



**CODA-CERVA**

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

GROESELBERG 99 – B 1180 BRUSSELS (UKKEL)

TEL: +32 (0)2 379 04 11

FAX : + 32 (0)2 379 06 70

HTTP: // WWW.CODA-CERVA.BE



172-PT

## **PROFICIENCY TESTING 2016**

***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: 07 NOVEMBER 2016**

**DATE REPORT: 08 FEBRUARY 2017**

## 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 ( $n = 3$ ; coded 'PT2016IBRgBSERNS1', 'PT2016IBRgBSERNS2' and 'PT2016IBRgBSERNS3') or containing detectable IBRgB-specific antibodies ( $n = 3$ ; coded 'PT2016IBRgBSERPS1', 'PT2016IBRgBSERPS2' and 'PT2016IBRgBSERPS3'), were used. In total, 220 aliquots were distributed to 11 participating laboratories. All participants received 20 aliquots: 1 aliquot of the reference serum sample PT2016IBRgBSERNS2, 3 aliquots of the reference serum samples PT2016IBRgBSERPS1 and PT2016IBRgBSERPS2, 4 aliquots of the reference serum samples PT2016IBRgBSERPS3 and PT2016IBRgBSERNS3 and 5 aliquots of the reference serum sample PT2016IBRgBSERNS1. 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 samples PT2016IBRgBSERNS1 and PT2016IBRgBSERNS2 were obtained from two animals from a Belgian I4-certified farm (IBR-free without vaccination). The reference serum sample PT2016IBRgBSERNS3 was obtained from a non-vaccinated, uninfected animal. The reference serum sample PT2016IBRgBSERPS1 was a 1/128 dilution of a sera from one vaccinated but uninfected animals. The reference serum samples PT2016IBRgBSERPS2 was a 1/32 dilution of a sera from one experimentally infected but non-vaccinated animal. The reference serum samples PT2016IBRgBSERPS3 was a 1/8 dilution of a sera obtained from a field infected animal. For each reference serum sample, the same qualitative result was obtained with all test methods used. Taken together, the reference serum samples PT2016IBRgBSERNS1, PT2016IBRgBSERNS2 and PT2016IBRgBSERNS3 were considered as negative sera, and the reference serum samples PT2016IBRgBSERPS1, PT2016IBRgBSERPS2 and PT2016IBRgBSERPS3 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 'PT2016IBRgESERNS1', 'PT2016IBRgESERNS2' and 'PT2016IBRgESERNS3') or containing detectable IBRgE-specific antibodies ( $n=3$ , coded 'PT2016IBRgESERPS1', 'PT2016IBRgESERPS2' and 'PT2016IBRgESERPS3'), were used. In total, 220 aliquots were distributed to 11 different participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference serum sample PT2016IBRgESERPS1, 3 aliquots of the reference serum samples PT2016IBRgESERNS1, PT2016IBRgESERNS2 and PT2016IBRgESERNS3, 4 aliquots of the reference serum sample PT2016IBRgESERPS2 and 5 aliquots of the reference serum sample PT2016IBRgESERPS3. 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 samples PT2016IBRgESERNS1 and PT2016IBRgESERNS2 were obtained from two animals from a Belgian I4-certified farm (IBR-free without vaccination) (=PT2016IBRgBSERNS1 and PT2016IBRgBSERNS2, respectively). The reference serum sample PT2016IBRgESERNS3 was obtained from a non-vaccinated, uninfected animal (=PT2016IBRgBSERNS3). The reference serum sample PT2016IBRgESERPS1 was a 1/16 dilution of a sera from one experimentally infected animal. The reference serum samples PT2016IBRgESERPS2 was a 1/8 dilution of a sera obtained from a field infected animal (=PT2016IBRgBSERPS3). The PT2016IBRgESERPS3 was obtained 12 days post experimental infection of a non-vaccinated animal. Taken together, the reference serum samples PT2016IBRgESERNS1, PT2016IBRgESERNS2 and PT2016IBRgESERNS3 were considered as negative sera, the reference serum samples PT2016IBRgESERPS1 and PT2016IBRgESERPS2 as (strong) positive sera, and the reference serum samples PT2016IBRgESERPS3 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 LAB10 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). LAB11 only participated in the PT IBRgB, whereas LAB12 only participated in the PT IBRgE. LAB11 and LAB12 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 7<sup>th</sup> of November 2016. LAB1, LAB6, LAB7, LAB8 and LAB10 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 8<sup>th</sup> (LAB5, LAB9 and LAB12) or 9<sup>th</sup> (LAB2, LAB3, LAB4 and LAB11) of November 2016. LAB9, LAB11 and LAB12 confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 7<sup>th</sup> of November and 1<sup>st</sup> of December 2016 (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 18<sup>th</sup> of November and 2<sup>nd</sup> of December 2016 (Table 1). All participants except LAB10 and LAB12 respected the deadline of 25<sup>th</sup> of November 2016.

**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	07/11/2016	14/11/2016	16/11/2016	22/11/2016
LAB2	09/11/2016	10/11/2016	09/11/2016	21/11/2016
LAB3	09/11/2016	15/11/2016 (#)	15/11/2016 (#)	22/11/2016
LAB4	09/11/2016	24/11/2016	16/11/2016 (##)	25/11/2016
LAB5	08/11/2016	17/11/2016	15/11/2016	21/11/2016
LAB6	07/11/2016	07/11/2016 and 17/11/2016 (°)	07/11/2016 and 21/11/2016 (#)	22/11/2016
LAB7	07/11/2016	10/11/2016	08/11/2016 (#)	22/11/2016
LAB8	07/11/2016	22/11/2016	22/11/2016	25/11/2016
LAB9	08/11/2016	10/11/2016	16/11/2016	22/11/2016
LAB10	07/11/2016	08/11/2016	09/11/2016	<b>02/12/2016</b>
LAB11	09/11/2016	16/11/2016	NA	18/11/2016
LAB12	08/11/2016	NA	01/12/2016	<b>02/12/2016</b>

**Legend:** NA = not applicable; (#) = this laboratory tested ELISA kits from 2 different producers; (##) = this laboratory tested ELISA kits from 3 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, LAB6 and LAB7 submitted 2 sets and LAB4 submitted 3 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 4 laboratories have been divided into different sublaboratories.

For PT IBRgB : LAB6 into LAB6.1 (Producer 1: Protocole 1) and LAB6.2 (Producer 1: Protocole 2), LAB3 into LAB3.1 (Producer 1) and LAB3.2 (Producer 2);

For PT IBRgE : LAB4 into LAB4.1 (Producer 1), LAB4.2 (Producer 2) and LAB4.3 (Producer 3), LAB3 into LAB3.1 (Producer 1) and LAB3.2 (Producer 2), LAB6 into LAB6.1 (Producer 1) and LAB6.3 (Producer 3) and LAB7 into LAB7.1 (Producer 1) and LAB7.3 (Producer 3). Producers 1, 2 or 3 are the same for the different laboratories.

