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PROFICIENCY TESTING 2015

INFECTIOUS BOVINE RHINOTRACHEITIS (IBR)

Detection of IBRgB- and IBRgE-specific antibodies in serum by Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 24 AUGUST 2015

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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 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 'PT2015IBRgBSERNS1' and 'PT2015IBRgBSERNS2') or containing detectable IBRgB-specific antibodies (n = 4; coded 'PT2015IBRgBSERPS1', 'PT2015IBRgBSERPS2', 'PT2015IBRgBSERPS3' and 'PT2015IBRgBSERPS4'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2015IBRgBSERNS1, PT2015IBRgBSERNS2, PT2015IBRgBSERPS1 and PT2015IBRgBSERPS3, and 4 aliquots of the reference serum samples PT2015IBRgBSERPS2 and PT2015IBRgBSERPS4. 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 available. 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 sample PT2015IBRgBSERNS1 was obtained from an animal from a Belgian I4-certified farm (IBR-free without vaccination). The reference serum sample PT2015IBRgBSERNS2 was obtained from a non-vaccinated, uninfected animal. The reference serum samples PT2015IBRgBSERPS1 and PT2015IBRgBSERPS2 were obtained from 2 different vaccinated but uninfected animals. The reference serum samples PT2015IBRgBSERPS3 and PT2015IBRgBSERPS4 were a 1/4 and a 1/16 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 PT2015IBRgBSERNS1 and PT2015IBRgBSERNS2 were considered as negative sera, and the reference serum samples PT2015IBRgBSERPS1, PT2015IBRgBSERPS2, PT2015IBRgBSERPS3 and PT2015IBRgBSERPS4 as strong positive sera in IBRgB ELISA.

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.

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 'PT2015IBRgESERNS1', 'PT2015IBRgESERNS2' and 'PT2015IBRgESERNS3') or containing detectable IBRgE-specific antibodies (n=3, coded 'PT2015IBRgESERPS1', 'PT2015IBRgESERPS2' and 'PT2015IBRgESERPS3'), were used. In total, 140 aliquots were distributed to 7 different participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2015IBRgESERNS1, PT2015IBRgESERNS2, PT2015IBRgESERNS3 and PT2015IBRgESERPS1, and 4 aliquots of the reference serum samples PT2015IBRgESERPS2 and PT2015IBRgESERPS3. 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 available. 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 PT2015IBRgESERNS1 was from an animal from a Belgian I4-certified farm (IBR-free without vaccination), whereas the reference serum samples PT2015IBRgESERNS2 and PT2015IBRgESERNS3 (=PT2015IBRgBSERPS2) were from vaccinated but uninfected animals. The reference serum sample PT2015IBRgESERNS2 was in fact a 1/128 dilution. The reference serum samples PT2015IBRgESERPS1 (=PT2015IBRgBSERPS4), PT2015IBRgESERPS2 and PT2015IBRgESERPS3 were derived from 2 different experimentally infected but non-vaccinated animals. Hereby, the reference serum sample PT2015IBRgESERPS1 was a 1/16 dilution of a serum collected from one animal, whereas the reference serum samples PT2015IBRgESERPS2 and PT2015IBRgESERPS3 were a 1/16 and a 1/32 dilution, respectively, of a serum obtained from another animal. Taken together, the reference serum samples PT2015IBRgESERNS1, PT2015IBRgESERNS2 and PT2015IBRgESERNS3 were considered as negative sera, the reference serum sample PT2015IBRgESERPS1 as a strong positive serum, and the reference serum samples PT2015IBRgESERPS2 and PT2015IBRgESERPS3 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 CODA-CERVA-Uccle.

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

LAB1 until LAB6 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). LAB7 and LAB8 only participated in the PT IBRgB, whereas LAB9 only participated in the PT IBRgE. LAB7, LAB8 and LAB9 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 24th of August 2015. LAB1, LAB2, LAB3 and LAB5 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 25th (LAB7) or 26th (LAB4, LAB6, LAB8 and LAB9) of August 2015. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 24th of August and 17th of September 2015 (Table 1).

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

Results from the participating laboratories were submitted to the CODA-CERVA-Uccle between 28th of August and 18th of September 2015 (Table 1). All participants hereby respected the deadline of 18th of September 2015.

