgBSERPS2	POS	POS	1
28	2	8	PT2013IBRgBSERNS1	NEG	NEG	1
29	2	9	PT2013IBRgBSERPS2	POS	POS	1
30	2	10	PT2013IBRgBSERNS2	NEG	NEG	1
31	2	11	PT2013IBRgBSERPS3	POS	POS	1
32	2	12	PT2013IBRgBSERNS2	NEG	NEG	1
33	2	13	PT2013IBRgBSERPS3	POS	POS	1
34	2	14	PT2013IBRgBSERNS2	NEG	NEG	1
35	2	15	PT2013IBRgBSERPS3	POS	POS	1
36	2	16	PT2013IBRgBSERNS2	NEG	NEG	1
37	2	17	PT2013IBRgBSERPS4	POS	POS	1
38	2	18	PT2013IBRgBSERPS1	POS	POS	1
39	2	19	PT2013IBRgBSERPS4	POS	POS	1
40	2	20	PT2013IBRgBSERPS1	POS	POS	1

Table 4. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3.1	1	PT2013IBRgBSERPS1	POS	POS	1
42	3.1	2	PT2013IBRgBSERPS4	POS	POS	1
43	3.1	3	PT2013IBRgBSERNS1	NEG	NEG	1
44	3.1	4	PT2013IBRgBSERPS1	POS	POS	1
45	3.1	5	PT2013IBRgBSERNS1	NEG	NEG	1
46	3.1	6	PT2013IBRgBSERPS2	POS	POS	1
47	3.1	7	PT2013IBRgBSERNS1	NEG	NEG	1
48	3.1	8	PT2013IBRgBSERPS2	POS	POS	1
49	3.1	9	PT2013IBRgBSERNS1	NEG	NEG	1
50	3.1	10	PT2013IBRgBSERPS2	POS	POS	1
51	3.1	11	PT2013IBRgBSERNS2	NEG	NEG	1
52	3.1	12	PT2013IBRgBSERPS3	POS	POS	1
53	3.1	13	PT2013IBRgBSERNS2	NEG	NEG	1
54	3.1	14	PT2013IBRgBSERPS3	POS	POS	1
55	3.1	15	PT2013IBRgBSERNS2	NEG	NEG	1
56	3.1	16	PT2013IBRgBSERPS3	POS	POS	1
57	3.1	17	PT2013IBRgBSERNS2	NEG	NEG	1
58	3.1	18	PT2013IBRgBSERPS4	POS	POS	1
59	3.1	19	PT2013IBRgBSERPS1	POS	POS	1
60	3.1	20	PT2013IBRgBSERPS4	POS	POS	1
61	3.2	1	PT2013IBRgBSERPS1	POS	POS	1
62	3.2	2	PT2013IBRgBSERPS4	POS	POS	1
63	3.2	3	PT2013IBRgBSERNS1	NEG	NEG	1
64	3.2	4	PT2013IBRgBSERPS1	POS	POS	1
65	3.2	5	PT2013IBRgBSERNS1	NEG	NEG	1
66	3.2	6	PT2013IBRgBSERPS2	POS	POS	1
67	3.2	7	PT2013IBRgBSERNS1	NEG	NEG	1
68	3.2	8	PT2013IBRgBSERPS2	POS	POS	1
69	3.2	9	PT2013IBRgBSERNS1	NEG	NEG	1
70	3.2	10	PT2013IBRgBSERPS2	POS	POS	1
71	3.2	11	PT2013IBRgBSERNS2	NEG	NEG	1
72	3.2	12	PT2013IBRgBSERPS3	POS	POS	1
73	3.2	13	PT2013IBRgBSERNS2	NEG	NEG	1
74	3.2	14	PT2013IBRgBSERPS3	POS	POS	1
75	3.2	15	PT2013IBRgBSERNS2	NEG	NEG	1
76	3.2	16	PT2013IBRgBSERPS3	POS	POS	1
77	3.2	17	PT2013IBRgBSERNS2	NEG	NEG	1
78	3.2	18	PT2013IBRgBSERPS4	POS	POS	1
79	3.2	19	PT2013IBRgBSERPS1	POS	POS	1
80	3.2	20	PT2013IBRgBSERPS4	POS	POS	1

Table 4. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.1	1	PT2013IBRgBSERPS4	POS	POS	1
82	4.1	2	PT2013IBRgBSERPS1	POS	POS	1
83	4.1	3	PT2013IBRgBSERPS4	POS	POS	1
84	4.1	4	PT2013IBRgBSERNS1	NEG	NEG	1
85	4.1	5	PT2013IBRgBSERPS1	POS	POS	1
86	4.1	6	PT2013IBRgBSERNS1	NEG	NEG	1
87	4.1	7	PT2013IBRgBSERPS2	POS	POS	1
88	4.1	8	PT2013IBRgBSERNS1	NEG	NEG	1
89	4.1	9	PT2013IBRgBSERPS2	POS	POS	1
90	4.1	10	PT2013IBRgBSERNS1	NEG	NEG	1
91	4.1	11	PT2013IBRgBSERPS2	POS	POS	1
92	4.1	12	PT2013IBRgBSERNS2	NEG	NEG	1
93	4.1	13	PT2013IBRgBSERPS3	POS	POS	1
94	4.1	14	PT2013IBRgBSERNS2	NEG	NEG	1
95	4.1	15	PT2013IBRgBSERPS3	POS	POS	1
96	4.1	16	PT2013IBRgBSERNS2	NEG	NEG	1
97	4.1	17	PT2013IBRgBSERPS3	POS	POS	1
98	4.1	18	PT2013IBRgBSERNS2	NEG	NEG	1
99	4.1	19	PT2013IBRgBSERPS4	POS	POS	1
100	4.1	20	PT2013IBRgBSERPS1	POS	POS	1
101	4.2	1	PT2013IBRgBSERPS4	POS	POS	1
102	4.2	2	PT2013IBRgBSERPS1	POS	POS	1
103	4.2	3	PT2013IBRgBSERPS4	POS	POS	1
104	4.2	4	PT2013IBRgBSERNS1	NEG	NEG	1
105	4.2	5	PT2013IBRgBSERPS1	POS	POS	1
106	4.2	6	PT2013IBRgBSERNS1	NEG	NEG	1
107	4.2	7	PT2013IBRgBSERPS2	POS	POS	1
108	4.2	8	PT2013IBRgBSERNS1	NEG	NEG	1
109	4.2	9	PT2013IBRgBSERPS2	POS	POS	1
110	4.2	10	PT2013IBRgBSERNS1	NEG	NEG	1
111	4.2	11	PT2013IBRgBSERPS2	POS	POS	1
112	4.2	12	PT2013IBRgBSERNS2	NEG	NEG	1
113	4.2	13	PT2013IBRgBSERPS3	POS	POS	1
114	4.2	14	PT2013IBRgBSERNS2	NEG	NEG	1
115	4.2	15	PT2013IBRgBSERPS3	POS	POS	1
116	4.2	16	PT2013IBRgBSERNS2	NEG	NEG	1
117	4.2	17	PT2013IBRgBSERPS3	POS	POS	1
118	4.2	18	PT2013IBRgBSERNS2	NEG	NEG	1
119	4.2	19	PT2013IBRgBSERPS4	POS	POS	1
120	4.2	20	PT2013IBRgBSERPS1	POS	POS	1

Table 4. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	5.1	1	PT2013IBRgBSERPS1	POS	POS	1
122	5.1	2	PT2013IBRgBSERPS4	POS	POS	1
123	5.1	3	PT2013IBRgBSERPS1	POS	POS	1
124	5.1	4	PT2013IBRgBSERPS4	POS	POS	1
125	5.1	5	PT2013IBRgBSERNS1	NEG	NEG	1
126	5.1	6	PT2013IBRgBSERPS1	POS	POS	1
127	5.1	7	PT2013IBRgBSERNS1	NEG	NEG	1
128	5.1	8	PT2013IBRgBSERPS2	POS	POS	1
129	5.1	9	PT2013IBRgBSERNS1	NEG	NEG	1
130	5.1	10	PT2013IBRgBSERPS2	POS	POS	1
131	5.1	11	PT2013IBRgBSERNS1	NEG	NEG	1
132	5.1	12	PT2013IBRgBSERPS2	POS	POS	1
133	5.1	13	PT2013IBRgBSERNS2	NEG	NEG	1
134	5.1	14	PT2013IBRgBSERPS3	POS	POS	1
135	5.1	15	PT2013IBRgBSERNS2	NEG	NEG	1
136	5.1	16	PT2013IBRgBSERPS3	POS	POS	1
137	5.1	17	PT2013IBRgBSERNS2	NEG	NEG	1
138	5.1	18	PT2013IBRgBSERPS3	POS	POS	1
139	5.1	19	PT2013IBRgBSERNS2	NEG	NEG	1
140	5.1	20	PT2013IBRgBSERPS4	POS	POS	1
141	5.2	1	PT2013IBRgBSERPS1	POS	POS	1
142	5.2	2	PT2013IBRgBSERPS4	POS	POS	1
143	5.2	3	PT2013IBRgBSERPS1	POS	POS	1
144	5.2	4	PT2013IBRgBSERPS4	POS	POS	1
145	5.2	5	PT2013IBRgBSERNS1	NEG	NEG	1
146	5.2	6	PT2013IBRgBSERPS1	POS	POS	1
147	5.2	7	PT2013IBRgBSERNS1	NEG	NEG	1
148	5.2	8	PT2013IBRgBSERPS2	POS	POS	1
149	5.2	9	PT2013IBRgBSERNS1	NEG	NEG	1
150	5.2	10	PT2013IBRgBSERPS2	POS	POS	1
151	5.2	11	PT2013IBRgBSERNS1	NEG	NEG	1
152	5.2	12	PT2013IBRgBSERPS2	POS	POS	1
153	5.2	13	PT2013IBRgBSERNS2	NEG	NEG	1
154	5.2	14	PT2013IBRgBSERPS3	POS	POS	1
155	5.2	15	PT2013IBRgBSERNS2	NEG	NEG	1
156	5.2	16	PT2013IBRgBSERPS3	POS	POS	1
157	5.2	17	PT2013IBRgBSERNS2	NEG	NEG	1
158	5.2	18	PT2013IBRgBSERPS3	POS	POS	1
159	5.2	19	PT2013IBRgBSERNS2	NEG	NEG	1
160	5.2	20	PT2013IBRgBSERPS4	POS	POS	1