##### IV.4.1. Level of agreement

Qualitative data analysis showed that:

- (i) For the detection of **IBRgB-specific antibodies**, all 11 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 ELISA kits from 2 different producers (Producer 1 and 2) and LAB6 used 2 different protocols of the same ELISA kit producer (Producer 1) (Table 2).
- (ii) For the detection of **IBRgE-specific antibodies**, all 11 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 ELISA kits from 2 different producers (Producer 1 and 2), LAB6 and LAB7 also used 2 ELISA kits from 2 different producers (Producer 1 and 3) and LAB 4 used 3 ELISA kits from 3 different producers (Producer 1, 2 and 3) (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	5	6.1	6.2	7	8
<b>Failure</b>	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)
<b>Success</b>	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)

	LABNR		
	9	10	11
<b>Failure</b>	0 (0.0)	0 (0.0)	0 (0.0)
<b>Success</b>	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	3.1	3.2	4.1	4.2	4.3	5	6.1	6.3
<b>Failure</b>	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)
<b>Success</b>	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)

	LABNR					
	7.1	7.3	8	9	10	12
<b>Failure</b>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Success</b>	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

#### IV.4.2. Variability among participating laboratories

No 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 LAB6 obtained identical qualitative results using 2 ELISA kits from different producers (LAB3) or 2 different protocols of the same ELISA kit (LAB6).
- (ii) For the detection of **IBRgE-specific antibodies**, no variability between laboratories could be observed since all participants correctly identified all reference serum samples. LAB3, LAB4, LAB6 and LAB7 obtained identical qualitative results using 2 or 3 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	PT2016IBRgBSERPS1	POS	POS	1
2	1	2	PT2016IBRgBSERNS2	NEG	NEG	1
3	1	3	PT2016IBRgBSERNS1	NEG	NEG	1
4	1	4	PT2016IBRgBSERPS2	POS	POS	1
5	1	5	PT2016IBRgBSERNS3	NEG	NEG	1
6	1	6	PT2016IBRgBSERPS3	POS	POS	1
7	1	7	PT2016IBRgBSERPS1	POS	POS	1
8	1	8	PT2016IBRgBSERPS3	POS	POS	1
9	1	9	PT2016IBRgBSERNS1	NEG	NEG	1
10	1	10	PT2016IBRgBSERPS3	POS	POS	1
11	1	11	PT2016IBRgBSERNS3	NEG	NEG	1
12	1	12	PT2016IBRgBSERPS2	POS	POS	1
13	1	13	PT2016IBRgBSERNS1	NEG	NEG	1
14	1	14	PT2016IBRgBSERPS3	POS	POS	1
15	1	15	PT2016IBRgBSERNS3	NEG	NEG	1
16	1	16	PT2016IBRgBSERNS1	NEG	NEG	1
17	1	17	PT2016IBRgBSERPS2	POS	POS	1
18	1	18	PT2016IBRgBSERPS1	POS	POS	1
19	1	19	PT2016IBRgBSERNS3	NEG	NEG	1
20	1	20	PT2016IBRgBSERNS1	NEG	NEG	1
21	2	1	PT2016IBRgBSERNS3	NEG	NEG	1
22	2	2	PT2016IBRgBSERNS1	NEG	NEG	1
23	2	3	PT2016IBRgBSERPS1	POS	POS	1
24	2	4	PT2016IBRgBSERPS2	POS	POS	1
25	2	5	PT2016IBRgBSERNS1	NEG	NEG	1
26	2	6	PT2016IBRgBSERPS3	POS	POS	1
27	2	7	PT2016IBRgBSERNS3	NEG	NEG	1
28	2	8	PT2016IBRgBSERPS3	POS	POS	1
29	2	9	PT2016IBRgBSERPS1	POS	POS	1
30	2	10	PT2016IBRgBSERPS2	POS	POS	1
31	2	11	PT2016IBRgBSERNS1	NEG	NEG	1
32	2	12	PT2016IBRgBSERPS1	POS	POS	1
33	2	13	PT2016IBRgBSERPS2	POS	POS	1
34	2	14	PT2016IBRgBSERNS1	NEG	NEG	1
35	2	15	PT2016IBRgBSERNS2	NEG	NEG	1
36	2	16	PT2016IBRgBSERNS3	NEG	NEG	1
37	2	17	PT2016IBRgBSERPS3	POS	POS	1
38	2	18	PT2016IBRgBSERNS1	NEG	NEG	1
39	2	19	PT2016IBRgBSERPS3	POS	POS	1
40	2	20	PT2016IBRgBSERNS3	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3.1	1	PT2016IBRgBSERPS1	POS	POS	1
42	3.1	2	PT2016IBRgBSERNS2	NEG	NEG	1
43	3.1	3	PT2016IBRgBSERNS1	NEG	NEG	1
44	3.1	4	PT2016IBRgBSERPS2	POS	POS	1
45	3.1	5	PT2016IBRgBSERNS3	NEG	NEG	1
46	3.1	6	PT2016IBRgBSERPS3	POS	POS	1
47	3.1	7	PT2016IBRgBSERPS1	POS	POS	1
48	3.1	8	PT2016IBRgBSERPS3	POS	POS	1
49	3.1	9	PT2016IBRgBSERNS1	NEG	NEG	1
50	3.1	10	PT2016IBRgBSERPS3	POS	POS	1
51	3.1	11	PT2016IBRgBSERNS3	NEG	NEG	1
52	3.1	12	PT2016IBRgBSERPS2	POS	POS	1
53	3.1	13	PT2016IBRgBSERNS1	NEG	NEG	1
54	3.1	14	PT2016IBRgBSERPS3	POS	POS	1
55	3.1	15	PT2016IBRgBSERNS3	NEG	NEG	1
56	3.1	16	PT2016IBRgBSERNS1	NEG	NEG	1
57	3.1	17	PT2016IBRgBSERPS2	POS	POS	1
58	3.1	18	PT2016IBRgBSERPS1	POS	POS	1
59	3.1	19	PT2016IBRgBSERNS3	NEG	NEG	1
60	3.1	20	PT2016IBRgBSERNS1	NEG	NEG	1
61	3.2	1	PT2016IBRgBSERPS1	POS	POS	1
62	3.2	2	PT2016IBRgBSERNS2	NEG	NEG	1
63	3.2	3	PT2016IBRgBSERNS1	NEG	NEG	1
64	3.2	4	PT2016IBRgBSERPS2	POS	POS	1
65	3.2	5	PT2016IBRgBSERNS3	NEG	NEG	1
66	3.2	6	PT2016IBRgBSERPS3	POS	POS	1
67	3.2	7	PT2016IBRgBSERPS1	POS	POS	1
68	3.2	8	PT2016IBRgBSERPS3	POS	POS	1
69	3.2	9	PT2016IBRgBSERNS1	NEG	NEG	1
70	3.2	10	PT2016IBRgBSERPS3	POS	POS	1
71	3.2	11	PT2016IBRgBSERNS3	NEG	NEG	1
72	3.2	12	PT2016IBRgBSERPS2	POS	POS	1
73	3.2	13	PT2016IBRgBSERNS1	NEG	NEG	1
74	3.2	14	PT2016IBRgBSERPS3	POS	POS	1
75	3.2	15	PT2016IBRgBSERNS3	NEG	NEG	1
76	3.2	16	PT2016IBRgBSERNS1	NEG	NEG	1
77	3.2	17	PT2016IBRgBSERPS2	POS	POS	1
78	3.2	18	PT2016IBRgBSERPS1	POS	POS	1
79	3.2	19	PT2016IBRgBSERNS3	NEG	NEG	1
80	3.2	20	PT2016IBRgBSERNS1	NEG	NEG	1