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

Laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	24/08/2015	27/08/2015	27/08/2015	28/08/2015
LAB2	24/08/2015	2/09/2015	2/09/2015	11/09/2015
LAB3	24/08/2015	24/08/2015 and 26/08/2015 (°)	24/08/2015	28/08/2015
LAB4	26/08/2015	26/08/2015 (#)	26/08/2015 (#)	04/09/2015
LAB5	24/08/2015	04/09/2015	02/09/2015	14/09/2015
LAB6	26/08/2015	17/09/2015	28/08/2015 and 04/09/2015 (#)	18/09/2015
LAB7	25/08/2015	10/09/2015	NA	14/09/2015
LAB8	26/08/2015	26/08/2015	NA	28/08/2015
LAB9	26/08/2015	NA	14/09/2015	18/09/2015

Legend: NA = not applicable; (#) = this laboratory tested ELISA kits from 2 different producers; (°) = this laboratory tested different protocols 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 and LAB6 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 protocols of the same ELISA kit. In order to analyse the provided data, these 3 laboratories have been divided into different sublaboratories, namely: LAB3 into LAB3.1 (Protocole 1 for PT IBRgB) and LAB3.2 (Protocole 2 for PT IBRgB), LAB4 into LAB4.1 (Producer 1 for PT IBRgB and PT IBRgE) and LAB4.2 (Producer 2 for PT IBRgB and PT IBRgE) and LAB6 into LAB6.1 (Producer 1 for PT IBRgE) and LAB6.2 (Producer 2 for PT IBRgE).

IV.4.1. Level of agreement

Qualitative data analysis showed that:

(i) For the detection of **IBRgB-specific antibodies**, all 8 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB3 used 2 different protocols of the same ELISA kit and LAB4 used 2 ELISA kits from different producers (Table 2).

(ii) For the detection of **IBRgE-specific antibodies**, 5 out of 7 participating laboratories (LAB2, LAB3, LAB5, LAB6 and LAB9) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). In contrast, LAB1 misclassified 1 aliquot (95% of agreement) and LAB4 misclassified 4 aliquots (80% of agreement) of reference serum samples. LAB4 and LAB6 each used 2 ELISA kits from different producers (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 the IBR reference laboratory of CODA-CERVA-Uccle. 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**	**6**	**7**	**8**
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)	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)	20 (100.0)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the **IBRgE** reference serum samples assigned by the IBR reference laboratory of CODA-CERVA-Uccle. 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**	**4.1**	**4.2**	**5**	**6.1**	**6.2**	**9**
Failure	1 (5.0)	0 (0.0)	0 (0.0)	4 (20.0)	4 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Success	19 (95.0)	20 (100.0)	20 (100.0)	16 (80.0)	16 (80.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

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 laboratories could be observed since all participants correctly identified all reference serum samples. LAB3 and LAB4 obtained identical qualitative results using 2 different protocols of the same ELISA kit (LAB3) or 2 ELISA kits from different producers (LAB4).

(ii) For the detection of **IBRgE-specific antibodies**, no variability between LAB2, LAB3, LAB5, LAB6 and LAB9 could be observed since these participants correctly identified all reference serum samples. LAB6 obtained identical qualitative results using 2 ELISA kits from different producers. In contrast, LAB1 misclassified 1 aliquots of the reference serum sample PT2015IBRgESERNS1 (POS instead of NEG), whereas LAB4 misclassified 2 out of 4 aliquots of the reference serum sample PT2015IBRgESERPS3 (2x NEG instead of POS), 1 out of 3 aliquots of the reference serum sample PT2015IBRgESERNS1 (POS instead of NEG) and 1 out of 3 aliquots of the reference serum sample PT2015IBRgESERNS2 (POS instead of NEG) using 2 ELISA kits from different producers.