Table 4. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	6	1	PT2013IBRgBSERPS4	POS	POS	1
162	6	2	PT2013IBRgBSERPS1	POS	POS	1
163	6	3	PT2013IBRgBSERPS4	POS	POS	1
164	6	4	PT2013IBRgBSERPS1	POS	POS	1
165	6	5	PT2013IBRgBSERPS4	POS	POS	1
166	6	6	PT2013IBRgBSERNS1	NEG	NEG	1
167	6	7	PT2013IBRgBSERPS1	POS	POS	1
168	6	8	PT2013IBRgBSERNS1	NEG	NEG	1
169	6	9	PT2013IBRgBSERPS2	POS	POS	1
170	6	10	PT2013IBRgBSERNS1	NEG	NEG	1
171	6	11	PT2013IBRgBSERPS2	POS	POS	1
172	6	12	PT2013IBRgBSERNS1	NEG	NEG	1
173	6	13	PT2013IBRgBSERPS2	POS	POS	1
174	6	14	PT2013IBRgBSERNS2	NEG	NEG	1
175	6	15	PT2013IBRgBSERPS3	POS	POS	1
176	6	16	PT2013IBRgBSERNS2	NEG	NEG	1
177	6	17	PT2013IBRgBSERPS3	POS	POS	1
178	6	18	PT2013IBRgBSERNS2	NEG	NEG	1
179	6	19	PT2013IBRgBSERPS3	POS	POS	1
180	6	20	PT2013IBRgBSERNS2	NEG	NEG	1
181	7	1	PT2013IBRgBSERNS2	NEG	NEG	1
182	7	2	PT2013IBRgBSERPS4	POS	POS	1
183	7	3	PT2013IBRgBSERPS1	**POS**	**NI**	**0**
184	7	4	PT2013IBRgBSERPS4	POS	POS	1
185	7	5	PT2013IBRgBSERPS1	**POS**	**NI**	**0**
186	7	6	PT2013IBRgBSERPS4	POS	POS	1
187	7	7	PT2013IBRgBSERNS1	NEG	NEG	1
188	7	8	PT2013IBRgBSERPS1	**POS**	**NI**	**0**
189	7	9	PT2013IBRgBSERNS1	NEG	NEG	1
190	7	10	PT2013IBRgBSERPS2	POS	POS	1
191	7	11	PT2013IBRgBSERNS1	NEG	NEG	1
192	7	12	PT2013IBRgBSERPS2	POS	POS	1
193	7	13	PT2013IBRgBSERNS1	NEG	NEG	1
194	7	14	PT2013IBRgBSERPS2	POS	POS	1
195	7	15	PT2013IBRgBSERNS2	NEG	NEG	1
196	7	16	PT2013IBRgBSERPS3	POS	POS	1
197	7	17	PT2013IBRgBSERNS2	NEG	NEG	1
198	7	18	PT2013IBRgBSERPS3	POS	POS	1
199	7	19	PT2013IBRgBSERNS2	NEG	NEG	1
200	7	20	PT2013IBRgBSERPS3	POS	POS	1

Table 4. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	8	1	PT2013IBRgBSERPS3	POS	POS	1
202	8	2	PT2013IBRgBSERNS2	NEG	NEG	1
203	8	3	PT2013IBRgBSERPS4	POS	POS	1
204	8	4	PT2013IBRgBSERPS1	POS	POS	1
205	8	5	PT2013IBRgBSERPS4	POS	POS	1
206	8	6	PT2013IBRgBSERPS1	POS	POS	1
207	8	7	PT2013IBRgBSERPS4	POS	POS	1
208	8	8	PT2013IBRgBSERNS1	NEG	NEG	1
209	8	9	PT2013IBRgBSERPS1	POS	POS	1
210	8	10	PT2013IBRgBSERNS1	NEG	NEG	1
211	8	11	PT2013IBRgBSERPS2	POS	POS	1
212	8	12	PT2013IBRgBSERNS1	NEG	NEG	1
213	8	13	PT2013IBRgBSERPS2	POS	POS	1
214	8	14	PT2013IBRgBSERNS1	NEG	NEG	1
215	8	15	PT2013IBRgBSERPS2	POS	POS	1
216	8	16	PT2013IBRgBSERNS2	NEG	NEG	1
217	8	17	PT2013IBRgBSERPS3	POS	POS	1
218	8	18	PT2013IBRgBSERNS2	NEG	NEG	1
219	8	19	PT2013IBRgBSERPS3	POS	POS	1
220	8	20	PT2013IBRgBSERNS2	NEG	NEG	1
221	9	1	PT2013IBRgBSERNS2	NEG	NEG	1
222	9	2	PT2013IBRgBSERPS3	POS	POS	1
223	9	3	PT2013IBRgBSERNS2	NEG	NEG	1
224	9	4	PT2013IBRgBSERPS4	POS	POS	1
225	9	5	PT2013IBRgBSERPS1	POS	POS	1
226	9	6	PT2013IBRgBSERPS4	POS	POS	1
227	9	7	PT2013IBRgBSERPS1	POS	POS	1
228	9	8	PT2013IBRgBSERPS4	POS	POS	1
229	9	9	PT2013IBRgBSERNS1	NEG	NEG	1
230	9	10	PT2013IBRgBSERPS1	POS	POS	1
231	9	11	PT2013IBRgBSERNS1	NEG	NEG	1
232	9	12	PT2013IBRgBSERPS2	POS	POS	1
233	9	13	PT2013IBRgBSERNS1	NEG	NEG	1
234	9	14	PT2013IBRgBSERPS2	**POS**	**NI**	**0**
235	9	15	PT2013IBRgBSERNS1	NEG	NEG	1
236	9	16	PT2013IBRgBSERPS2	**POS**	**NEG**	**0**
237	9	17	PT2013IBRgBSERNS2	NEG	NEG	1
238	9	18	PT2013IBRgBSERPS3	POS	POS	1
239	9	19	PT2013IBRgBSERNS2	NEG	NEG	1
240	9	20	PT2013IBRgBSERPS3	POS	POS	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **IBRgE** reference serum samples (SAMPLE), the positions of the IBRgE 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	PT2013IBRgESERNS1	NEG	NEG	1
2	1	2	PT2013IBRgESERPS1	POS	POS	1
3	1	3	PT2013IBRgESERNS1	NEG	NEG	1
4	1	4	PT2013IBRgESERPS1	POS	POS	1
5	1	5	PT2013IBRgESERNS1	NEG	NEG	1
6	1	6	PT2013IBRgESERPS1	POS	POS	1
7	1	7	PT2013IBRgESERNS2	NEG	NEG	1
8	1	8	PT2013IBRgESERPS1	POS	POS	1
9	1	9	PT2013IBRgESERNS2	NEG	NEG	1
10	1	10	PT2013IBRgESERPS2	POS	POS	1
11	1	11	PT2013IBRgESERNS2	NEG	NEG	1
12	1	12	PT2013IBRgESERPS2	POS	POS	1
13	1	13	PT2013IBRgESERNS3	NEG	NEG	1
14	1	14	PT2013IBRgESERPS2	POS	POS	1
15	1	15	PT2013IBRgESERNS3	NEG	NEG	1
16	1	16	PT2013IBRgESERPS2	POS	POS	1
17	1	17	PT2013IBRgESERNS3	NEG	NEG	1
18	1	18	PT2013IBRgESERPS3	POS	POS	1
19	1	19	PT2013IBRgESERPS3	POS	POS	1
20	1	20	PT2013IBRgESERPS3	POS	POS	1
21	2	1	PT2013IBRgESERPS3	POS	POS	1
22	2	2	PT2013IBRgESERNS1	NEG	NEG	1
23	2	3	PT2013IBRgESERPS1	POS	POS	1
24	2	4	PT2013IBRgESERNS1	NEG	NEG	1
25	2	5	PT2013IBRgESERPS1	POS	POS	1
26	2	6	PT2013IBRgESERNS1	NEG	NEG	1
27	2	7	PT2013IBRgESERPS1	POS	POS	1
28	2	8	PT2013IBRgESERNS2	NEG	NEG	1
29	2	9	PT2013IBRgESERPS1	POS	POS	1
30	2	10	PT2013IBRgESERNS2	NEG	NEG	1
31	2	11	PT2013IBRgESERPS2	POS	POS	1
32	2	12	PT2013IBRgESERNS2	NEG	NEG	1
33	2	13	PT2013IBRgESERPS2	POS	POS	1
34	2	14	PT2013IBRgESERNS3	NEG	NEG	1
35	2	15	PT2013IBRgESERPS2	POS	POS	1
36	2	16	PT2013IBRgESERNS3	NEG	NEG	1
37	2	17	PT2013IBRgESERPS2	POS	POS	1
38	2	18	PT2013IBRgESERNS3	NEG	NEG	1
39	2	19	PT2013IBRgESERPS3	POS	POS	1
40	2	20	PT2013IBRgESERPS3	POS	POS	1

Table 5. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013IBRgESERPS3	POS	POS	1
42	3	2	PT2013IBRgESERPS3	POS	POS	1
43	3	3	PT2013IBRgESERNS1	NEG	NEG	1
44	3	4	PT2013IBRgESERPS1	POS	POS	1
45	3	5	PT2013IBRgESERNS1	NEG	NEG	1
46	3	6	PT2013IBRgESERPS1	POS	POS	1
47	3	7	PT2013IBRgESERNS1	NEG	NEG	1
48	3	8	PT2013IBRgESERPS1	POS	POS	1
49	3	9	PT2013IBRgESERNS2	NEG	NEG	1
50	3	10	PT2013IBRgESERPS1	POS	POS	1
51	3	11	PT2013IBRgESERNS2	NEG	NEG	1
52	3	12	PT2013IBRgESERPS2	POS	POS	1
53	3	13	PT2013IBRgESERNS2	NEG	NEG	1
54	3	14	PT2013IBRgESERPS2	POS	POS	1
55	3	15	PT2013IBRgESERNS3	NEG	NEG	1
56	3	16	PT2013IBRgESERPS2	POS	POS	1
57	3	17	PT2013IBRgESERNS3	NEG	NEG	1
58	3	18	PT2013IBRgESERPS2	POS	POS	1
59	3	19	PT2013IBRgESERNS3	NEG	NEG	1
60	3	20	PT2013IBRgESERPS3	POS	POS	1
61	4	1	PT2013IBRgESERPS3	POS	POS	1
62	4	2	PT2013IBRgESERPS3	POS	POS	1
63	4	3	PT2013IBRgESERPS3	POS	POS	1
64	4	4	PT2013IBRgESERNS1	NEG	NEG	1
65	4	5	PT2013IBRgESERPS1	POS	POS	1
66	4	6	PT2013IBRgESERNS1	NEG	NEG	1
67	4	7	PT2013IBRgESERPS1	POS	POS	1
68	4	8	PT2013IBRgESERNS1	NEG	NEG	1
69	4	9	PT2013IBRgESERPS1	POS	POS	1
70	4	10	PT2013IBRgESERNS2	NEG	NEG	1
71	4	11	PT2013IBRgESERPS1	POS	POS	1
72	4	12	PT2013IBRgESERNS2	NEG	NEG	1
73	4	13	PT2013IBRgESERPS2	POS	POS	1
74	4	14	PT2013IBRgESERNS2	NEG	NEG	1
75	4	15	PT2013IBRgESERPS2	POS	POS	1
76	4	16	PT2013IBRgESERNS3	NEG	NEG	1
77	4	17	PT2013IBRgESERPS2	POS	POS	1
78	4	18	PT2013IBRgESERNS3	NEG	NEG	1
79	4	19	PT2013IBRgESERPS2	POS	POS	1
80	4	20	PT2013IBRgESERNS3	NEG	NEG	1