(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4	1	PT2016IBRgBSERNS3	NEG	NEG	1
82	4	2	PT2016IBRgBSERNS1	NEG	NEG	1
83	4	3	PT2016IBRgBSERPS1	POS	POS	1
84	4	4	PT2016IBRgBSERPS2	POS	POS	1
85	4	5	PT2016IBRgBSERNS1	NEG	NEG	1
86	4	6	PT2016IBRgBSERPS3	POS	POS	1
87	4	7	PT2016IBRgBSERNS3	NEG	NEG	1
88	4	8	PT2016IBRgBSERPS3	POS	POS	1
89	4	9	PT2016IBRgBSERPS1	POS	POS	1
90	4	10	PT2016IBRgBSERPS2	POS	POS	1
91	4	11	PT2016IBRgBSERNS1	NEG	NEG	1
92	4	12	PT2016IBRgBSERPS1	POS	POS	1
93	4	13	PT2016IBRgBSERPS2	POS	POS	1
94	4	14	PT2016IBRgBSERNS1	NEG	NEG	1
95	4	15	PT2016IBRgBSERNS2	NEG	NEG	1
96	4	16	PT2016IBRgBSERNS3	NEG	NEG	1
97	4	17	PT2016IBRgBSERPS3	POS	POS	1
98	4	18	PT2016IBRgBSERNS1	NEG	NEG	1
99	4	19	PT2016IBRgBSERPS3	POS	POS	1
100	4	20	PT2016IBRgBSERNS3	NEG	NEG	1
101	5	1	PT2016IBRgBSERPS1	POS	POS	1
102	5	2	PT2016IBRgBSERNS2	NEG	NEG	1
103	5	3	PT2016IBRgBSERNS1	NEG	NEG	1
104	5	4	PT2016IBRgBSERPS2	POS	POS	1
105	5	5	PT2016IBRgBSERNS3	NEG	NEG	1
106	5	6	PT2016IBRgBSERPS3	POS	POS	1
107	5	7	PT2016IBRgBSERPS1	POS	POS	1
108	5	8	PT2016IBRgBSERPS3	POS	POS	1
109	5	9	PT2016IBRgBSERNS1	NEG	NEG	1
110	5	10	PT2016IBRgBSERPS3	POS	POS	1
111	5	11	PT2016IBRgBSERNS3	NEG	NEG	1
112	5	12	PT2016IBRgBSERPS2	POS	POS	1
113	5	13	PT2016IBRgBSERNS1	NEG	NEG	1
114	5	14	PT2016IBRgBSERPS3	POS	POS	1
115	5	15	PT2016IBRgBSERNS3	NEG	NEG	1
116	5	16	PT2016IBRgBSERNS1	NEG	NEG	1
117	5	17	PT2016IBRgBSERPS2	POS	POS	1
118	5	18	PT2016IBRgBSERPS1	POS	POS	1
119	5	19	PT2016IBRgBSERNS3	NEG	NEG	1
120	5	20	PT2016IBRgBSERNS1	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	6.1	1	PT2016IBRgBSERNS3	NEG	NEG	1
122	6.1	2	PT2016IBRgBSERNS1	NEG	NEG	1
123	6.1	3	PT2016IBRgBSERPS1	POS	POS	1
124	6.1	4	PT2016IBRgBSERPS2	POS	POS	1
125	6.1	5	PT2016IBRgBSERNS1	NEG	NEG	1
126	6.1	6	PT2016IBRgBSERPS3	POS	POS	1
127	6.1	7	PT2016IBRgBSERNS3	NEG	NEG	1
128	6.1	8	PT2016IBRgBSERPS3	POS	POS	1
129	6.1	9	PT2016IBRgBSERPS1	POS	POS	1
130	6.1	10	PT2016IBRgBSERPS2	POS	POS	1
131	6.1	11	PT2016IBRgBSERNS1	NEG	NEG	1
132	6.1	12	PT2016IBRgBSERPS1	POS	POS	1
133	6.1	13	PT2016IBRgBSERPS2	POS	POS	1
134	6.1	14	PT2016IBRgBSERNS1	NEG	NEG	1
135	6.1	15	PT2016IBRgBSERNS2	NEG	NEG	1
136	6.1	16	PT2016IBRgBSERNS3	NEG	NEG	1
137	6.1	17	PT2016IBRgBSERPS3	POS	POS	1
138	6.1	18	PT2016IBRgBSERNS1	NEG	NEG	1
139	6.1	19	PT2016IBRgBSERPS3	POS	POS	1
140	6.1	20	PT2016IBRgBSERNS3	NEG	NEG	1
141	6.2	1	PT2016IBRgBSERNS3	NEG	NEG	1
142	6.2	2	PT2016IBRgBSERNS1	NEG	NEG	1
143	6.2	3	PT2016IBRgBSERPS1	POS	POS	1
144	6.2	4	PT2016IBRgBSERPS2	POS	POS	1
145	6.2	5	PT2016IBRgBSERNS1	NEG	NEG	1
146	6.2	6	PT2016IBRgBSERPS3	POS	POS	1
147	6.2	7	PT2016IBRgBSERNS3	NEG	NEG	1
148	6.2	8	PT2016IBRgBSERPS3	POS	POS	1
149	6.2	9	PT2016IBRgBSERPS1	POS	POS	1
150	6.2	10	PT2016IBRgBSERPS2	POS	POS	1
151	6.2	11	PT2016IBRgBSERNS1	NEG	NEG	1
152	6.2	12	PT2016IBRgBSERPS1	POS	POS	1
153	6.2	13	PT2016IBRgBSERPS2	POS	POS	1
154	6.2	14	PT2016IBRgBSERNS1	NEG	NEG	1
155	6.2	15	PT2016IBRgBSERNS2	NEG	NEG	1
156	6.2	16	PT2016IBRgBSERNS3	NEG	NEG	1
157	6.2	17	PT2016IBRgBSERPS3	POS	POS	1
158	6.2	18	PT2016IBRgBSERNS1	NEG	NEG	1
159	6.2	19	PT2016IBRgBSERPS3	POS	POS	1
160	6.2	20	PT2016IBRgBSERNS3	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	7	1	PT2016IBRgBSERPS1	POS	POS	1
162	7	2	PT2016IBRgBSERNS2	NEG	NEG	1
163	7	3	PT2016IBRgBSERNS1	NEG	NEG	1
164	7	4	PT2016IBRgBSERPS2	POS	POS	1
165	7	5	PT2016IBRgBSERNS3	NEG	NEG	1
166	7	6	PT2016IBRgBSERPS3	POS	POS	1
167	7	7	PT2016IBRgBSERPS1	POS	POS	1
168	7	8	PT2016IBRgBSERPS3	POS	POS	1
169	7	9	PT2016IBRgBSERNS1	NEG	NEG	1
170	7	10	PT2016IBRgBSERPS3	POS	POS	1
171	7	11	PT2016IBRgBSERNS3	NEG	NEG	1
172	7	12	PT2016IBRgBSERPS2	POS	POS	1
173	7	13	PT2016IBRgBSERNS1	NEG	NEG	1
174	7	14	PT2016IBRgBSERPS3	POS	POS	1
175	7	15	PT2016IBRgBSERNS3	NEG	NEG	1
176	7	16	PT2016IBRgBSERNS1	NEG	NEG	1
177	7	17	PT2016IBRgBSERPS2	POS	POS	1
178	7	18	PT2016IBRgBSERPS1	POS	POS	1
179	7	19	PT2016IBRgBSERNS3	NEG	NEG	1
180	7	20	PT2016IBRgBSERNS1	NEG	NEG	1
181	8	1	PT2016IBRgBSERNS3	NEG	NEG	1
182	8	2	PT2016IBRgBSERNS1	NEG	NEG	1
183	8	3	PT2016IBRgBSERPS1	POS	POS	1
184	8	4	PT2016IBRgBSERPS2	POS	POS	1
185	8	5	PT2016IBRgBSERNS1	NEG	NEG	1
186	8	6	PT2016IBRgBSERPS3	POS	POS	1
187	8	7	PT2016IBRgBSERNS3	NEG	NEG	1
188	8	8	PT2016IBRgBSERPS3	POS	POS	1
189	8	9	PT2016IBRgBSERPS1	POS	POS	1
190	8	10	PT2016IBRgBSERPS2	POS	POS	1
191	8	11	PT2016IBRgBSERNS1	NEG	NEG	1
192	8	12	PT2016IBRgBSERPS1	POS	POS	1
193	8	13	PT2016IBRgBSERPS2	POS	POS	1
194	8	14	PT2016IBRgBSERNS1	NEG	NEG	1
195	8	15	PT2016IBRgBSERNS2	NEG	NEG	1
196	8	16	PT2016IBRgBSERNS3	NEG	NEG	1
197	8	17	PT2016IBRgBSERPS3	POS	POS	1
198	8	18	PT2016IBRgBSERNS1	NEG	NEG	1
199	8	19	PT2016IBRgBSERPS3	POS	POS	1
200	8	20	PT2016IBRgBSERNS3	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	9	1	PT2016IBRgBSERPS1	POS	POS	1
202	9	2	PT2016IBRgBSERNS2	NEG	NEG	1
203	9	3	PT2016IBRgBSERNS1	NEG	NEG	1
204	9	4	PT2016IBRgBSERPS2	POS	POS	1
205	9	5	PT2016IBRgBSERNS3	NEG	NEG	1
206	9	6	PT2016IBRgBSERPS3	POS	POS	1
207	9	7	PT2016IBRgBSERPS1	POS	POS	1
208	9	8	PT2016IBRgBSERPS3	POS	POS	1
209	9	9	PT2016IBRgBSERNS1	NEG	NEG	1
210	9	10	PT2016IBRgBSERPS3	POS	POS	1
211	9	11	PT2016IBRgBSERNS3	NEG	NEG	1
212	9	12	PT2016IBRgBSERPS2	POS	POS	1
213	9	13	PT2016IBRgBSERNS1	NEG	NEG	1
214	9	14	PT2016IBRgBSERPS3	POS	POS	1
215	9	15	PT2016IBRgBSERNS3	NEG	NEG	1
216	9	16	PT2016IBRgBSERNS1	NEG	NEG	1
217	9	17	PT2016IBRgBSERPS2	POS	POS	1
218	9	18	PT2016IBRgBSERPS1	POS	POS	1
219	9	19	PT2016IBRgBSERNS3	NEG	NEG	1
220	9	20	PT2016IBRgBSERNS1	NEG	NEG	1
221	10	1	PT2016IBRgBSERNS3	NEG	NEG	1
222	10	2	PT2016IBRgBSERNS1	NEG	NEG	1
223	10	3	PT2016IBRgBSERPS1	POS	POS	1
224	10	4	PT2016IBRgBSERPS2	POS	POS	1
225	10	5	PT2016IBRgBSERNS1	NEG	NEG	1
226	10	6	PT2016IBRgBSERPS3	POS	POS	1
227	10	7	PT2016IBRgBSERNS3	NEG	NEG	1
228	10	8	PT2016IBRgBSERPS3	POS	POS	1
229	10	9	PT2016IBRgBSERPS1	POS	POS	1
230	10	10	PT2016IBRgBSERPS2	POS	POS	1