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015IBRgBSERNS1	NEG	NEG	1
2	1	2	PT2015IBRgBSERPS1	POS	POS	1
3	1	3	PT2015IBRgBSERPS2	POS	POS	1
4	1	4	PT2015IBRgBSERNS2	NEG	NEG	1
5	1	5	PT2015IBRgBSERPS1	POS	POS	1
6	1	6	PT2015IBRgBSERPS2	POS	POS	1
7	1	7	PT2015IBRgBSERPS2	POS	POS	1
8	1	8	PT2015IBRgBSERPS3	POS	POS	1
9	1	9	PT2015IBRgBSERNS1	NEG	NEG	1
10	1	10	PT2015IBRgBSERPS4	POS	POS	1
11	1	11	PT2015IBRgBSERPS3	POS	POS	1
12	1	12	PT2015IBRgBSERNS2	NEG	NEG	1
13	1	13	PT2015IBRgBSERPS4	POS	POS	1
14	1	14	PT2015IBRgBSERPS3	POS	POS	1
15	1	15	PT2015IBRgBSERNS1	NEG	NEG	1
16	1	16	PT2015IBRgBSERPS4	POS	POS	1
17	1	17	PT2015IBRgBSERNS2	NEG	NEG	1
18	1	18	PT2015IBRgBSERPS2	POS	POS	1
19	1	19	PT2015IBRgBSERPS4	POS	POS	1
20	1	20	PT2015IBRgBSERPS1	POS	POS	1
21	2	1	PT2015IBRgBSERPS2	POS	POS	1
22	2	2	PT2015IBRgBSERNS1	NEG	NEG	1
23	2	3	PT2015IBRgBSERPS3	POS	POS	1
24	2	4	PT2015IBRgBSERPS4	POS	POS	1
25	2	5	PT2015IBRgBSERPS2	POS	POS	1
26	2	6	PT2015IBRgBSERNS2	NEG	NEG	1
27	2	7	PT2015IBRgBSERPS1	POS	POS	1
28	2	8	PT2015IBRgBSERNS2	NEG	NEG	1
29	2	9	PT2015IBRgBSERPS4	POS	POS	1
30	2	10	PT2015IBRgBSERPS3	POS	POS	1
31	2	11	PT2015IBRgBSERPS4	POS	POS	1
32	2	12	PT2015IBRgBSERPS4	POS	POS	1
33	2	13	PT2015IBRgBSERNS1	NEG	NEG	1
34	2	14	PT2015IBRgBSERPS3	POS	POS	1
35	2	15	PT2015IBRgBSERPS2	POS	POS	1
36	2	16	PT2015IBRgBSERNS2	NEG	NEG	1
37	2	17	PT2015IBRgBSERPS2	POS	POS	1
38	2	18	PT2015IBRgBSERPS1	POS	POS	1
39	2	19	PT2015IBRgBSERPS1	POS	POS	1
40	2	20	PT2015IBRgBSERNS1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3.1	1	PT2015IBRgBSERNS1	NEG	NEG	1
42	3.1	2	PT2015IBRgBSERPS1	POS	POS	1
43	3.1	3	PT2015IBRgBSERPS2	POS	POS	1
44	3.1	4	PT2015IBRgBSERNS2	NEG	NEG	1
45	3.1	5	PT2015IBRgBSERPS1	POS	POS	1
46	3.1	6	PT2015IBRgBSERPS2	POS	POS	1
47	3.1	7	PT2015IBRgBSERPS2	POS	POS	1
48	3.1	8	PT2015IBRgBSERPS3	POS	POS	1
49	3.1	9	PT2015IBRgBSERNS1	NEG	NEG	1
50	3.1	10	PT2015IBRgBSERPS4	POS	POS	1
51	3.1	11	PT2015IBRgBSERPS3	POS	POS	1
52	3.1	12	PT2015IBRgBSERNS2	NEG	NEG	1
53	3.1	13	PT2015IBRgBSERPS4	POS	POS	1
54	3.1	14	PT2015IBRgBSERPS3	POS	POS	1
55	3.1	15	PT2015IBRgBSERNS1	NEG	NEG	1
56	3.1	16	PT2015IBRgBSERPS4	POS	POS	1
57	3.1	17	PT2015IBRgBSERNS2	NEG	NEG	1
58	3.1	18	PT2015IBRgBSERPS2	POS	POS	1
59	3.1	19	PT2015IBRgBSERPS4	POS	POS	1
60	3.1	20	PT2015IBRgBSERPS1	POS	POS	1
61	3.2	1	PT2015IBRgBSERNS1	NEG	NEG	1
62	3.2	2	PT2015IBRgBSERPS1	POS	POS	1
63	3.2	3	PT2015IBRgBSERPS2	POS	POS	1
64	3.2	4	PT2015IBRgBSERNS2	NEG	NEG	1
65	3.2	5	PT2015IBRgBSERPS1	POS	POS	1
66	3.2	6	PT2015IBRgBSERPS2	POS	POS	1
67	3.2	7	PT2015IBRgBSERPS2	POS	POS	1
68	3.2	8	PT2015IBRgBSERPS3	POS	POS	1
69	3.2	9	PT2015IBRgBSERNS1	NEG	NEG	1
70	3.2	10	PT2015IBRgBSERPS4	POS	POS	1
71	3.2	11	PT2015IBRgBSERPS3	POS	POS	1
72	3.2	12	PT2015IBRgBSERNS2	NEG	NEG	1
73	3.2	13	PT2015IBRgBSERPS4	POS	POS	1
74	3.2	14	PT2015IBRgBSERPS3	POS	POS	1
75	3.2	15	PT2015IBRgBSERNS1	NEG	NEG	1
76	3.2	16	PT2015IBRgBSERPS4	POS	POS	1
77	3.2	17	PT2015IBRgBSERNS2	NEG	NEG	1
78	3.2	18	PT2015IBRgBSERPS2	POS	POS	1
79	3.2	19	PT2015IBRgBSERPS4	POS	POS	1
80	3.2	20	PT2015IBRgBSERPS1	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.1	1	PT2015IBRgBSERPS2	POS	POS	1