Table 5. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5.1	1	PT2013IBRgESERNS3	NEG	NEG	1
82	5.1	2	PT2013IBRgESERPS3	POS	POS	1
83	5.1	3	PT2013IBRgESERPS3	POS	POS	1
84	5.1	4	PT2013IBRgESERPS3	POS	POS	1
85	5.1	5	PT2013IBRgESERNS1	NEG	NEG	1
86	5.1	6	PT2013IBRgESERPS1	POS	POS	1
87	5.1	7	PT2013IBRgESERNS1	NEG	NEG	1
88	5.1	8	PT2013IBRgESERPS1	POS	POS	1
89	5.1	9	PT2013IBRgESERNS1	NEG	NEG	1
90	5.1	10	PT2013IBRgESERPS1	POS	POS	1
91	5.1	11	PT2013IBRgESERNS2	NEG	NEG	1
92	5.1	12	PT2013IBRgESERPS1	POS	POS	1
93	5.1	13	PT2013IBRgESERNS2	NEG	NEG	1
94	5.1	14	PT2013IBRgESERPS2	POS	POS	1
95	5.1	15	PT2013IBRgESERNS2	NEG	NEG	1
96	5.1	16	PT2013IBRgESERPS2	POS	POS	1
97	5.1	17	PT2013IBRgESERNS3	NEG	NEG	1
98	5.1	18	PT2013IBRgESERPS2	POS	POS	1
99	5.1	19	PT2013IBRgESERNS3	NEG	NEG	1
100	5.1	20	PT2013IBRgESERPS2	POS	POS	1
101	5.2	1	PT2013IBRgESERNS3	NEG	NEG	1
102	5.2	2	PT2013IBRgESERPS3	POS	POS	1
103	5.2	3	PT2013IBRgESERPS3	POS	POS	1
104	5.2	4	PT2013IBRgESERPS3	POS	POS	1
105	5.2	5	PT2013IBRgESERNS1	NEG	NEG	1
106	5.2	6	PT2013IBRgESERPS1	POS	POS	1
107	5.2	7	PT2013IBRgESERNS1	NEG	NEG	1
108	5.2	8	PT2013IBRgESERPS1	POS	POS	1
109	5.2	9	PT2013IBRgESERNS1	NEG	NEG	1
110	5.2	10	PT2013IBRgESERPS1	POS	POS	1
111	5.2	11	PT2013IBRgESERNS2	NEG	NEG	1
112	5.2	12	PT2013IBRgESERPS1	POS	POS	1
113	5.2	13	PT2013IBRgESERNS2	NEG	NEG	1
114	5.2	14	PT2013IBRgESERPS2	POS	POS	1
115	5.2	15	PT2013IBRgESERNS2	NEG	NEG	1
116	5.2	16	PT2013IBRgESERPS2	POS	POS	1
117	5.2	17	PT2013IBRgESERNS3	NEG	NEG	1
118	5.2	18	PT2013IBRgESERPS2	POS	POS	1
119	5.2	19	PT2013IBRgESERNS3	NEG	NEG	1
120	5.2	20	PT2013IBRgESERPS2	POS	POS	1

Table 5. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	6	1	PT2013IBRgESERPS2	POS	POS	1
122	6	2	PT2013IBRgESERNS3	NEG	NEG	1
123	6	3	PT2013IBRgESERPS3	POS	POS	1
124	6	4	PT2013IBRgESERPS3	POS	POS	1
125	6	5	PT2013IBRgESERPS3	POS	POS	1
126	6	6	PT2013IBRgESERNS1	NEG	NEG	1
127	6	7	PT2013IBRgESERPS1	POS	POS	1
128	6	8	PT2013IBRgESERNS1	NEG	NEG	1
129	6	9	PT2013IBRgESERPS1	POS	POS	1
130	6	10	PT2013IBRgESERNS1	NEG	NEG	1
131	6	11	PT2013IBRgESERPS1	POS	POS	1
132	6	12	PT2013IBRgESERNS2	NEG	NEG	1
133	6	13	PT2013IBRgESERPS1	POS	POS	1
134	6	14	PT2013IBRgESERNS2	NEG	NEG	1
135	6	15	PT2013IBRgESERPS2	POS	POS	1
136	6	16	PT2013IBRgESERNS2	NEG	NEG	1
137	6	17	PT2013IBRgESERPS2	POS	POS	1
138	6	18	PT2013IBRgESERNS3	NEG	NEG	1
139	6	19	PT2013IBRgESERPS2	POS	POS	1
140	6	20	PT2013IBRgESERNS3	NEG	NEG	1
141	7.1	1	PT2013IBRgESERNS3	NEG	NEG	1
142	7.1	2	PT2013IBRgESERPS2	POS	POS	1
143	7.1	3	PT2013IBRgESERNS3	NEG	NEG	1
144	7.1	4	PT2013IBRgESERPS3	POS	POS	1
145	7.1	5	PT2013IBRgESERPS3	POS	POS	1
146	7.1	6	PT2013IBRgESERPS3	POS	POS	1
147	7.1	7	PT2013IBRgESERNS1	NEG	NEG	1
148	7.1	8	PT2013IBRgESERPS1	POS	POS	1
149	7.1	9	PT2013IBRgESERNS1	NEG	NEG	1
150	7.1	10	PT2013IBRgESERPS1	POS	POS	1
151	7.1	11	PT2013IBRgESERNS1	NEG	NEG	1
152	7.1	12	PT2013IBRgESERPS1	POS	POS	1
153	7.1	13	PT2013IBRgESERNS2	NEG	NEG	1
154	7.1	14	PT2013IBRgESERPS1	POS	POS	1
155	7.1	15	PT2013IBRgESERNS2	NEG	NEG	1
156	7.1	16	PT2013IBRgESERPS2	POS	POS	1
157	7.1	17	PT2013IBRgESERNS2	NEG	NEG	1
158	7.1	18	PT2013IBRgESERPS2	POS	POS	1
159	7.1	19	PT2013IBRgESERNS3	NEG	NEG	1
160	7.1	20	PT2013IBRgESERPS2	POS	POS	1

Table 5. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	7.2	1	PT2013IBRgESERNS3	NEG	NEG	1
162	7.2	2	PT2013IBRgESERPS2	POS	POS	1
163	7.2	3	PT2013IBRgESERNS3	NEG	NEG	1
164	7.2	4	PT2013IBRgESERPS3	POS	POS	1
165	7.2	5	PT2013IBRgESERPS3	POS	POS	1
166	7.2	6	PT2013IBRgESERPS3	POS	POS	1
167	7.2	7	PT2013IBRgESERNS1	NEG	NEG	1
168	7.2	8	PT2013IBRgESERPS1	POS	POS	1
169	7.2	9	PT2013IBRgESERNS1	NEG	NEG	1
170	7.2	10	PT2013IBRgESERPS1	POS	POS	1
171	7.2	11	PT2013IBRgESERNS1	NEG	NEG	1
172	7.2	12	PT2013IBRgESERPS1	POS	POS	1
173	7.2	13	PT2013IBRgESERNS2	NEG	NEG	1
174	7.2	14	PT2013IBRgESERPS1	POS	POS	1
175	7.2	15	PT2013IBRgESERNS2	NEG	NEG	1
176	7.2	16	PT2013IBRgESERPS2	POS	POS	1
177	7.2	17	PT2013IBRgESERNS2	NEG	NEG	1
178	7.2	18	PT2013IBRgESERPS2	POS	POS	1
179	7.2	19	PT2013IBRgESERNS3	NEG	NEG	1
180	7.2	20	PT2013IBRgESERPS2	POS	POS	1
181	8	1	PT2013IBRgESERPS2	POS	POS	1
182	8	2	PT2013IBRgESERNS3	NEG	NEG	1
183	8	3	PT2013IBRgESERPS2	POS	POS	1
184	8	4	PT2013IBRgESERNS3	NEG	NEG	1
185	8	5	PT2013IBRgESERPS3	POS	POS	1
186	8	6	PT2013IBRgESERPS3	POS	POS	1
187	8	7	PT2013IBRgESERPS3	POS	POS	1
188	8	8	PT2013IBRgESERNS1	NEG	NEG	1
189	8	9	PT2013IBRgESERPS1	POS	POS	1
190	8	10	PT2013IBRgESERNS1	NEG	NEG	1
191	8	11	PT2013IBRgESERPS1	POS	POS	1
192	8	12	PT2013IBRgESERNS1	NEG	NEG	1
193	8	13	PT2013IBRgESERPS1	POS	POS	1
194	8	14	PT2013IBRgESERNS2	NEG	NEG	1
195	8	15	PT2013IBRgESERPS1	POS	POS	1
196	8	16	PT2013IBRgESERNS2	NEG	NEG	1
197	8	17	PT2013IBRgESERPS2	POS	POS	1
198	8	18	PT2013IBRgESERNS2	NEG	NEG	1
199	8	19	PT2013IBRgESERPS2	POS	POS	1
200	8	20	PT2013IBRgESERNS3	NEG	NEG	1

Table 5. (CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	10	1	PT2013IBRgESERNS3	NEG	NEG	1
202	10	2	PT2013IBRgESERPS2	POS	POS	1
203	10	3	PT2013IBRgESERNS3	NEG	NEG	1
204	10	4	PT2013IBRgESERPS2	POS	POS	1
205	10	5	PT2013IBRgESERNS3	NEG	NEG	1
206	10	6	PT2013IBRgESERPS3	POS	POS	1
207	10	7	PT2013IBRgESERPS3	POS	POS	1
208	10	8	PT2013IBRgESERPS3	POS	POS	1
209	10	9	PT2013IBRgESERNS1	NEG	NEG	1
210	10	10	PT2013IBRgESERPS1	POS	POS	1
211	10	11	PT2013IBRgESERNS1	NEG	NEG	1
212	10	12	PT2013IBRgESERPS1	POS	POS	1
213	10	13	PT2013IBRgESERNS1	NEG	NEG	1
214	10	14	PT2013IBRgESERPS1	POS	POS	1
215	10	15	PT2013IBRgESERNS2	NEG	NEG	1
216	10	16	PT2013IBRgESERPS1	POS	POS	1
217	10	17	PT2013IBRgESERNS2	NEG	NEG	1
218	10	18	PT2013IBRgESERPS2	POS	POS	1
219	10	19	PT2013IBRgESERNS2	NEG	NEG	1
220	10	20	PT2013IBRgESERPS2	POS	POS	1

V. Discussion

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

For the detection of IBRgB-specific antibodies in reference serum samples, 7 out of 9 participating laboratories (LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB8) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). Both LAB3, LAB4 and LAB5 analysed the reference serum samples using 2 ELISA kits from different producers, hereby obtaining identical qualitative results. In contrast, LAB7 misclassified 3 aliquots of the reference serum sample PT2013IBRgBSERPS1 (85% of agreement), whereas LAB9 misclassified 2 aliquots of the reference serum sample PT2013IBRgBSERPS2 (90% of agreement) (Table 2 and Table 4). One participating laboratory used an in-house developed IBRgB antibody ELISA kit, whereas the other participants used IBRgB antibody ELISA kits from 4 different commercial kit producers. Hereby, different kits from the same producer and different batches from the same ELISA kit were used: IDEXX (HerdChek IBR gB X2 Ab Test [IDEXX Switzerland AG] - 2 batches: A321, A001; IBR gB Blocking Ab Test [IDEXX Montpellier SAS] - 1 batch: 2223), Synbiotics Europe (1 batch: 13SIBR1BN211), LSI (1 batch: IBRG-005) and QIAGEN (1 batch: 12-11-7BHV). LAB1, LAB2, LAB3.1, LAB4.1 and LAB5.1 used the same IBRgB ELISA kit, hereby all performing the long incubation protocol. In addition, LAB1, LAB2, LAB3.1 and LAB4.1 used the same batch. Furthermore, also LAB3.2, LAB4.2 and LAB6 used the same IBRgB ELISA kit, all performing the short incubation protocol of the same batch.