231	10	11	PT2016IBRgBSERNS1	NEG	NEG	1
232	10	12	PT2016IBRgBSERPS1	POS	POS	1
233	10	13	PT2016IBRgBSERPS2	POS	POS	1
234	10	14	PT2016IBRgBSERNS1	NEG	NEG	1
235	10	15	PT2016IBRgBSERNS2	NEG	NEG	1
236	10	16	PT2016IBRgBSERNS3	NEG	NEG	1
237	10	17	PT2016IBRgBSERPS3	POS	POS	1
238	10	18	PT2016IBRgBSERNS1	NEG	NEG	1
239	10	19	PT2016IBRgBSERPS3	POS	POS	1
240	10	20	PT2016IBRgBSERNS3	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
241	11	1	PT2016IBRgBSERPS1	POS	POS	1
242	11	2	PT2016IBRgBSERNS2	NEG	NEG	1
243	11	3	PT2016IBRgBSERNS1	NEG	NEG	1
244	11	4	PT2016IBRgBSERPS2	POS	POS	1
245	11	5	PT2016IBRgBSERNS3	NEG	NEG	1
246	11	6	PT2016IBRgBSERPS3	POS	POS	1
247	11	7	PT2016IBRgBSERPS1	POS	POS	1
248	11	8	PT2016IBRgBSERPS3	POS	POS	1
249	11	9	PT2016IBRgBSERNS1	NEG	NEG	1
250	11	10	PT2016IBRgBSERPS3	POS	POS	1
251	11	11	PT2016IBRgBSERNS3	NEG	NEG	1
252	11	12	PT2016IBRgBSERPS2	POS	POS	1
253	11	13	PT2016IBRgBSERNS1	NEG	NEG	1
254	11	14	PT2016IBRgBSERPS3	POS	POS	1
255	11	15	PT2016IBRgBSERNS3	NEG	NEG	1
256	11	16	PT2016IBRgBSERNS1	NEG	NEG	1
257	11	17	PT2016IBRgBSERPS2	POS	POS	1
258	11	18	PT2016IBRgBSERPS1	POS	POS	1
259	11	19	PT2016IBRgBSERNS3	NEG	NEG	1
260	11	20	PT2016IBRgBSERNS1	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	PT2016IBRgESERPS2	POS	POS	1
2	1	2	PT2016IBRgESERNS3	NEG	NEG	1
3	1	3	PT2016IBRgESERNS1	NEG	NEG	1
4	1	4	PT2016IBRgESERPS3	POS	POS	1
5	1	5	PT2016IBRgESERNS2	NEG	NEG	1
6	1	6	PT2016IBRgESERPS2	POS	POS	1
7	1	7	PT2016IBRgESERPS1	POS	POS	1
8	1	8	PT2016IBRgESERNS3	NEG	NEG	1
9	1	9	PT2016IBRgESERNS1	NEG	NEG	1
10	1	10	PT2016IBRgESERNS2	NEG	NEG	1
11	1	11	PT2016IBRgESERPS3	POS	POS	1
12	1	12	PT2016IBRgESERPS1	POS	POS	1
13	1	13	PT2016IBRgESERPS3	POS	POS	1
14	1	14	PT2016IBRgESERNS3	NEG	NEG	1
15	1	15	PT2016IBRgESERPS3	POS	POS	1
16	1	16	PT2016IBRgESERPS2	POS	POS	1
17	1	17	PT2016IBRgESERPS3	POS	POS	1
18	1	18	PT2016IBRgESERNS1	NEG	NEG	1
19	1	19	PT2016IBRgESERNS2	NEG	NEG	1
20	1	20	PT2016IBRgESERPS2	POS	POS	1
21	2	1	PT2016IBRgESERNS1	NEG	NEG	1
22	2	2	PT2016IBRgESERPS2	POS	POS	1
23	2	3	PT2016IBRgESERNS3	NEG	NEG	1
24	2	4	PT2016IBRgESERNS2	NEG	NEG	1
25	2	5	PT2016IBRgESERPS3	POS	POS	1
26	2	6	PT2016IBRgESERPS1	POS	POS	1
27	2	7	PT2016IBRgESERNS3	NEG	NEG	1
28	2	8	PT2016IBRgESERNS1	NEG	NEG	1
29	2	9	PT2016IBRgESERPS2	POS	POS	1
30	2	10	PT2016IBRgESERPS3	POS	POS	1
31	2	11	PT2016IBRgESERPS3	POS	POS	1
32	2	12	PT2016IBRgESERNS2	NEG	NEG	1
33	2	13	PT2016IBRgESERPS1	POS	POS	1
34	2	14	PT2016IBRgESERPS3	POS	POS	1
35	2	15	PT2016IBRgESERNS1	NEG	NEG	1
36	2	16	PT2016IBRgESERPS2	POS	POS	1
37	2	17	PT2016IBRgESERPS3	POS	POS	1
38	2	18	PT2016IBRgESERNS3	NEG	NEG	1
39	2	19	PT2016IBRgESERPS2	POS	POS	1
40	2	20	PT2016IBRgESERNS2	NEG	NEG	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3.1	1	PT2016IBRgESERPS2	POS	POS	1
42	3.1	2	PT2016IBRgESERNS3	NEG	NEG	1
43	3.1	3	PT2016IBRgESERNS1	NEG	NEG	1
44	3.1	4	PT2016IBRgESERPS3	POS	POS	1
45	3.1	5	PT2016IBRgESERNS2	NEG	NEG	1
46	3.1	6	PT2016IBRgESERPS2	POS	POS	1
47	3.1	7	PT2016IBRgESERPS1	POS	POS	1
48	3.1	8	PT2016IBRgESERNS3	NEG	NEG	1
49	3.1	9	PT2016IBRgESERNS1	NEG	NEG	1
50	3.1	10	PT2016IBRgESERNS2	NEG	NEG	1
51	3.1	11	PT2016IBRgESERPS3	POS	POS	1
52	3.1	12	PT2016IBRgESERPS1	POS	POS	1
53	3.1	13	PT2016IBRgESERPS3	POS	POS	1
54	3.1	14	PT2016IBRgESERNS3	NEG	NEG	1
55	3.1	15	PT2016IBRgESERPS3	POS	POS	1
56	3.1	16	PT2016IBRgESERPS2	POS	POS	1
57	3.1	17	PT2016IBRgESERPS3	POS	POS	1
58	3.1	18	PT2016IBRgESERNS1	NEG	NEG	1
59	3.1	19	PT2016IBRgESERNS2	NEG	NEG	1
60	3.1	20	PT2016IBRgESERPS2	POS	POS	1
61	3.2	1	PT2016IBRgESERPS2	POS	POS	1
62	3.2	2	PT2016IBRgESERNS3	NEG	NEG	1
63	3.2	3	PT2016IBRgESERNS1	NEG	NEG	1
64	3.2	4	PT2016IBRgESERPS3	POS	POS	1
65	3.2	5	PT2016IBRgESERNS2	NEG	NEG	1
66	3.2	6	PT2016IBRgESERPS2	POS	POS	1
67	3.2	7	PT2016IBRgESERPS1	POS	POS	1
68	3.2	8	PT2016IBRgESERNS3	NEG	NEG	1
69	3.2	9	PT2016IBRgESERNS1	NEG	NEG	1
70	3.2	10	PT2016IBRgESERNS2	NEG	NEG	1
71	3.2	11	PT2016IBRgESERPS3	POS	POS	1
72	3.2	12	PT2016IBRgESERPS1	POS	POS	1
73	3.2	13	PT2016IBRgESERPS3	POS	POS	1
74	3.2	14	PT2016IBRgESERNS3	NEG	NEG	1
75	3.2	15	PT2016IBRgESERPS3	POS	POS	1
76	3.2	16	PT2016IBRgESERPS2	POS	POS	1
77	3.2	17	PT2016IBRgESERPS3	POS	POS	1
78	3.2	18	PT2016IBRgESERNS1	NEG	NEG	1
79	3.2	19	PT2016IBRgESERNS2	NEG	NEG	1
80	3.2	20	PT2016IBRgESERPS2	POS	POS	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.1	1	PT2016IBRgESERNS1	NEG	NEG	1
82	4.1	2	PT2016IBRgESERPS2	POS	POS	1
83	4.1	3	PT2016IBRgESERNS3	NEG	NEG	1
84	4.1	4	PT2016IBRgESERNS2	NEG	NEG	1
85	4.1	5	PT2016IBRgESERPS3	POS	POS	1
86	4.1	6	PT2016IBRgESERPS1	POS	POS	1
87	4.1	7	PT2016IBRgESERNS3	NEG	NEG	1
88	4.1	8	PT2016IBRgESERNS1	NEG	NEG	1
89	4.1	9	PT2016IBRgESERPS2	POS	POS	1
90	4.1	10	PT2016IBRgESERPS3	POS	POS	1
91	4.1	11	PT2016IBRgESERPS3	POS	POS	1
92	4.1	12	PT2016IBRgESERNS2	NEG	NEG	1
93	4.1	13	PT2016IBRgESERPS1	POS	POS	1
94	4.1	14	PT2016IBRgESERPS3	POS	POS	1
95	4.1	15	PT2016IBRgESERNS1	NEG	NEG	1
96	4.1	16	PT2016IBRgESERPS2	POS	POS	1
97	4.1	17	PT2016IBRgESERPS3	POS	POS	1
98	4.1	18	PT2016IBRgESERNS3	NEG	NEG	1
99	4.1	19	PT2016IBRgESERPS2	POS	POS	1
100	4.1	20	PT2016IBRgESERNS2	NEG	NEG	1
101	4.2	1	PT2016IBRgESERNS1	NEG	NEG	1
102	4.2	2	PT2016IBRgESERPS2	POS	POS	1
103	4.2	3	PT2016IBRgESERNS3	NEG	NEG	1
104	4.2	4	PT2016IBRgESERNS2	NEG	NEG	1
105	4.2	5	PT2016IBRgESERPS3	POS	POS	1
106	4.2	6	PT2016IBRgESERPS1	POS	POS	1
107	4.2	7	PT2016IBRgESERNS3	NEG	NEG	1
108	4.2	8	PT2016IBRgESERNS1	NEG	NEG	1
109	4.2	9	PT2016IBRgESERPS2	POS	POS	1
110	4.2	10	PT2016IBRgESERPS3	POS	POS	1
111	4.2	11	PT2016IBRgESERPS3	POS	POS	1
112	4.2	12	PT2016IBRgESERNS2	NEG	NEG	1
113	4.2	13	PT2016IBRgESERPS1	POS	POS	1
114	4.2	14	PT2016IBRgESERPS3	POS	POS	1
115	4.2	15	PT2016IBRgESERNS1	NEG	NEG	1
116	4.2	16	PT2016IBRgESERPS2	POS	POS	1
117	4.2	17	PT2016IBRgESERPS3	POS	POS	1
118	4.2	18	PT2016IBRgESERNS3	NEG	NEG	1
119	4.2	19	PT2016IBRgESERPS2	POS	POS	1
120	4.2	20	PT2016IBRgESERNS2	NEG	NEG	1