82	4.1	2	PT2015IBRgBSERNS1	NEG	NEG	1
83	4.1	3	PT2015IBRgBSERPS3	POS	POS	1
84	4.1	4	PT2015IBRgBSERPS4	POS	POS	1
85	4.1	5	PT2015IBRgBSERPS2	POS	POS	1
86	4.1	6	PT2015IBRgBSERNS2	NEG	NEG	1
87	4.1	7	PT2015IBRgBSERPS1	POS	POS	1
88	4.1	8	PT2015IBRgBSERNS2	NEG	NEG	1
89	4.1	9	PT2015IBRgBSERPS4	POS	POS	1
90	4.1	10	PT2015IBRgBSERPS3	POS	POS	1
91	4.1	11	PT2015IBRgBSERPS4	POS	POS	1
92	4.1	12	PT2015IBRgBSERPS4	POS	POS	1
93	4.1	13	PT2015IBRgBSERNS1	NEG	NEG	1
94	4.1	14	PT2015IBRgBSERPS3	POS	POS	1
95	4.1	15	PT2015IBRgBSERPS2	POS	POS	1
96	4.1	16	PT2015IBRgBSERNS2	NEG	NEG	1
97	4.1	17	PT2015IBRgBSERPS2	POS	POS	1
98	4.1	18	PT2015IBRgBSERPS1	POS	POS	1
99	4.1	19	PT2015IBRgBSERPS1	POS	POS	1
100	4.1	20	PT2015IBRgBSERNS1	NEG	NEG	1
101	4.2	1	PT2015IBRgBSERPS2	POS	POS	1
102	4.2	2	PT2015IBRgBSERNS1	NEG	NEG	1
103	4.2	3	PT2015IBRgBSERPS3	POS	POS	1
104	4.2	4	PT2015IBRgBSERPS4	POS	POS	1
105	4.2	5	PT2015IBRgBSERPS2	POS	POS	1
106	4.2	6	PT2015IBRgBSERNS2	NEG	NEG	1
107	4.2	7	PT2015IBRgBSERPS1	POS	POS	1
108	4.2	8	PT2015IBRgBSERNS2	NEG	NEG	1
109	4.2	9	PT2015IBRgBSERPS4	POS	POS	1
110	4.2	10	PT2015IBRgBSERPS3	POS	POS	1
111	4.2	11	PT2015IBRgBSERPS4	POS	POS	1
112	4.2	12	PT2015IBRgBSERPS4	POS	POS	1
113	4.2	13	PT2015IBRgBSERNS1	NEG	NEG	1
114	4.2	14	PT2015IBRgBSERPS3	POS	POS	1
115	4.2	15	PT2015IBRgBSERPS2	POS	POS	1
116	4.2	16	PT2015IBRgBSERNS2	NEG	NEG	1
117	4.2	17	PT2015IBRgBSERPS2	POS	POS	1
118	4.2	18	PT2015IBRgBSERPS1	POS	POS	1
119	4.2	19	PT2015IBRgBSERPS1	POS	POS	1
120	4.2	20	PT2015IBRgBSERNS1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	5	1	PT2015IBRgBSERNS1	NEG	NEG	1
122	5	2	PT2015IBRgBSERPS1	POS	POS	1
123	5	3	PT2015IBRgBSERPS2	POS	POS	1
124	5	4	PT2015IBRgBSERNS2	NEG	NEG	1
125	5	5	PT2015IBRgBSERPS1	POS	POS	1
126	5	6	PT2015IBRgBSERPS2	POS	POS	1
127	5	7	PT2015IBRgBSERPS2	POS	POS	1
128	5	8	PT2015IBRgBSERPS3	POS	POS	1
129	5	9	PT2015IBRgBSERNS1	NEG	NEG	1
130	5	10	PT2015IBRgBSERPS4	POS	POS	1
131	5	11	PT2015IBRgBSERPS3	POS	POS	1
132	5	12	PT2015IBRgBSERNS2	NEG	NEG	1
133	5	13	PT2015IBRgBSERPS4	POS	POS	1
134	5	14	PT2015IBRgBSERPS3	POS	POS	1
135	5	15	PT2015IBRgBSERNS1	NEG	NEG	1
136	5	16	PT2015IBRgBSERPS4	POS	POS	1
137	5	17	PT2015IBRgBSERNS2	NEG	NEG	1
138	5	18	PT2015IBRgBSERPS2	POS	POS	1
139	5	19	PT2015IBRgBSERPS4	POS	POS	1
140	5	20	PT2015IBRgBSERPS1	POS	POS	1
141	6	1	PT2015IBRgBSERPS2	POS	POS	1
142	6	2	PT2015IBRgBSERNS1	NEG	NEG	1
143	6	3	PT2015IBRgBSERPS3	POS	POS	1
144	6	4	PT2015IBRgBSERPS4	POS	POS	1
145	6	5	PT2015IBRgBSERPS2	POS	POS	1
146	6	6	PT2015IBRgBSERNS2	NEG	NEG	1
147	6	7	PT2015IBRgBSERPS1	POS	POS	1
148	6	8	PT2015IBRgBSERNS2	NEG	NEG	1
149	6	9	PT2015IBRgBSERPS4	POS	POS	1
150	6	10	PT2015IBRgBSERPS3	POS	POS	1
151	6	11	PT2015IBRgBSERPS4	POS	POS	1
152	6	12	PT2015IBRgBSERPS4	POS	POS	1
153	6	13	PT2015IBRgBSERNS1	NEG	NEG	1
154	6	14	PT2015IBRgBSERPS3	POS	POS	1
155	6	15	PT2015IBRgBSERPS2	POS	POS	1
156	6	16	PT2015IBRgBSERNS2	NEG	NEG	1
157	6	17	PT2015IBRgBSERPS2	POS	POS	1
158	6	18	PT2015IBRgBSERPS1	POS	POS	1
159	6	19	PT2015IBRgBSERPS1	POS	POS	1
160	6	20	PT2015IBRgBSERNS1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	7	1	PT2015IBRgBSERNS1	NEG	NEG	1
162	7	2	PT2015IBRgBSERPS1	POS	POS	1