For the detection of IBRgE-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). Both LAB5 and LAB7 obtained identical qualitative results using 2 different batches of the same ELISA kit (Table 3 and Table 5).
The IBRgE participating laboratories used ELISA kits from 3 different producers as well as different batches from the same ELISA kit: IDEXX (5 batches: KH521, AJ888, CJ201, AJ894, CJ220), LSI (1 batch: IBRGE-001) and ID.VET (1 batch: 466). LAB1, LAB2, LAB3, LAB4, LAB5.1, LAB5.2, LAB7.1, LAB7.2 and LAB8 used the same IBRgE ELISA kit. In addition, LAB1 and LAB8 on the one hand, and LAB2, LAB3, LAB4 and LAB5.1 on the other hand, used the same batch.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 95% (PT IBRgB) or at least 90% (PT IBRgE) 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.). As a consequence: (i) 7 out of 9 laboratories that participated in the PT IBRgB achieved a satisfactory performance for the detection of IBRgB-specific antibodies in reference serum samples of bovine origin by ELISA with all ELISA kits used, and (ii) all laboratories that participated in the PT IBRgE achieved a satisfactory performance for the detection of IBRgE-specific antibodies in reference serum samples of bovine origin with all batches used. For the PT IBRgB, LAB7 and LAB9 did not reach the required 95% of agreement.

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) (Sophia-Antipolis, France)

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Friedrich-Loeffler-Institut (FLI) (Greifswald-Insel-Riems, Germany)

Innovative Diagnostics (ID.VET) (Montpellier, France)

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

Laboratoire Service International (LSI) (Lissieu, France)

State Veterinary Institute Zvolen (Zvolen, Slovakia)

Synbiotics Europe (Lyon, 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 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- (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.

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 IBRgB and the PT IBRgE are shown in Figure 1 and Figure 2, respectively.

Remark: To calculate the percentages blocking, the PT provider used the formula [1- (OD_{Sample} / mean OD $_{Negative\ Kit\ Controls}$)] x 100 for both the PT IBRgB and the PT IBRgE. Because LAB7 calculated the percentages blocking for the PT IBRgB using the formula [(mean $OD_{Negative\ Kit\ Controls}$ - OD_{Sample}) / (mean $OD_{Negative\ Kit\ Controls}$ - mean $OD_{Positive\ Kit\ Controls}$)] x100, the cut-offs for the IBRgB antibody ELISA kit used by LAB7 were adapted accordingly (26,4-44,0% instead of 30-50%).

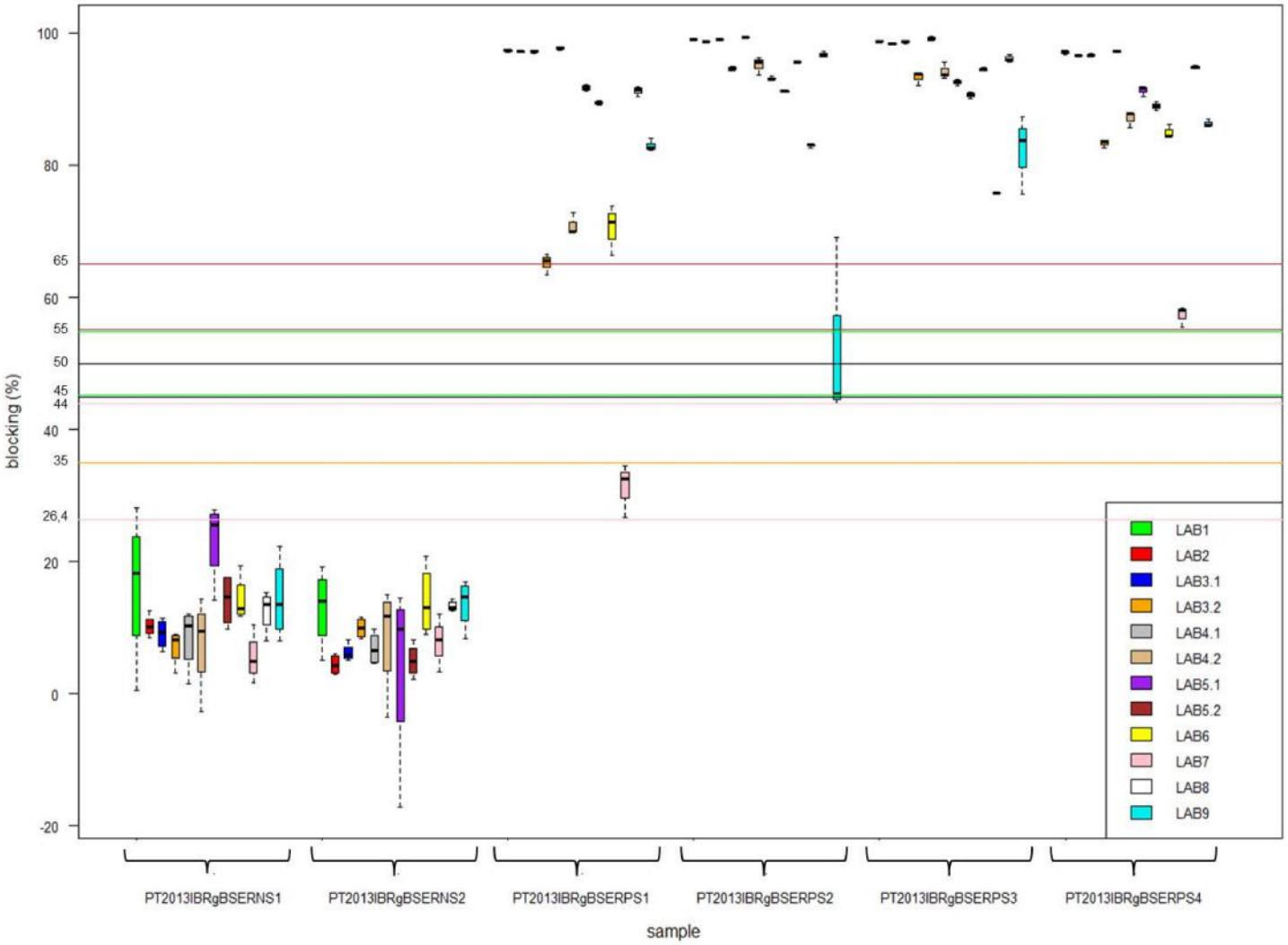

Figure 1. Box plots showing the percentage blocking per IBRgB reference serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are shown in pink (26,4-44%; LAB7), orange (35%; LAB3.2, LAB4.2, LAB6), black (45-50%; LAB8, LAB9), green (45-55%; LAB1, LAB2, LAB3.1, LAB4.1, LAB5.1), brown (55-65%; LAB5.2), respectively. LAB1, LAB2, LAB3.1, LAB4.1 and LAB5.1 performed the same incubation protocol of the same IBRgB ELISA kit (2 different batches; LAB1, LAB2, LAB3.1 and LAB4.1 used the same batch). Also LAB3.2, LAB4.2 and LAB6 performed the same incubation protocol of the same IBRgB ELISA kit (1 batch).

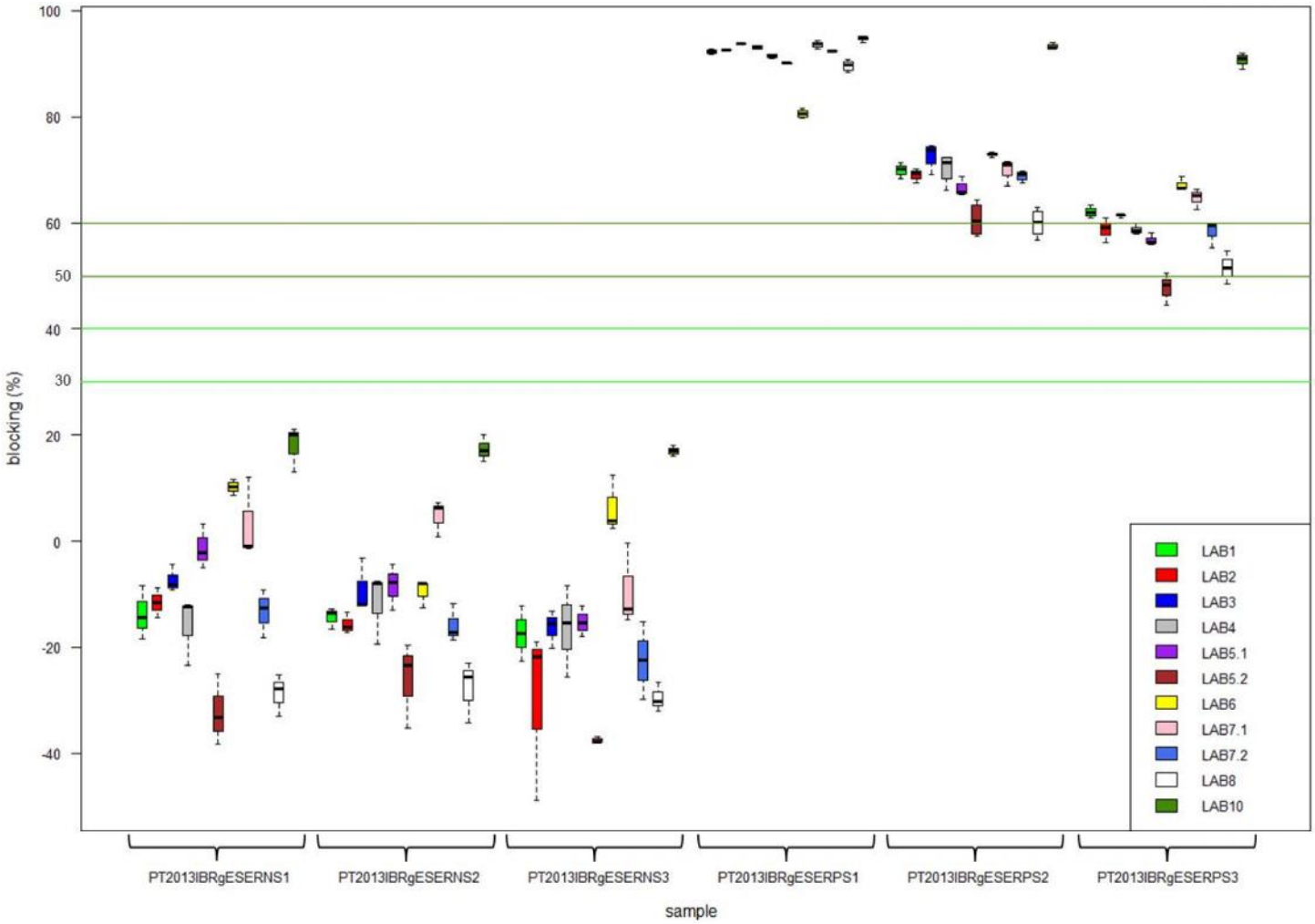

Figure 2. Box plots showing the percentage blocking per IBRgE reference serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in light green (30-40%; all participants except LAB10) and dark green (50-60%; LAB10), respectively. LAB1, LAB2, LAB3, LAB4, LAB5.1, LAB5.2, LAB7.1, LAB7.2 and LAB8 used the same IBRgE ELISA kit (same incubation protocol). In addition, LAB1 and LAB8 on the one hand, and LAB2, LAB3, LAB4 and LAB5.1 on the other hand, used the same batch.