(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	4.3	1	PT2016IBRgESERNS1	NEG	NEG	1
122	4.3	2	PT2016IBRgESERPS2	POS	POS	1
123	4.3	3	PT2016IBRgESERNS3	NEG	NEG	1
124	4.3	4	PT2016IBRgESERNS2	NEG	NEG	1
125	4.3	5	PT2016IBRgESERPS3	POS	POS	1
126	4.3	6	PT2016IBRgESERPS1	POS	POS	1
127	4.3	7	PT2016IBRgESERNS3	NEG	NEG	1
128	4.3	8	PT2016IBRgESERNS1	NEG	NEG	1
129	4.3	9	PT2016IBRgESERPS2	POS	POS	1
130	4.3	10	PT2016IBRgESERPS3	POS	POS	1
131	4.3	11	PT2016IBRgESERPS3	POS	POS	1
132	4.3	12	PT2016IBRgESERNS2	NEG	NEG	1
133	4.3	13	PT2016IBRgESERPS1	POS	POS	1
134	4.3	14	PT2016IBRgESERPS3	POS	POS	1
135	4.3	15	PT2016IBRgESERNS1	NEG	NEG	1
136	4.3	16	PT2016IBRgESERPS2	POS	POS	1
137	4.3	17	PT2016IBRgESERPS3	POS	POS	1
138	4.3	18	PT2016IBRgESERNS3	NEG	NEG	1
139	4.3	19	PT2016IBRgESERPS2	POS	POS	1
140	4.3	20	PT2016IBRgESERNS2	NEG	NEG	1
141	5	1	PT2016IBRgESERPS2	POS	POS	1
142	5	2	PT2016IBRgESERNS3	NEG	NEG	1
143	5	3	PT2016IBRgESERNS1	NEG	NEG	1
144	5	4	PT2016IBRgESERPS3	POS	POS	1
145	5	5	PT2016IBRgESERNS2	NEG	NEG	1
146	5	6	PT2016IBRgESERPS2	POS	POS	1
147	5	7	PT2016IBRgESERPS1	POS	POS	1
148	5	8	PT2016IBRgESERNS3	NEG	NEG	1
149	5	9	PT2016IBRgESERNS1	NEG	NEG	1
150	5	10	PT2016IBRgESERNS2	NEG	NEG	1
151	5	11	PT2016IBRgESERPS3	POS	POS	1
152	5	12	PT2016IBRgESERPS1	POS	POS	1
153	5	13	PT2016IBRgESERPS3	POS	POS	1
154	5	14	PT2016IBRgESERNS3	NEG	NEG	1
155	5	15	PT2016IBRgESERPS3	POS	POS	1
156	5	16	PT2016IBRgESERPS2	POS	POS	1
157	5	17	PT2016IBRgESERPS3	POS	POS	1
158	5	18	PT2016IBRgESERNS1	NEG	NEG	1
159	5	19	PT2016IBRgESERNS2	NEG	NEG	1
160	5	20	PT2016IBRgESERPS2	POS	POS	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	6.1	1	PT2016IBRgESERNS1	NEG	NEG	1
162	6.1	2	PT2016IBRgESERPS2	POS	POS	1
163	6.1	3	PT2016IBRgESERNS3	NEG	NEG	1
164	6.1	4	PT2016IBRgESERNS2	NEG	NEG	1
165	6.1	5	PT2016IBRgESERPS3	POS	POS	1
166	6.1	6	PT2016IBRgESERPS1	POS	POS	1
167	6.1	7	PT2016IBRgESERNS3	NEG	NEG	1
168	6.1	8	PT2016IBRgESERNS1	NEG	NEG	1
169	6.1	9	PT2016IBRgESERPS2	POS	POS	1
170	6.1	10	PT2016IBRgESERPS3	POS	POS	1
171	6.1	11	PT2016IBRgESERPS3	POS	POS	1
172	6.1	12	PT2016IBRgESERNS2	NEG	NEG	1
173	6.1	13	PT2016IBRgESERPS1	POS	POS	1
174	6.1	14	PT2016IBRgESERPS3	POS	POS	1
175	6.1	15	PT2016IBRgESERNS1	NEG	NEG	1
176	6.1	16	PT2016IBRgESERPS2	POS	POS	1
177	6.1	17	PT2016IBRgESERPS3	POS	POS	1
178	6.1	18	PT2016IBRgESERNS3	NEG	NEG	1
179	6.1	19	PT2016IBRgESERPS2	POS	POS	1
180	6.1	20	PT2016IBRgESERNS2	NEG	NEG	1
181	6.3	1	PT2016IBRgESERNS1	NEG	NEG	1
182	6.3	2	PT2016IBRgESERPS2	POS	POS	1
183	6.3	3	PT2016IBRgESERNS3	NEG	NEG	1
184	6.3	4	PT2016IBRgESERNS2	NEG	NEG	1
185	6.3	5	PT2016IBRgESERPS3	POS	POS	1
186	6.3	6	PT2016IBRgESERPS1	POS	POS	1
187	6.3	7	PT2016IBRgESERNS3	NEG	NEG	1
188	6.3	8	PT2016IBRgESERNS1	NEG	NEG	1
189	6.3	9	PT2016IBRgESERPS2	POS	POS	1
190	6.3	10	PT2016IBRgESERPS3	POS	POS	1
191	6.3	11	PT2016IBRgESERPS3	POS	POS	1
192	6.3	12	PT2016IBRgESERNS2	NEG	NEG	1
193	6.3	13	PT2016IBRgESERPS1	POS	POS	1
194	6.3	14	PT2016IBRgESERPS3	POS	POS	1
195	6.3	15	PT2016IBRgESERNS1	NEG	NEG	1
196	6.3	16	PT2016IBRgESERPS2	POS	POS	1
197	6.3	17	PT2016IBRgESERPS3	POS	POS	1
198	6.3	18	PT2016IBRgESERNS3	NEG	NEG	1
199	6.3	19	PT2016IBRgESERPS2	POS	POS	1
200	6.3	20	PT2016IBRgESERNS2	NEG	NEG	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	7.1	1	PT2016IBRgESERPS2	POS	POS	1
202	7.1	2	PT2016IBRgESERNS3	NEG	NEG	1
203	7.1	3	PT2016IBRgESERNS1	NEG	NEG	1
204	7.1	4	PT2016IBRgESERPS3	POS	POS	1
205	7.1	5	PT2016IBRgESERNS2	NEG	NEG	1
206	7.1	6	PT2016IBRgESERPS2	POS	POS	1
207	7.1	7	PT2016IBRgESERPS1	POS	POS	1
208	7.1	8	PT2016IBRgESERNS3	NEG	NEG	1
209	7.1	9	PT2016IBRgESERNS1	NEG	NEG	1
210	7.1	10	PT2016IBRgESERNS2	NEG	NEG	1
211	7.1	11	PT2016IBRgESERPS3	POS	POS	1
212	7.1	12	PT2016IBRgESERPS1	POS	POS	1
213	7.1	13	PT2016IBRgESERPS3	POS	POS	1
214	7.1	14	PT2016IBRgESERNS3	NEG	NEG	1
215	7.1	15	PT2016IBRgESERPS3	POS	POS	1
216	7.1	16	PT2016IBRgESERPS2	POS	POS	1
217	7.1	17	PT2016IBRgESERPS3	POS	POS	1
218	7.1	18	PT2016IBRgESERNS1	NEG	NEG	1
219	7.1	19	PT2016IBRgESERNS2	NEG	NEG	1
220	7.1	20	PT2016IBRgESERPS2	POS	POS	1
221	7.3	1	PT2016IBRgESERPS2	POS	POS	1
222	7.3	2	PT2016IBRgESERNS3	NEG	NEG	1
223	7.3	3	PT2016IBRgESERNS1	NEG	NEG	1
224	7.3	4	PT2016IBRgESERPS3	POS	POS	1
225	7.3	5	PT2016IBRgESERNS2	NEG	NEG	1
226	7.3	6	PT2016IBRgESERPS2	POS	POS	1
227	7.3	7	PT2016IBRgESERPS1	POS	POS	1
228	7.3	8	PT2016IBRgESERNS3	NEG	NEG	1
229	7.3	9	PT2016IBRgESERNS1	NEG	NEG	1
230	7.3	10	PT2016IBRgESERNS2	NEG	NEG	1
231	7.3	11	PT2016IBRgESERPS3	POS	POS	1
232	7.3	12	PT2016IBRgESERPS1	POS	POS	1
233	7.3	13	PT2016IBRgESERPS3	POS	POS	1
234	7.3	14	PT2016IBRgESERNS3	NEG	NEG	1
235	7.3	15	PT2016IBRgESERPS3	POS	POS	1
236	7.3	16	PT2016IBRgESERPS2	POS	POS	1
237	7.3	17	PT2016IBRgESERPS3	POS	POS	1
238	7.3	18	PT2016IBRgESERNS1	NEG	NEG	1
239	7.3	19	PT2016IBRgESERNS2	NEG	NEG	1
240	7.3	20	PT2016IBRgESERPS2	POS	POS	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
241	8	1	PT2016IBRgESERNS1	NEG	NEG	1
242	8	2	PT2016IBRgESERPS2	POS	POS	1
243	8	3	PT2016IBRgESERNS3	NEG	NEG	1
244	8	4	PT2016IBRgESERNS2	NEG	NEG	1
245	8	5	PT2016IBRgESERPS3	POS	POS	1
246	8	6	PT2016IBRgESERPS1	POS	POS	1
247	8	7	PT2016IBRgESERNS3	NEG	NEG	1
248	8	8	PT2016IBRgESERNS1	NEG	NEG	1
249	8	9	PT2016IBRgESERPS2	POS	POS	1
250	8	10	PT2016IBRgESERPS3	POS	POS	1
251	8	11	PT2016IBRgESERPS3	POS	POS	1
252	8	12	PT2016IBRgESERNS2	NEG	NEG	1
253	8	13	PT2016IBRgESERPS1	POS	POS	1
254	8	14	PT2016IBRgESERPS3	POS	POS	1
255	8	15	PT2016IBRgESERNS1	NEG	NEG	1
256	8	16	PT2016IBRgESERPS2	POS	POS	1
257	8	17	PT2016IBRgESERPS3	POS	POS	1
258	8	18	PT2016IBRgESERNS3	NEG	NEG	1
259	8	19	PT2016IBRgESERPS2	POS	POS	1
260	8	20	PT2016IBRgESERNS2	NEG	NEG	1
261	9	1	PT2016IBRgESERPS2	POS	POS	1
262	9	2	PT2016IBRgESERNS3	NEG	NEG	1
263	9	3	PT2016IBRgESERNS1	NEG	NEG	1
264	9	4	PT2016IBRgESERPS3	POS	POS	1
265	9	5	PT2016IBRgESERNS2	NEG	NEG	1
266	9	6	PT2016IBRgESERPS2	POS	POS	1
267	9	7	PT2016IBRgESERPS1	POS	POS	1
268	9	8	PT2016IBRgESERNS3	NEG	NEG	1
269	9	9	PT2016IBRgESERNS1	NEG	NEG	1
270	9	10	PT2016IBRgESERNS2	NEG	NEG	1
271	9	11	PT2016IBRgESERPS3	POS	POS	1
272	9	12	PT2016IBRgESERPS1	POS	POS	1
273	9	13	PT2016IBRgESERPS3	POS	POS	1
274	9	14	PT2016IBRgESERNS3	NEG	NEG	1
275	9	15	PT2016IBRgESERPS3	POS	POS	1
276	9	16	PT2016IBRgESERPS2	POS	POS	1
277	9	17	PT2016IBRgESERPS3	POS	POS	1
278	9	18	PT2016IBRgESERNS1	NEG	NEG	1
279	9	19	PT2016IBRgESERNS2	NEG	NEG	1
280	9	20	PT2016IBRgESERPS2	POS	POS	1