163	7	3	PT2015IBRgBSERPS2	POS	POS	1
164	7	4	PT2015IBRgBSERNS2	NEG	NEG	1
165	7	5	PT2015IBRgBSERPS1	POS	POS	1
166	7	6	PT2015IBRgBSERPS2	POS	POS	1
167	7	7	PT2015IBRgBSERPS2	POS	POS	1
168	7	8	PT2015IBRgBSERPS3	POS	POS	1
169	7	9	PT2015IBRgBSERNS1	NEG	NEG	1
170	7	10	PT2015IBRgBSERPS4	POS	POS	1
171	7	11	PT2015IBRgBSERPS3	POS	POS	1
172	7	12	PT2015IBRgBSERNS2	NEG	NEG	1
173	7	13	PT2015IBRgBSERPS4	POS	POS	1
174	7	14	PT2015IBRgBSERPS3	POS	POS	1
175	7	15	PT2015IBRgBSERNS1	NEG	NEG	1
176	7	16	PT2015IBRgBSERPS4	POS	POS	1
177	7	17	PT2015IBRgBSERNS2	NEG	NEG	1
178	7	18	PT2015IBRgBSERPS2	POS	POS	1
179	7	19	PT2015IBRgBSERPS4	POS	POS	1
180	7	20	PT2015IBRgBSERPS1	POS	POS	1
181	8	1	PT2015IBRgBSERPS2	POS	POS	1
182	8	2	PT2015IBRgBSERNS1	NEG	NEG	1
183	8	3	PT2015IBRgBSERPS3	POS	POS	1
184	8	4	PT2015IBRgBSERPS4	POS	POS	1
185	8	5	PT2015IBRgBSERPS2	POS	POS	1
186	8	6	PT2015IBRgBSERNS2	NEG	NEG	1
187	8	7	PT2015IBRgBSERPS1	POS	POS	1
188	8	8	PT2015IBRgBSERNS2	NEG	NEG	1
189	8	9	PT2015IBRgBSERPS4	POS	POS	1
190	8	10	PT2015IBRgBSERPS3	POS	POS	1
191	8	11	PT2015IBRgBSERPS4	POS	POS	1
192	8	12	PT2015IBRgBSERPS4	POS	POS	1
193	8	13	PT2015IBRgBSERNS1	NEG	NEG	1
194	8	14	PT2015IBRgBSERPS3	POS	POS	1
195	8	15	PT2015IBRgBSERPS2	POS	POS	1
196	8	16	PT2015IBRgBSERNS2	NEG	NEG	1
197	8	17	PT2015IBRgBSERPS2	POS	POS	1
198	8	18	PT2015IBRgBSERPS1	POS	POS	1
199	8	19	PT2015IBRgBSERPS1	POS	POS	1
200	8	20	PT2015IBRgBSERNS1	NEG	NEG	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 the IBR reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015IBRgESERNS1	NEG	POS	0
2	1	2	PT2015IBRgESERNS3	NEG	NEG	1
3	1	3	PT2015IBRgESERNS3	NEG	NEG	1
4	1	4	PT2015IBRgESERPS2	POS	POS	1
5	1	5	PT2015IBRgESERNS2	NEG	NEG	1
6	1	6	PT2015IBRgESERNS1	NEG	NEG	1
7	1	7	PT2015IBRgESERPS2	POS	POS	1
8	1	8	PT2015IBRgESERPS1	POS	POS	1
9	1	9	PT2015IBRgESERNS2	NEG	NEG	1
10	1	10	PT2015IBRgESERPS3	POS	POS	1
11	1	11	PT2015IBRgESERNS3	NEG	NEG	1
12	1	12	PT2015IBRgESERPS2	POS	POS	1
13	1	13	PT2015IBRgESERPS3	POS	POS	1
14	1	14	PT2015IBRgESERNS1	NEG	NEG	1
15	1	15	PT2015IBRgESERPS3	POS	POS	1
16	1	16	PT2015IBRgESERPS3	POS	POS	1
17	1	17	PT2015IBRgESERNS2	NEG	NEG	1
18	1	18	PT2015IBRgESERPS2	POS	POS	1
19	1	19	PT2015IBRgESERNS3	NEG	NEG	1
20	1	20	PT2015IBRgESERPS1	POS	POS	1
21	2	1	PT2015IBRgESERPS2	POS	POS	1
22	2	2	PT2015IBRgESERNS3	NEG	NEG	1
23	2	3	PT2015IBRgESERPS3	POS	POS	1
24	2	4	PT2015IBRgESERPS1	POS	POS	1
25	2	5	PT2015IBRgESERNS2	NEG	NEG	1
26	2	6	PT2015IBRgESERPS2	POS	POS	1
27	2	7	PT2015IBRgESERNS1	NEG	NEG	1
28	2	8	PT2015IBRgESERPS1	POS	POS	1
29	2	9	PT2015IBRgESERPS2	POS	POS	1
30	2	10	PT2015IBRgESERNS2	NEG	NEG	1
31	2	11	PT2015IBRgESERPS3	POS	POS	1
32	2	12	PT2015IBRgESERNS1	NEG	NEG	1
33	2	13	PT2015IBRgESERPS3	POS	POS	1
34	2	14	PT2015IBRgESERNS3	NEG	NEG	1
35	2	15	PT2015IBRgESERNS2	NEG	NEG	1
36	2	16	PT2015IBRgESERPS3	POS	POS	1
37	2	17	PT2015IBRgESERNS3	NEG	NEG	1
38	2	18	PT2015IBRgESERPS2	POS	POS	1
39	2	19	PT2015IBRgESERPS1	POS	POS	1
40	2	20	PT2015IBRgESERNS1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2015IBRgESERPS1	POS	POS	1
42	3	2	PT2015IBRgESERNS1	NEG	NEG	1
43	3	3	PT2015IBRgESERNS3	NEG	NEG	1
44	3	4	PT2015IBRgESERPS2	POS	POS	1