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 1,83 for the PT IBRgB (p=12) and 1,82 for the PT IBRgE (p=11). For the PT IBRgB, the maximum value for Mandel's k-statistic is 1,69 for the reference serum samples PT2013IBRgBSERPS1, PT2013IBRgBSERPS2, PT2013IBRgBSERPS3 and PT2013IBRgBSERPS4 (p=12 and n=3) and 1,58 for the reference serum samples PT2013IBRgBSERNS1 and PT2013IBRgBSERNS2 (p=12 and n=4). For the PT IBRgE, the maximum value for Mandel's k-statistic is 1,69 for the reference serum samples PT2013IBRgESERNS1, PT2013IBRgESERNS2, PT2013IBRgESERNS3 and PT2013IBRgESERPS3 (p=11 and n=3) and 1,58 for the reference serum samples PT2013IBRgESERPS1 and PT2013IBRgESERPS2 (p=11 and n=4).

For the detection of IBRgB-specific antibodies, 9 out of 12 participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB5.1, LAB7 and LAB9 which showed an increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB5.1 for the negative reference serum sample PT2013IBRgBSERNS1 (h=2,29), LAB7 for the positive reference serum samples PT2013IBRgBSERPS1 (h=-2,56), PT2013IBRgBSERPS3 (h=-2,38) and PT2013IBRgBSERPS4 (h=-2,81), and LAB9 for the positive reference serum sample PT2013IBRgBSERPS2 (h=-2,97). LAB1, LAB2, LAB3.1, LAB4.1 and LAB5.1 on the one hand, and LAB3.2, LAB4.2 and LAB6 on the other hand, performed the same incubation protocol of the same IBRgB ELISA kit. Hereby, LAB1, LAB2, LAB3.1 and LAB4.1 on the one hand, and LAB3.2, LAB4.2 and LAB6 on the other hand used the same batch.
Furthermore, 7 out of 12 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB1, LAB5.1, LAB6, LAB7 and LAB9 which showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB1 for the negative reference serum sample PT2013IBRgBSERNS1 (k=2,12), LAB5.1 for the negative reference serum sample PT2013IBRgBSERNS2 (k=2,53), LAB6 for the positive reference serum sample PT2013IBRgBSERPS1 (k=2,14), LAB7 for the positive reference serum samples PT2013IBRgBSERPS1 (k=2,25), and PT2013IBRgBSERPS4 (k=2,02), and LAB9 for the positive reference serum samples PT2013IBRgBSERPS2 (k=3,44) and PT2013IBRgBSERPS3 (k=3,29).

For the detection of IBRgE-specific antibodies, 9 out of 11 participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB6 and LAB10, which showed an increased increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB6 for the positive reference serum sample PT2013IBRgESERPS1 (h=-2,77) and LAB10 for the negative reference serum samples PT2013IBRgESERNS2 (h=2,19) and PT2013IBRgESERNS3 (h=2,05) and for the positive reference serum samples PT2013IBRgESERPS2 (h=2,62) and PT2013IBRgESERPS3 (h=2,62). LAB1, LAB2, LAB3, LAB4, LAB5.1, LAB5.2, LAB7.1, LAB7.2 and LAB8 used the same IBRgE ELISA kit (same incubation protocol). In addition, LAB1 and LAB8 on the one hand, and LAB2, LAB3, LAB4 and LAB5.1 on the other hand, used the same batch.
Furthermore, 8 out of 11 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB2, LAB5.2 and LAB8, which showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB2 for the negative reference serum sample PT2013IBRgESERNS3 (k=2,33), LAB5.2 for the negative reference serum sample PT2013IBRgESERNS2 (k=1,75) and for the positive reference serum sample PT2013IBRgESERPS2 (k=1,65), and LAB8 for the positive reference serum sample PT2013IBRgESERPS1 (k=2,07).

All data used for the calculations of Mandel's h- and k-statistics for the PT IBRgB and the PT IBRgE 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 (α=0,05) between participating laboratories and between different kits or batches used at the same laboratory. 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. IBRgB

For the PT IBRgB, no statistically significant differences were observed between laboratories or between different kits used at the same laboratory at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were only observed for the positive reference serum samples. LAB7 reported percentages blocking that were significantly lower than those reported by the other participants. LAB4.1, LAB1, LAB3.1 and LAB2 reported percentages blocking that were significantly higher than those reported by LAB3.2, LAB9 and LAB7. Of those laboratories that tested 2 different IBRgB ELISA kits (LAB3, LAB4 and LAB5), only a significant difference was observed between LAB3.1 and LAB3.2, with lower percentages blocking for the latter.

III.2. IBRgE

For the PT IBRgE, no statistically significant differences were observed between laboratories or between different batches used at the same laboratory 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. For the negative reference serum samples, LAB10 reported percentages blocking that were significantly higher than those reported by the other laboratories. For the positive reference serum samples, LAB10 reported percentages blocking that were significantly higher than those reported by LAB8 and LAB5.2. The percentages blocking obtained by LAB5 and LAB7 using 2 different batches of the same IBRgE ELISA kit (LAB5.1 and LAB5.2, LAB7.1 and LAB7.2, respectively) did result in significant differences for the negative but not for the positive reference serum samples. The percentages blocking for the negative reference serum samples obtained by LAB5.1 and LAB7.1 were significantly higher than those obtained by LAB5.2 and LAB7.2, respectively.

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

A. IBRgB

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgBSERNS1	1	4	133,24	16,33	11,94	0,05	5,43	5,58	1,25	0,89	2,12	70,67
PT2013IBRgBSERNS1	2	4	2,82	10,28	11,94	0,05	5,43	5,58	1,25	-0,33	0,31	16,33
PT2013IBRgBSERNS1	3.1	4	5,33	9,07	11,94	0,05	5,43	5,58	1,25	-0,58	0,42	25,45
PT2013IBRgBSERNS1	3.2	4	7,28	7,11	11,94	0,05	5,43	5,58	1,25	-0,97	0,50	37,95
PT2013IBRgBSERNS1	4.1	4	23,71	8,51	11,94	0,05	5,43	5,58	1,25	-0,69	0,90	57,19
PT2013IBRgBSERNS1	4.2	4	53,74	7,67	11,94	0,05	5,43	5,58	1,25	-0,86	1,35	95,52
PT2013IBRgBSERNS1	5.1	4	38,67	23,29	11,94	0,05	5,43	5,58	1,25	2,29	1,14	26,70
PT2013IBRgBSERNS1	5.2	4	15,59	14,18	11,94	0,05	5,43	5,58	1,25	0,45	0,73	27,84
PT2013IBRgBSERNS1	6	4	12,47	14,27	11,94	0,05	5,43	5,58	1,25	0,47	0,65	24,75
PT2013IBRgBSERNS1	7	4	13,32	5,51	11,94	0,05	5,43	5,58	1,25	-1,30	0,67	66,20
PT2013IBRgBSERNS1	8	4	10,20	12,63	11,94	0,05	5,43	5,58	1,25	0,14	0,59	25,28
PT2013IBRgBSERNS1	9	4	38,06	14,37	11,94	0,05	5,43	5,58	1,25	0,49	1,14	42,94
PT2013IBRgBSERNS2	1	4	35,66	13,05	8,95	0,02	5,70	5,75	0,73	1,09	1,05	45,78
PT2013IBRgBSERNS2	2	4	2,59	4,44	8,95	0,02	5,70	5,75	0,73	-1,21	0,28	36,29
PT2013IBRgBSERNS2	3.1	4	1,81	6,27	8,95	0,02	5,70	5,75	0,73	-0,72	0,24	21,45
PT2013IBRgBSERNS2	3.2	4	2,42	9,98	8,95	0,02	5,70	5,75	0,73	0,27	0,27	15,59
PT2013IBRgBSERNS2	4.1	4	6,12	6,85	8,95	0,02	5,70	5,75	0,73	-0,56	0,43	36,10
PT2013IBRgBSERNS2	4.2	4	70,02	8,70	8,95	0,02	5,70	5,75	0,73	-0,07	1,47	96,18
PT2013IBRgBSERNS2	5.1	4	208,74	4,25	8,95	0,02	5,70	5,75	0,73	-1,26	2,53	340,17
PT2013IBRgBSERNS2	5.2	4	6,32	5,04	8,95	0,02	5,70	5,75	0,73	-1,04	0,44	49,88
PT2013IBRgBSERNS2	6	4	28,80	13,98	8,95	0,02	5,70	5,75	0,73	1,34	0,94	38,37
PT2013IBRgBSERNS2	7	4	12,74	7,95	8,95	0,02	5,70	5,75	0,73	-0,27	0,63	44,89
PT2013IBRgBSERNS2	8	4	0,63	13,30	8,95	0,02	5,70	5,75	0,73	1,16	0,14	5,99