(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
281	10	1	PT2016IBRgESERNS1	NEG	NEG	1
282	10	2	PT2016IBRgESERPS2	POS	POS	1
283	10	3	PT2016IBRgESERNS3	NEG	NEG	1
284	10	4	PT2016IBRgESERNS2	NEG	NEG	1
285	10	5	PT2016IBRgESERPS3	POS	POS	1
286	10	6	PT2016IBRgESERPS1	POS	POS	1
287	10	7	PT2016IBRgESERNS3	NEG	NEG	1
288	10	8	PT2016IBRgESERNS1	NEG	NEG	1
289	10	9	PT2016IBRgESERPS2	POS	POS	1
290	10	10	PT2016IBRgESERPS3	POS	POS	1
291	10	11	PT2016IBRgESERPS3	POS	POS	1
292	10	12	PT2016IBRgESERNS2	NEG	NEG	1
293	10	13	PT2016IBRgESERPS1	POS	POS	1
294	10	14	PT2016IBRgESERPS3	POS	POS	1
295	10	15	PT2016IBRgESERNS1	NEG	NEG	1
296	10	16	PT2016IBRgESERPS2	POS	POS	1
297	10	17	PT2016IBRgESERPS3	POS	POS	1
298	10	18	PT2016IBRgESERNS3	NEG	NEG	1
299	10	19	PT2016IBRgESERPS2	POS	POS	1
300	10	20	PT2016IBRgESERNS2	NEG	NEG	1
301	12	1	PT2016IBRgESERPS2	POS	POS	1
302	12	2	PT2016IBRgESERNS3	NEG	NEG	1
303	12	3	PT2016IBRgESERNS1	NEG	NEG	1
304	12	4	PT2016IBRgESERPS3	POS	POS	1
305	12	5	PT2016IBRgESERNS2	NEG	NEG	1
306	12	6	PT2016IBRgESERPS2	POS	POS	1
307	12	7	PT2016IBRgESERPS1	POS	POS	1
308	12	8	PT2016IBRgESERNS3	NEG	NEG	1
309	12	9	PT2016IBRgESERNS1	NEG	NEG	1
310	12	10	PT2016IBRgESERNS2	NEG	NEG	1
311	12	11	PT2016IBRgESERPS3	POS	POS	1
312	12	12	PT2016IBRgESERPS1	POS	POS	1
313	12	13	PT2016IBRgESERPS3	POS	POS	1
314	12	14	PT2016IBRgESERNS3	NEG	NEG	1
315	12	15	PT2016IBRgESERPS3	POS	POS	1
316	12	16	PT2016IBRgESERPS2	POS	POS	1
317	12	17	PT2016IBRgESERPS3	POS	POS	1
318	12	18	PT2016IBRgESERNS1	NEG	NEG	1
319	12	19	PT2016IBRgESERNS2	NEG	NEG	1
320	12	20	PT2016IBRgESERPS2	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 ELISA kits from different producers and LAB6 used 2 different protocols of the same ELISA kit, hereby obtaining identical qualitative results (Table 2 and Table 4).