45	3	5	PT2015IBRgESERNS2	NEG	NEG	1
46	3	6	PT2015IBRgESERNS1	NEG	NEG	1
47	3	7	PT2015IBRgESERPS2	POS	POS	1
48	3	8	PT2015IBRgESERPS1	POS	POS	1
49	3	9	PT2015IBRgESERNS2	NEG	NEG	1
50	3	10	PT2015IBRgESERPS3	POS	POS	1
51	3	11	PT2015IBRgESERNS3	NEG	NEG	1
52	3	12	PT2015IBRgESERPS2	POS	POS	1
53	3	13	PT2015IBRgESERPS3	POS	POS	1
54	3	14	PT2015IBRgESERNS1	NEG	NEG	1
55	3	15	PT2015IBRgESERPS3	POS	POS	1
56	3	16	PT2015IBRgESERPS3	POS	POS	1
57	3	17	PT2015IBRgESERNS2	NEG	NEG	1
58	3	18	PT2015IBRgESERPS2	POS	POS	1
59	3	19	PT2015IBRgESERNS3	NEG	NEG	1
60	3	20	PT2015IBRgESERPS1	POS	POS	1
61	4.1	1	PT2015IBRgESERPS2	POS	POS	1
62	4.1	2	PT2015IBRgESERNS3	NEG	NEG	1
63	4.1	3	PT2015IBRgESERPS3	POS	POS	1
64	4.1	4	PT2015IBRgESERPS1	POS	POS	1
65	4.1	5	PT2015IBRgESERNS2	NEG	NEG	1
66	4.1	6	PT2015IBRgESERPS2	POS	POS	1
67	4.1	7	PT2015IBRgESERNS1	NEG	NEG	1
68	4.1	8	PT2015IBRgESERPS1	POS	POS	1
69	4.1	9	PT2015IBRgESERPS2	POS	POS	1
70	4.1	10	PT2015IBRgESERNS2	NEG	NEG	1
71	4.1	11	PT2015IBRgESERPS3	**POS**	**NEG**	**0**
72	4.1	12	PT2015IBRgESERNS1	**NEG**	**POS**	**0**
73	4.1	13	PT2015IBRgESERPS3	POS	POS	1
74	4.1	14	PT2015IBRgESERNS3	NEG	NEG	1
75	4.1	15	PT2015IBRgESERNS2	**NEG**	**POS**	**0**
76	4.1	16	PT2015IBRgESERPS3	**POS**	**NEG**	**0**
77	4.1	17	PT2015IBRgESERNS3	NEG	NEG	1
78	4.1	18	PT2015IBRgESERPS2	POS	POS	1
79	4.1	19	PT2015IBRgESERPS1	POS	POS	1
80	4.1	20	PT2015IBRgESERNS1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.2	1	PT2015IBRgESERPS2	POS	POS	1
82	4.2	2	PT2015IBRgESERNS3	NEG	NEG	1
83	4.2	3	PT2015IBRgESERPS3	POS	POS	1
84	4.2	4	PT2015IBRgESERPS1	POS	POS	1
85	4.2	5	PT2015IBRgESERNS2	NEG	NEG	1
86	4.2	6	PT2015IBRgESERPS2	POS	POS	1
87	4.2	7	PT2015IBRgESERNS1	NEG	NEG	1
88	4.2	8	PT2015IBRgESERPS1	POS	POS	1
89	4.2	9	PT2015IBRgESERPS2	POS	POS	1
90	4.2	10	PT2015IBRgESERNS2	NEG	NEG	1
91	4.2	11	PT2015IBRgESERPS3	**POS**	**NEG**	**0**
92	4.2	12	PT2015IBRgESERNS1	**NEG**	**POS**	**0**
93	4.2	13	PT2015IBRgESERPS3	POS	POS	1
94	4.2	14	PT2015IBRgESERNS3	NEG	NEG	1
95	4.2	15	PT2015IBRgESERNS2	**NEG**	**POS**	**0**
96	4.2	16	PT2015IBRgESERPS3	**POS**	**NEG**	**0**
97	4.2	17	PT2015IBRgESERNS3	NEG	NEG	1
98	4.2	18	PT2015IBRgESERPS2	POS	POS	1
99	4.2	19	PT2015IBRgESERPS1	POS	POS	1
100	4.2	20	PT2015IBRgESERNS1	NEG	NEG	1
101	5	1	PT2015IBRgESERPS1	POS	POS	1
102	5	2	PT2015IBRgESERNS1	NEG	NEG	1
103	5	3	PT2015IBRgESERNS3	NEG	NEG	1
104	5	4	PT2015IBRgESERPS2	POS	POS	1
105	5	5	PT2015IBRgESERNS2	NEG	NEG	1
106	5	6	PT2015IBRgESERNS1	NEG	NEG	1
107	5	7	PT2015IBRgESERPS2	POS	POS	1
108	5	8	PT2015IBRgESERPS1	POS	POS	1
109	5	9	PT2015IBRgESERNS2	NEG	NEG	1
110	5	10	PT2015IBRgESERPS3	POS	POS	1
111	5	11	PT2015IBRgESERNS3	NEG	NEG	1
112	5	12	PT2015IBRgESERPS2	POS	POS	1
113	5	13	PT2015IBRgESERPS3	POS	POS	1
114	5	14	PT2015IBRgESERNS1	NEG	NEG	1
115	5	15	PT2015IBRgESERPS3	POS	POS	1
116	5	16	PT2015IBRgESERPS3	POS	POS	1
117	5	17	PT2015IBRgESERNS2	NEG	NEG	1
118	5	18	PT2015IBRgESERPS2	POS	POS	1
119	5	19	PT2015IBRgESERNS3	NEG	NEG	1
120	5	20	PT2015IBRgESERPS1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	6.1	1	PT2015IBRgESERPS2	POS	POS	1
122	6.1	2	PT2015IBRgESERNS3	NEG	NEG	1
123	6.1	3	PT2015IBRgESERPS3	POS	POS	1