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgBSERNS2	9	4	14,37	13,63	8,95	0,02	5,70	5,75	0,73	1,25	0,66	27,81
PT2013IBRgBSERPS1	1	3	0,04	97,30	81,88	0,92	1,81	6,22	5,95	0,78	0,11	0,21
PT2013IBRgBSERPS1	2	3	0,04	97,20	81,88	0,92	1,81	6,22	5,95	0,78	0,11	0,21
PT2013IBRgBSERPS1	3.1	3	0,05	97,20	81,88	0,92	1,81	6,22	5,95	0,78	0,13	0,24
PT2013IBRgBSERPS1	3.2	3	2,65	65,18	81,88	0,92	1,81	6,22	5,95	-0,84	0,90	2,50
PT2013IBRgBSERPS1	4.1	3	0,09	97,67	81,88	0,92	1,81	6,22	5,95	0,80	0,17	0,31
PT2013IBRgBSERPS1	4.2	3	2,94	70,88	81,88	0,92	1,81	6,22	5,95	-0,56	0,95	2,42
PT2013IBRgBSERPS1	5.1	3	0,27	91,70	81,88	0,92	1,81	6,22	5,95	0,50	0,29	0,57
PT2013IBRgBSERPS1	5.2	3	0,12	89,44	81,88	0,92	1,81	6,22	5,95	0,38	0,19	0,38
PT2013IBRgBSERPS1	**6**	3	15,00	70,51	81,88	0,92	1,81	6,22	5,95	-0,58	**2,14**	5,49
PT2013IBRgBSERPS1	**7**	3	16,69	31,23	81,88	0,92	1,81	6,22	5,95	**-2,56**	**2,25**	13,08
PT2013IBRgBSERPS1	8	3	0,55	91,24	81,88	0,92	1,81	6,22	5,95	0,47	0,41	0,82
PT2013IBRgBSERPS1	9	3	0,95	82,94	81,88	0,92	1,81	6,22	5,95	0,05	0,54	1,18
PT2013IBRgBSERPS2	1	3	0,01	98,99	91,51	0,47	4,10	5,63	3,86	0,57	0,02	0,08
PT2013IBRgBSERPS2	2	3	0,00	98,63	91,51	0,47	4,10	5,63	3,86	0,55	0,00	0,01
PT2013IBRgBSERPS2	3.1	3	0,01	99,03	91,51	0,47	4,10	5,63	3,86	0,58	0,02	0,10
PT2013IBRgBSERPS2	3.2	3	0,08	94,69	91,51	0,47	4,10	5,63	3,86	0,24	0,07	0,30
PT2013IBRgBSERPS2	4.1	3	0,00	99,31	91,51	0,47	4,10	5,63	3,86	0,60	0,01	0,05
PT2013IBRgBSERPS2	4.2	3	1,81	95,14	91,51	0,47	4,10	5,63	3,86	0,28	0,33	1,42
PT2013IBRgBSERPS2	5.1	3	0,10	93,10	91,51	0,47	4,10	5,63	3,86	0,12	0,08	0,33
PT2013IBRgBSERPS2	5.2	3	0,01	91,15	91,51	0,47	4,10	5,63	3,86	-0,03	0,02	0,10
PT2013IBRgBSERPS2	6	3	0,00	95,61	91,51	0,47	4,10	5,63	3,86	0,31	0,01	0,05
PT2013IBRgBSERPS2	7	3	0,06	82,95	91,51	0,47	4,10	5,63	3,86	-0,66	0,06	0,29
PT2013IBRgBSERPS2	8	3	0,20	96,71	91,51	0,47	4,10	5,63	3,86	0,40	0,11	0,46
PT2013IBRgBSERPS2	**9**	3	199,87	52,81	91,51	0,47	4,10	5,63	3,86	**-2,97**	**3,44**	26,77
PT2013IBRgBSERPS3	1	3	0,01	98,78	92,85	0,58	1,81	2,80	2,14	0,83	0,06	0,11
PT2013IBRgBSERPS3	2	3	0,00	98,36	92,85	0,58	1,81	2,80	2,14	0,77	0,02	0,04

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgBSERPS3	3.1	3	0,06	98,68	92,85	0,58	1,81	2,80	2,14	0,81	0,13	0,24
PT2013IBRgBSERPS3	3.2	3	1,11	93,28	92,85	0,58	1,81	2,80	2,14	0,06	0,58	1,13
PT2013IBRgBSERPS3	4.1	3	0,08	99,17	92,85	0,58	1,81	2,80	2,14	0,88	0,16	0,29
PT2013IBRgBSERPS3	4.2	3	1,69	94,18	92,85	0,58	1,81	2,80	2,14	0,19	0,72	1,38
PT2013IBRgBSERPS3	5.1	3	0,29	92,55	92,85	0,58	1,81	2,80	2,14	-0,04	0,30	0,58
PT2013IBRgBSERPS3	5.2	3	0,18	90,62	92,85	0,58	1,81	2,80	2,14	-0,31	0,24	0,47
PT2013IBRgBSERPS3	6	3	0,09	94,49	92,85	0,58	1,81	2,80	2,14	0,23	0,17	0,32
PT2013IBRgBSERPS3	**7**	3	0,02	75,77	92,85	0,58	1,81	2,80	2,14	**-2,38**	0,08	0,20
PT2013IBRgBSERPS3	8	3	0,34	96,11	92,85	0,58	1,81	2,80	2,14	0,45	0,32	0,60
PT2013IBRgBSERPS3	**9**	3	35,46	82,23	92,85	0,58	1,81	2,80	2,14	-1,48	**3,29**	7,24
PT2013IBRgBSERPS4	1	3	0,08	97,00	88,45	0,95	0,77	3,42	3,33	0,77	0,37	0,29
PT2013IBRgBSERPS4	2	3	0,01	96,56	88,45	0,95	0,77	3,42	3,33	0,73	0,14	0,11
PT2013IBRgBSERPS4	3.1	3	0,05	96,64	88,45	0,95	0,77	3,42	3,33	0,74	0,28	0,22
PT2013IBRgBSERPS4	3.2	3	0,41	83,29	88,45	0,95	0,77	3,42	3,33	-0,47	0,83	0,77
PT2013IBRgBSERPS4	4.1	3	0,04	97,23	88,45	0,95	0,77	3,42	3,33	0,79	0,27	0,21
PT2013IBRgBSERPS4	4.2	3	1,58	87,07	88,45	0,95	0,77	3,42	3,33	-0,12	1,64	1,44
PT2013IBRgBSERPS4	5.1	3	0,47	91,26	88,45	0,95	0,77	3,42	3,33	0,25	0,90	0,75
PT2013IBRgBSERPS4	5.2	3	0,36	88,91	88,45	0,95	0,77	3,42	3,33	0,04	0,79	0,68
PT2013IBRgBSERPS4	6	3	1,19	84,97	88,45	0,95	0,77	3,42	3,33	-0,31	1,42	1,28
PT2013IBRgBSERPS4	**7**	3	2,40	57,29	88,45	0,95	0,77	3,42	3,33	**-2,81**	**2,02**	2,70
PT2013IBRgBSERPS4	8	3	0,08	94,81	88,45	0,95	0,77	3,42	3,33	0,57	0,37	0,30
PT2013IBRgBSERPS4	9	3	0,36	86,35	88,45	0,95	0,77	3,42	3,33	-0,19	0,78	0,69

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; 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. IBRgE

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgESERNS1	1	3	24,92	-13,75	-8,43	0,49	4,85	6,79	4,75	-0,35	1,03	-36,31
PT2013IBRgESERNS1	2	3	7,79	-11,63	-8,43	0,49	4,85	6,79	4,75	-0,21	0,58	-24,00
PT2013IBRgESERNS1	3	3	6,51	-7,32	-8,43	0,49	4,85	6,79	4,75	0,07	0,53	-34,84
PT2013IBRgESERNS1	4	3	41,96	-15,96	-8,43	0,49	4,85	6,79	4,75	-0,49	1,33	-40,58
PT2013IBRgESERNS1	5.1	3	17,77	-1,31	-8,43	0,49	4,85	6,79	4,75	0,47	0,87	-322,08
PT2013IBRgESERNS1	5.2	3	43,81	-32,21	-8,43	0,49	4,85	6,79	4,75	-1,56	1,36	-20,55
PT2013IBRgESERNS1	6	3	2,43	10,19	-8,43	0,49	4,85	6,79	4,75	1,22	0,32	15,30
PT2013IBRgESERNS1	7.1	3	58,05	3,31	-8,43	0,49	4,85	6,79	4,75	0,77	1,57	229,85
PT2013IBRgESERNS1	7.2	3	21,03	-13,32	-8,43	0,49	4,85	6,79	4,75	-0,32	0,95	-34,43
PT2013IBRgESERNS1	8	3	15,73	-28,77	-8,43	0,49	4,85	6,79	4,75	-1,33	0,82	-13,79
PT2013IBRgESERNS1	10	3	19,00	18,00	-8,43	0,49	4,85	6,79	4,75	1,73	0,90	24,22
PT2013IBRgESERNS2	1	3	4,30	-14,35	-10,56	0,42	4,63	6,08	3,94	-0,30	0,45	-14,44
PT2013IBRgESERNS2	2	3	3,75	-15,66	-10,56	0,42	4,63	6,08	3,94	-0,40	0,42	-12,37
PT2013IBRgESERNS2	3	3	26,50	-9,10	-10,56	0,42	4,63	6,08	3,94	0,11	1,11	-56,59
PT2013IBRgESERNS2	4	3	44,78	-11,60	-10,56	0,42	4,63	6,08	3,94	-0,08	1,45	-57,69
PT2013IBRgESERNS2	5.1	3	18,70	-8,39	-10,56	0,42	4,63	6,08	3,94	0,17	0,93	-51,56
PT2013IBRgESERNS2	5.2	3	65,37	-26,08	-10,56	0,42	4,63	6,08	3,94	-1,22	1,75	-31,00
PT2013IBRgESERNS2	6	3	7,48	-9,50	-10,56	0,42	4,63	6,08	3,94	0,08	0,59	-28,78
PT2013IBRgESERNS2	7.1	3	11,65	4,70	-10,56	0,42	4,63	6,08	3,94	1,20	0,74	72,56
PT2013IBRgESERNS2	7.2	3	12,39	-15,86	-10,56	0,42	4,63	6,08	3,94	-0,42	0,76	-22,19
PT2013IBRgESERNS2	8	3	34,11	-27,68	-10,56	0,42	4,63	6,08	3,94	-1,34	1,26	-21,10
PT2013IBRgESERNS2	10	3	6,33	17,33	-10,56	0,42	4,63	6,08	3,94	2,19	0,54	14,52
PT2013IBRgESERNS3	1	3	27,01	-17,43	-15,55	0,32	7,05	8,56	4,85	-0,12	0,74	-29,82
PT2013IBRgESERNS3	2	3	270,27	-29,92	-15,55	0,32	7,05	8,56	4,85	-0,91	2,33	-54,94
PT2013IBRgESERNS3	3	3	12,66	-16,32	-15,55	0,32	7,05	8,56	4,85	-0,05	0,50	-21,80
PT2013IBRgESERNS3	4	3	74,07	-16,48	-15,55	0,32	7,05	8,56	4,85	-0,06	1,22	-52,21