One participating laboratory used an in-house developed IBRgB antibody ELISA kit (Home Made ELISA (1 batch: 28/9/16)), whereas the other participants used IBRgB antibody ELISA kits from 4 different commercial kit producers: IDEXX (5 batches: F221, G281, G651, G671, G701), IDVET (1 batch: 932), Synbiotics Europe (1 batch: 16ZEA003) and QIAGEN (1 batch: 254111122). LAB1, LAB3.1, LAB5, LAB6.1, LAB6.2, LAB7, LAB8, LAB9 and LAB10 used the same IBRgB ELISA kit producer, hereby LAB1, LAB3.1, LAB6.2, LAB7, LAB8 and LAB10 performed the long incubation protocol and LAB5, LAB6.1 and LAB9 the short incubation protocol. In addition, LAB6.1, LAB6.2, LAB7, LAB8 and LAB10 used 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). LAB3, LAB4, LAB6 and LAB7 obtained identical qualitative results using 2 or 3 ELISA kits from different producers (Table 3 and Table 5).

The IBRgE participating laboratories used ELISA kits from 4 different producers: IDEXX (4 batches: KL403, ML601, CM965, FM298), Qiagen (2 batches: 254130904, 254110207), IDVET (2 batches: A70, 934) and Biovet Inc (1 batch : 7561110). LAB1, LAB3.1, LAB4.1, LAB5, LAB6.1, LAB7.1, LAB8, LAB9 and LAB10 used the same IBRgE ELISA kit producer. In addition, LAB1, LAB3.1 and LAB10 used the same batch. LAB4.1, LAB5 and LAB9 used an other same batch and LAB6.1 and LAB7.1 used also an other 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 batches and protocols of 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 of ELISA kits used.