124	6.1	4	PT2015IBRgESERPS1	POS	POS	1
125	6.1	5	PT2015IBRgESERNS2	NEG	NEG	1
126	6.1	6	PT2015IBRgESERPS2	POS	POS	1
127	6.1	7	PT2015IBRgESERNS1	NEG	NEG	1
128	6.1	8	PT2015IBRgESERPS1	POS	POS	1
129	6.1	9	PT2015IBRgESERPS2	POS	POS	1
130	6.1	10	PT2015IBRgESERNS2	NEG	NEG	1
131	6.1	11	PT2015IBRgESERPS3	POS	POS	1
132	6.1	12	PT2015IBRgESERNS1	NEG	NEG	1
133	6.1	13	PT2015IBRgESERPS3	POS	POS	1
134	6.1	14	PT2015IBRgESERNS3	NEG	NEG	1
135	6.1	15	PT2015IBRgESERNS2	NEG	NEG	1
136	6.1	16	PT2015IBRgESERPS3	POS	POS	1
137	6.1	17	PT2015IBRgESERNS3	NEG	NEG	1
138	6.1	18	PT2015IBRgESERPS2	POS	POS	1
139	6.1	19	PT2015IBRgESERPS1	POS	POS	1
140	6.1	20	PT2015IBRgESERNS1	NEG	NEG	1
141	6.2	1	PT2015IBRgESERPS2	POS	POS	1
142	6.2	2	PT2015IBRgESERNS3	NEG	NEG	1
143	6.2	3	PT2015IBRgESERPS3	POS	POS	1
144	6.2	4	PT2015IBRgESERPS1	POS	POS	1
145	6.2	5	PT2015IBRgESERNS2	NEG	NEG	1
146	6.2	6	PT2015IBRgESERPS2	POS	POS	1
147	6.2	7	PT2015IBRgESERNS1	NEG	NEG	1
148	6.2	8	PT2015IBRgESERPS1	POS	POS	1
149	6.2	9	PT2015IBRgESERPS2	POS	POS	1
150	6.2	10	PT2015IBRgESERNS2	NEG	NEG	1
151	6.2	11	PT2015IBRgESERPS3	POS	POS	1
152	6.2	12	PT2015IBRgESERNS1	NEG	NEG	1
153	6.2	13	PT2015IBRgESERPS3	POS	POS	1
154	6.2	14	PT2015IBRgESERNS3	NEG	NEG	1
155	6.2	15	PT2015IBRgESERNS2	NEG	NEG	1
156	6.2	16	PT2015IBRgESERPS3	POS	POS	1
157	6.2	17	PT2015IBRgESERNS3	NEG	NEG	1
158	6.2	18	PT2015IBRgESERPS2	POS	POS	1
159	6.2	19	PT2015IBRgESERPS1	POS	POS	1
160	6.2	20	PT2015IBRgESERNS1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	9	1	PT2015IBRgESERPS1	POS	POS	1
162	9	2	PT2015IBRgESERNS1	NEG	NEG	1
163	9	3	PT2015IBRgESERNS3	NEG	NEG	1
164	9	4	PT2015IBRgESERPS2	POS	POS	1
165	9	5	PT2015IBRgESERNS2	NEG	NEG	1
166	9	6	PT2015IBRgESERNS1	NEG	NEG	1
167	9	7	PT2015IBRgESERPS2	POS	POS	1
168	9	8	PT2015IBRgESERPS1	POS	POS	1
169	9	9	PT2015IBRgESERNS2	NEG	NEG	1
170	9	10	PT2015IBRgESERPS3	POS	POS	1
171	9	11	PT2015IBRgESERNS3	NEG	NEG	1
172	9	12	PT2015IBRgESERPS2	POS	POS	1
173	9	13	PT2015IBRgESERPS3	POS	POS	1
174	9	14	PT2015IBRgESERNS1	NEG	NEG	1
175	9	15	PT2015IBRgESERPS3	POS	POS	1
176	9	16	PT2015IBRgESERPS3	POS	POS	1
177	9	17	PT2015IBRgESERNS2	NEG	NEG	1
178	9	18	PT2015IBRgESERPS2	POS	POS	1
179	9	19	PT2015IBRgESERNS3	NEG	NEG	1
180	9	20	PT2015IBRgESERPS1	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, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB3 used 2 different protocols of the same ELISA kit and LAB4 used 2 ELISA kits from different producers, hereby obtaining identical qualitative results (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. The IBRgB participating laboratories used ELISA kits from 5 different producers as well as different batches from the same ELISA kit: IDEXX (2 batches: E191, E541), IDVET (1 batch: 729), Synbiotics Europe (1 batch: 14SIBR1BN217), QIAGEN (1 batch: 251130402) and Home Made ELISA (1 batch 15/7/15). LAB1, LAB2, LAB3, LAB4.1 and LAB5 used the same IBRgB ELISA kit, hereby LAB1, LAB2, LAB4.1 and LAB5 performed the long incubation protocol and LAB3 the long and the short incubation protocol. In addition, LAB1, LAB3 and LAB5 on the one hand, and LAB2 and LAB4.1 on the other hand, used the same batch.