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgESERNS3	5.1	3	8,90	-15,22	-15,55	0,32	7,05	8,56	4,85	0,02	0,42	-19,60
PT2013IBRgESERNS3	5.2	3	0,39	-37,52	-15,55	0,32	7,05	8,56	4,85	-1,38	0,09	-1,67
PT2013IBRgESERNS3	6	3	29,96	6,26	-15,55	0,32	7,05	8,56	4,85	1,38	0,78	87,44
PT2013IBRgESERNS3	7.1	3	61,42	-9,30	-15,55	0,32	7,05	8,56	4,85	0,39	1,11	-84,25
PT2013IBRgESERNS3	7.2	3	53,82	-22,49	-15,55	0,32	7,05	8,56	4,85	-0,44	1,04	-32,62
PT2013IBRgESERNS3	8	3	7,16	-29,66	-15,55	0,32	7,05	8,56	4,85	-0,89	0,38	-9,02
PT2013IBRgESERNS3	10	3	1,00	17,00	-15,55	0,32	7,05	8,56	4,85	2,05	0,14	5,88
PT2013IBRgESERPS1	1	4	0,18	92,25	91,30	0,86	0,49	1,32	1,22	0,24	0,86	0,45
PT2013IBRgESERPS1	2	4	0,00	92,56	91,30	0,86	0,49	1,32	1,22	0,32	0,00	0,00
PT2013IBRgESERPS1	3	4	0,02	93,77	91,30	0,86	0,49	1,32	1,22	0,64	0,25	0,13
PT2013IBRgESERPS1	4	4	0,09	93,11	91,30	0,86	0,49	1,32	1,22	0,47	0,62	0,32
PT2013IBRgESERPS1	5.1	4	0,14	91,51	91,30	0,86	0,49	1,32	1,22	0,05	0,78	0,41
PT2013IBRgESERPS1	5.2	4	0,01	90,10	91,30	0,86	0,49	1,32	1,22	-0,31	0,22	0,12
PT2013IBRgESERPS1	6	4	0,54	80,57	91,30	0,86	0,49	1,32	1,22	-2,77	1,51	0,91
PT2013IBRgESERPS1	7.1	4	0,36	93,64	91,30	0,86	0,49	1,32	1,22	0,61	1,23	0,64
PT2013IBRgESERPS1	7.2	4	0,00	92,42	91,30	0,86	0,49	1,32	1,22	0,29	0,11	0,06
PT2013IBRgESERPS1	8	4	1,02	89,63	91,30	0,86	0,49	1,32	1,22	-0,43	2,07	1,13
PT2013IBRgESERPS1	10	4	0,25	94,75	91,30	0,86	0,49	1,32	1,22	0,89	1,03	0,53
PT2013IBRgESERPS2	1	4	1,43	70,03	70,40	0,65	1,99	3,38	2,74	-0,04	0,60	1,71
PT2013IBRgESERPS2	2	4	1,37	69,07	70,40	0,65	1,99	3,38	2,74	-0,15	0,59	1,69
PT2013IBRgESERPS2	3	4	6,28	72,83	70,40	0,65	1,99	3,38	2,74	0,28	1,26	3,44
PT2013IBRgESERPS2	4	4	8,24	70,28	70,40	0,65	1,99	3,38	2,74	-0,01	1,44	4,08
PT2013IBRgESERPS2	5.1	4	2,39	66,40	70,40	0,65	1,99	3,38	2,74	-0,46	0,78	2,33
PT2013IBRgESERPS2	5.2	4	10,86	60,66	70,40	0,65	1,99	3,38	2,74	-1,12	1,65	5,43
PT2013IBRgESERPS2	6	4	0,17	72,96	70,40	0,65	1,99	3,38	2,74	0,29	0,20	0,56
PT2013IBRgESERPS2	7.1	4	4,53	70,05	70,40	0,65	1,99	3,38	2,74	-0,04	1,07	3,04
PT2013IBRgESERPS2	7.2	4	0,94	68,85	70,40	0,65	1,99	3,38	2,74	-0,18	0,49	1,41

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgESERPS2	8	4	7,20	59,99	70,40	0,65	1,99	3,38	2,74	-1,20	1,35	4,47
PT2013IBRgESERPS2	**10**	4	0,25	93,25	70,40	0,65	1,99	3,38	2,74	**2,62**	0,25	0,54
PT2013IBRgESERPS3	1	3	1,64	62,06	61,62	0,76	1,97	4,01	3,49	0,04	0,65	2,06
PT2013IBRgESERPS3	2	3	5,48	58,76	61,62	0,76	1,97	4,01	3,49	-0,26	1,19	3,98
PT2013IBRgESERPS3	3	3	0,16	61,35	61,62	0,76	1,97	4,01	3,49	-0,02	0,20	0,65
PT2013IBRgESERPS3	4	3	1,19	58,76	61,62	0,76	1,97	4,01	3,49	-0,26	0,56	1,86
PT2013IBRgESERPS3	5.1	3	1,36	56,76	61,62	0,76	1,97	4,01	3,49	-0,44	0,59	2,05
PT2013IBRgESERPS3	5.2	3	9,08	47,73	61,62	0,76	1,97	4,01	3,49	-1,25	1,53	6,31
PT2013IBRgESERPS3	6	3	1,62	67,26	61,62	0,76	1,97	4,01	3,49	0,51	0,65	1,90
PT2013IBRgESERPS3	7.1	3	3,74	64,69	61,62	0,76	1,97	4,01	3,49	0,28	0,98	2,99
PT2013IBRgESERPS3	7.2	3	6,05	58,24	61,62	0,76	1,97	4,01	3,49	-0,30	1,25	4,22
PT2013IBRgESERPS3	8	3	9,84	51,53	61,62	0,76	1,97	4,01	3,49	-0,91	1,60	6,09
PT2013IBRgESERPS3	**10**	3	2,33	90,67	61,62	0,76	1,97	4,01	3,49	**2,62**	0,78	1,68

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; 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).

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

For the PT IBRgB, 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 IBRgB-specific antibodies in serum, was sent to the participating laboratories along with the regular IBRgB 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 IBRgB ELISA kits by the participating laboratories. This part was out of scope of this PT according to ISO 17043, 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 IBRgB serum samples

In total, 108 aliquots of these additional serum samples were distributed to the 9 laboratories participating in the PT IBRgB. All participants received 3 aliquots of 4 different serum samples of bovine origin (PT2013IBRgBSER-S1A, PT2013IBRgBSER-S1B, PT2013IBRgBSER-S1C, PT2013IBRgBSER-S1D), i.e. 12 aliquots in total. The positions of the serum samples in the sent blocks were randomized for each participant.

The serum samples PT2013IBRgBSER-S1A, PT2013IBRgBSER-S1B, PT2013IBRgBSER-S1C and PT2013IBRgBSER-S1D were a 1/32, a 1/64, a 1/128 and a 1/256 dilution, respectively, of the reference serum sample PT2013IBRgBSERPS2 (see III.2.1). During pre-verification, all 4 additional serum samples were negative using SN and the indirect ELISA test from LSI (LSIVET serum IBR screening), whereas variable results were obtained using the HerdChek IBRgB antibody ELISA test from IDEXX (POS - POS - NI - NEG, respectively).

After aliquoting the different serum samples, a homogeneity check was performed on 10 aliquots of each serum sample using the HerdChek IBRgB antibody ELISA test from IDEXX. 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 HerdChek IBRgB antibody ELISA test from IDEXX.

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 12 aliquots of additional IBRgB serum samples.

	LAB1	LAB2	LAB3.1	LAB3.2	LAB4.1	LAB4.2	LAB5.1	LAB5.2	LAB6	LAB7	LAB8	LAB9	TOTAL	GRAND TOTAL
PT2013IBRgBSER-S1A														
POS	3	3	3	3	3	3	3	3	3	0	3	3	33	
NI	0	0	0	0	0	0	0	0	0	3	0	0	3	36
NEG	0	0	0	0	0	0	0	0	0	0	0	0	0	
PT2013IBRgBSER-S1B														
POS	3	3	3	0	3	0	3	3	2	0	3	3	26	
NI	0	0	0	0	0	0	0	0	0	0	0	0	0	36
NEG	0	0	0	3	0	3	0	0	1	3	0	0	10	
PT2013IBRgBSER-S1C														
POS	1	1	0	0	0	0	0	1	0	0	0	0	3	
NI	2	2	3	0	2	0	1	0	0	0	0	0	10	36
NEG	0	0	0	3	1	3	2	2	3	3	3	3	23	
PT2013IBRgBSER-S1D														
POS	0	0	0	0	0	0	0	0	0	0	0	0	0	
NI	0	0	0	0	0	0	0	0	0	0	0	0	0	36
NEG	3	3	3	3	3	3	3	3	3	3	3	3	36	
TOTAL	12	12	12	12	12	12	12	12	12	12	12	12		
POS	7	7	6	3	6	3	6	7	5	0	6	6	62	
NI	2	2	3	0	2	0	1	0	0	3	0	0	13	144
NEG	3	3	3	9	4	9	5	5	7	9	6	6	69	

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 IBRgB serum sample and per participating (sub)laboratory were made using the statistical software R and are shown in Figure 1.

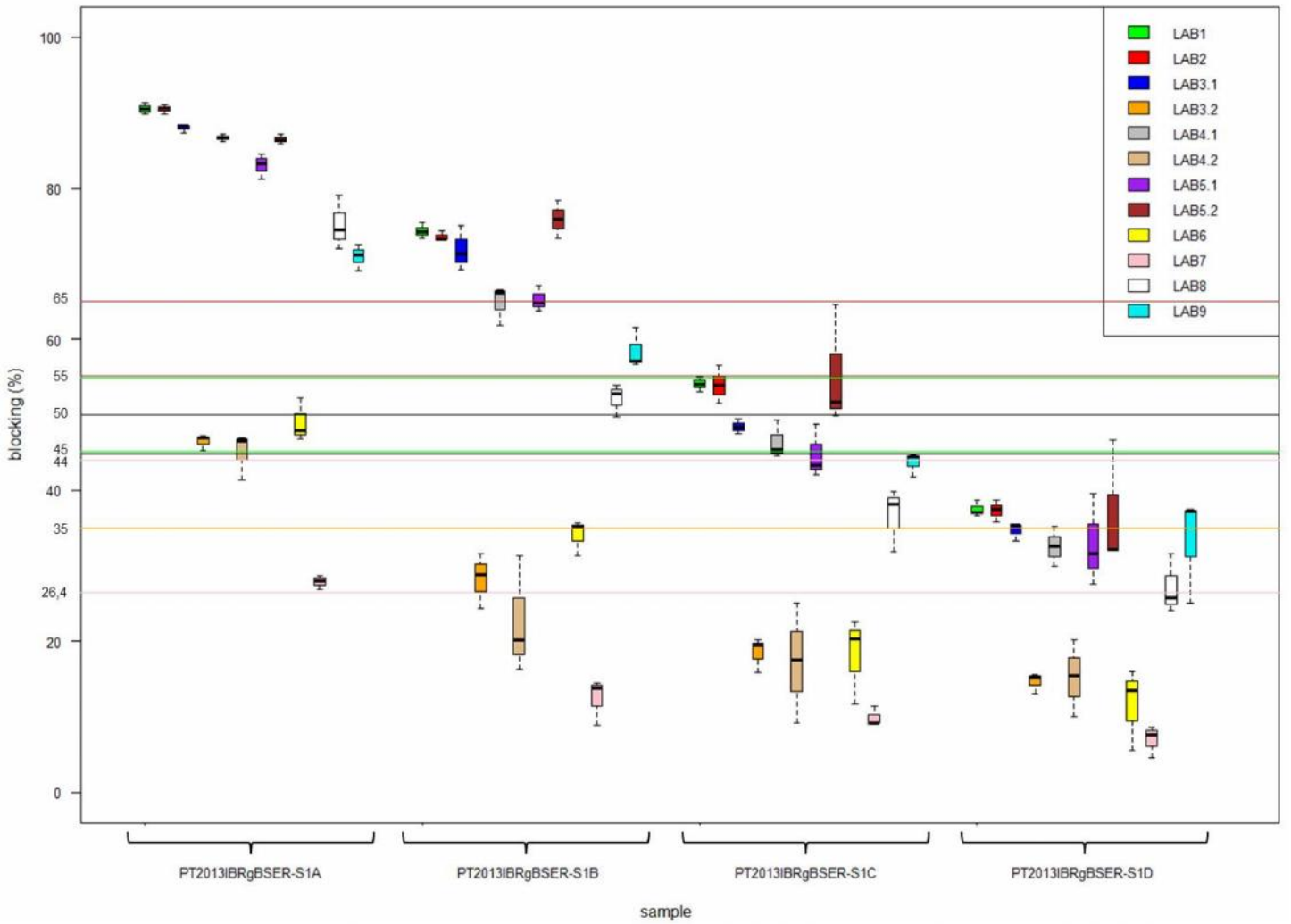

Figure 1. Box plots showing the percentage blocking per additional IBRgB serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are shown in pink (26,4-44%; LAB7), orange (35%; LAB3.2, LAB4.2, LAB6), black (45-50%; LAB8, LAB9), green (45-55%; LAB1, LAB2, LAB3.1, LAB4.1, LAB5.1), brown (55-65%; LAB5.2), respectively. LAB1, LAB2, LAB3.1, LAB4.1 and LAB5.1 performed the same incubation protocol of the same IBRgB ELISA kit (2 different batches; LAB1, LAB2, LAB3.1 and LAB4.1 used the same batch). Also LAB3.2, LAB4.2 and LAB6 performed the same incubation protocol of the same IBRgB ELISA kit (1 batch).

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 1,83 (p=12) and the maximum value for Mandel's k-statistic is 1,69 for all serum samples (p=12 and n=3).

For the detection of IBRgB-specific antibodies in the additional serum samples, 11 out of 12 participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all serum samples. This was not the case for LAB7 which showed an increased value for Mandel's h-statistic for the serum sample PT2013IBRgBSER-S1A (h=-1,92).
Furthermore, 9 out of 12 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all serum samples. This was not the case for LAB4.2, LAB5.2 and LAB8 which showed an increased value for Mandel's k-statistic for at least 1 serum sample: LAB4.2 for the serum samples PT2013IBRgBSER-S1A (k=1,71), PT2013IBRgBSER-S1B (k=2,40) and PT2013IBRgBSER-S1C (k=1,88), LABB5.2 for the serum samples PT2013IBRgBSER-S1C (k=1,90) and PT2013IBRgBSER-S1D (k=1,86), and LAB8 for serum sample PT2013IBRgBSER-S1A (k=1,95).

III.3 ANOVA

For the additional IBRgB serum samples, there was a significant difference between the participating laboratories at a global level. LAB7 reported percentages blocking that were significantly lower than those reported by LAB1, LAB2, LAB3.1, LAB4.1, LAB5.1, LAB5.2, LAB8 and LAB9. Of those laboratories that tested 2 different IBRgB ELISA kits (LAB3, LAB4 and LAB5), significant differences were observed for LAB3 and LAB4, but not for LAB5. LAB3.1 and LAB4.1 obtained percentages blocking that were significantly higher than those obtained by LAB3.2 and LAB4.2, respectively.

Table 2. Calculations of Mandel's h- and k-statistics (based on % blocking) for the panel additional IBRgB 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
PT2013IBRgBSER-S1A	1	3	0,63	90,54	69,98	0,93	1,84	6,87	6,62	0,94	0,43	0,88
PT2013IBRgBSER-S1A	2	3	0,36	90,52	69,98	0,93	1,84	6,87	6,62	0,93	0,33	0,66
PT2013IBRgBSER-S1A	3.1	3	0,33	88,01	69,98	0,93	1,84	6,87	6,62	0,82	0,31	0,65
PT2013IBRgBSER-S1A	3.2	3	1,14	46,49	69,98	0,93	1,84	6,87	6,62	-1,07	0,58	2,30
PT2013IBRgBSER-S1A	4.1	3	0,25	86,71	69,98	0,93	1,84	6,87	6,62	0,76	0,27	0,57
PT2013IBRgBSER-S1A	**4.2**	3	9,89	44,95	69,98	0,93	1,84	6,87	6,62	-1,14	**1,71**	7,00
PT2013IBRgBSER-S1A	5.1	3	2,75	83,01	69,98	0,93	1,84	6,87	6,62	0,59	0,90	2,00
PT2013IBRgBSER-S1A	5.2	3	0,42	86,51	69,98	0,93	1,84	6,87	6,62	0,75	0,35	0,75
PT2013IBRgBSER-S1A	6	3	8,19	49,00	69,98	0,93	1,84	6,87	6,62	-0,95	1,55	5,84
PT2013IBRgBSER-S1A	**7**	3	0,89	27,86	69,98	0,93	1,84	6,87	6,62	**-1,92**	0,51	3,39
PT2013IBRgBSER-S1A	**8**	3	12,90	75,21	69,98	0,93	1,84	6,87	6,62	0,24	**1,95**	4,78
PT2013IBRgBSER-S1A	9	3	2,92	70,94	69,98	0,93	1,84	6,87	6,62	0,04	0,93	2,41
PT2013IBRgBSER-S1B	1	3	1,17	74,35	52,80	0,81	3,27	7,54	6,79	0,95	0,33	1,46
PT2013IBRgBSER-S1B	2	3	0,52	73,60	52,80	0,81	3,27	7,54	6,79	0,92	0,22	0,98
PT2013IBRgBSER-S1B	3.1	3	8,88	71,83	52,80	0,81	3,27	7,54	6,79	0,84	0,91	4,15
PT2013IBRgBSER-S1B	3.2	3	13,05	28,22	52,80	0,81	3,27	7,54	6,79	-1,09	1,11	12,80
PT2013IBRgBSER-S1B	4.1	3	7,02	64,84	52,80	0,81	3,27	7,54	6,79	0,53	0,81	4,09
PT2013IBRgBSER-S1B	**4.2**	3	61,54	22,62	52,80	0,81	3,27	7,54	6,79	-1,33	**2,40**	34,68
PT2013IBRgBSER-S1B	5.1	3	2,81	65,23	52,80	0,81	3,27	7,54	6,79	0,55	0,51	2,57
PT2013IBRgBSER-S1B	5.2	3	6,36	75,87	52,80	0,81	3,27	7,54	6,79	1,02	0,77	3,32
PT2013IBRgBSER-S1B	6	3	5,62	34,06	52,80	0,81	3,27	7,54	6,79	-0,83	0,73	6,96
PT2013IBRgBSER-S1B	7	3	9,18	12,40	52,80	0,81	3,27	7,54	6,79	-1,79	0,93	24,42
PT2013IBRgBSER-S1B	8	3	4,77	52,13	52,80	0,81	3,27	7,54	6,79	-0,03	0,67	4,19
PT2013IBRgBSER-S1B	9	3	7,12	58,45	52,80	0,81	3,27	7,54	6,79	0,25	0,82	4,56
PT2013IBRgBSER-S1C	1	3	0,94	54,05	37,23	0,58	4,23	6,53	4,98	1,01	0,23	1,79
PT2013IBRgBSER-S1C	2	3	6,12	53,95	37,23	0,58	4,23	6,53	4,98	1,00	0,59	4,59

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

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013IBRgBSER-S1C	3.1	3	0,99	48,43	37,23	0,58	4,23	6,53	4,98	0,67	0,24	2,06
PT2013IBRgBSER-S1C	3.2	3	5,31	18,46	37,23	0,58	4,23	6,53	4,98	-1,13	0,55	12,49
PT2013IBRgBSER-S1C	4.1	3	6,39	46,37	37,23	0,58	4,23	6,53	4,98	0,55	0,60	5,45
PT2013IBRgBSER-S1C	**4.2**	3	63,01	17,24	37,23	0,58	4,23	6,53	4,98	-1,20	**1,88**	46,06
PT2013IBRgBSER-S1C	5.1	3	12,59	44,71	37,23	0,58	4,23	6,53	4,98	0,45	0,84	7,94
PT2013IBRgBSER-S1C	5.2	3	64,43	55,36	37,23	0,58	4,23	6,53	4,98	1,09	**1,90**	14,50
PT2013IBRgBSER-S1C	6	3	32,51	18,16	37,23	0,58	4,23	6,53	4,98	-1,14	1,35	31,41
PT2013IBRgBSER-S1C	7	3	1,76	9,88	37,23	0,58	4,23	6,53	4,98	-1,64	0,31	13,43
PT2013IBRgBSER-S1C	8	3	17,63	36,57	37,23	0,58	4,23	6,53	4,98	-0,04	0,99	11,48
PT2013IBRgBSER-S1C	9	3	2,60	43,63	37,23	0,58	4,23	6,53	4,98	0,38	0,38	3,69
PT2013IBRgBSER-S1D	1	3	1,21	37,51	26,72	0,35	4,54	5,63	3,32	0,95	0,24	2,93
PT2013IBRgBSER-S1D	2	3	2,05	37,30	26,72	0,35	4,54	5,63	3,32	0,93	0,31	3,84
PT2013IBRgBSER-S1D	3.1	3	1,46	34,70	26,72	0,35	4,54	5,63	3,32	0,71	0,27	3,48
PT2013IBRgBSER-S1D	3.2	3	1,80	14,63	26,72	0,35	4,54	5,63	3,32	-1,07	0,30	9,16
PT2013IBRgBSER-S1D	4.1	3	7,06	32,56	26,72	0,35	4,54	5,63	3,32	0,52	0,59	8,16
PT2013IBRgBSER-S1D	4.2	3	25,71	15,16	26,72	0,35	4,54	5,63	3,32	-1,02	1,12	33,45
PT2013IBRgBSER-S1D	5.1	3	36,78	32,89	26,72	0,35	4,54	5,63	3,32	0,54	1,34	18,44
PT2013IBRgBSER-S1D	**5.2**	3	71,04	36,92	26,72	0,35	4,54	5,63	3,32	0,90	**1,86**	22,83
PT2013IBRgBSER-S1D	6	3	30,00	11,67	26,72	0,35	4,54	5,63	3,32	-1,33	1,21	46,95
PT2013IBRgBSER-S1D	7	3	4,47	6,95	26,72	0,35	4,54	5,63	3,32	-1,75	0,47	30,42
PT2013IBRgBSER-S1D	8	3	15,94	27,15	26,72	0,35	4,54	5,63	3,32	0,04	0,88	14,71
PT2013IBRgBSER-S1D	9	3	50,06	33,23	26,72	0,35	4,54	5,63	3,32	0,58	1,56	21,29

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; 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).