For the detection of IBRgE-specific antibodies in reference serum samples, 5 out of 7 participating laboratories (LAB2, LAB3, LAB5, LAB6 and LAB9) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB6 obtained identical qualitative results using 2 different batches of the same ELISA kit. In contrast, LAB1 misclassified 1 aliquot of reference serum sample PT2015IBRgESERNS1 (95% of agreement), whereas LAB4 misclassified 2 out of 4 aliquots of the reference serum sample PT2015IBRgESERPS3 (2x NEG instead of POS), 1 out of 3 aliquots of the reference serum sample PT2015IBRgESERNS1 (POS instead of NEG) and 1 out of 3 aliquots of the reference serum sample PT2015IBRgESERNS2 (POS instead of NEG) using 2 ELISA kits from different producers (80% of agreement) (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 (4 batches: KK833, LK999, JK690, DL553), Qiagen (1 batch: 28111021) and ID.VET (2 batch: 815, 868). LAB1, LAB2, LAB3, LAB4.1, LAB5, and LAB6.1 used the same IBRgE ELISA kit. In addition, LAB1, LAB4.1 and LAB6.1 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-Uccle (see III.3.3.). As a consequence: (i) all 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) 6 out of 7 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 IBRgE, LAB4 did not reach the required 90% of agreement.

Coordinator proficiency tests

Katia Knapen

Appendix

Names of the participating laboratories

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

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

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

Synbiotics Europe (Lyon, France)

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