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)  
ELDC BV/Biovet-Europe (Kerkrade, Nederland)  
Friedrich-Loeffler-Institut (FLI) (Greifswald-Insel-Riems, Germany)  
ID.VET (Grabels, France)  
Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)  
Laboratoire National de Contrôle des Reproducteurs (LNCR) / ACSEDIATE (Maisons-Alfort, France)  
Lavetan NV (Turnhout, Belgium)  
State Veterinary and Food Institute, Veterinyry Institute in Zvolen (Zvolen, Slovakia)  
Synbiotics Europe (Lyon, France)  
Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)

## Annex 1: Quantitative data analysis (Box plots)

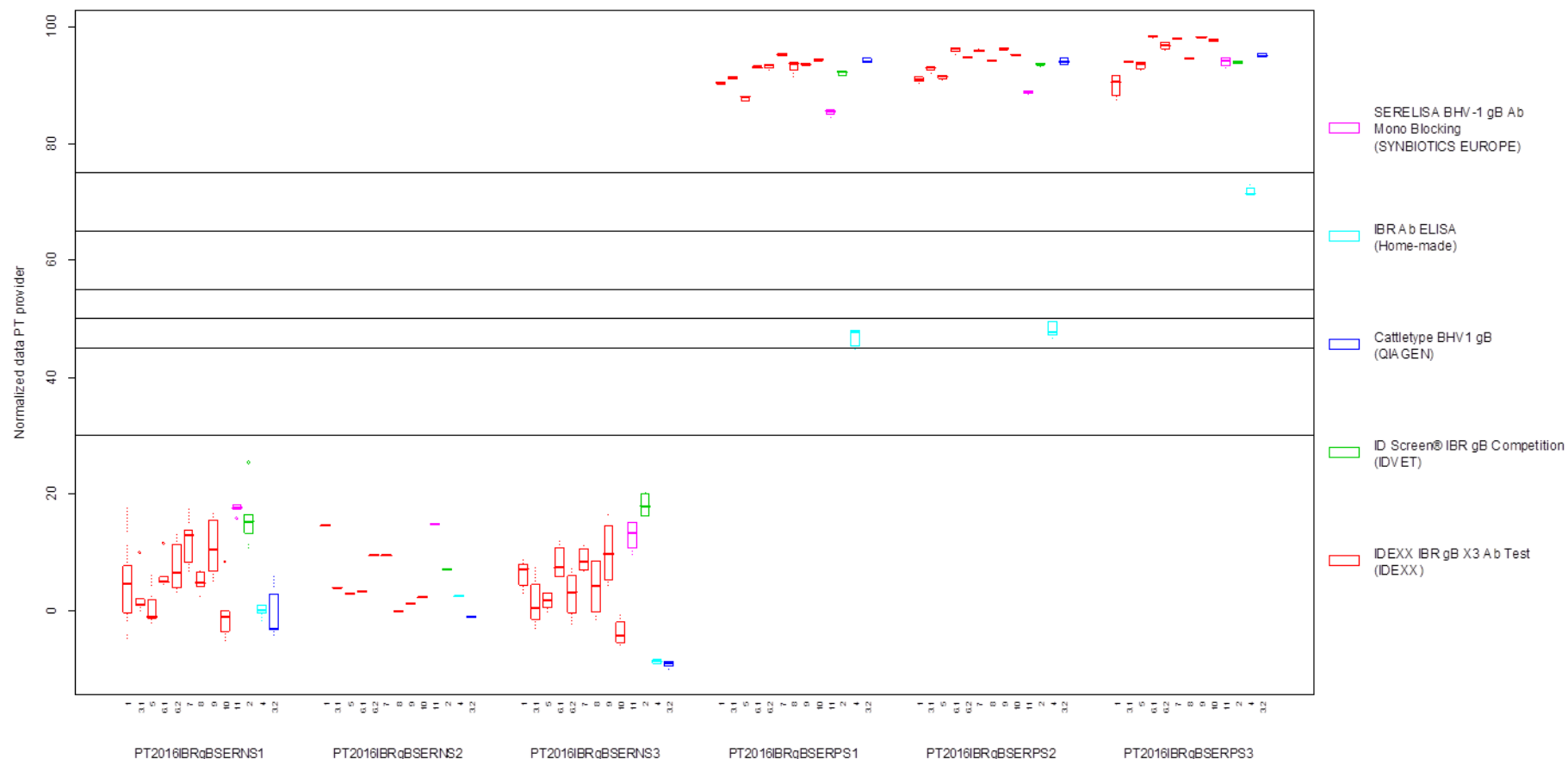
Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R. All quantitative data analyses, except for LAB12, were performed on normalized data, namely the percentages blocking calculated according to the instructions of the PT provider:  $[1 - \text{OD sample} / \text{mean (OD negative kit controls)}] * 100$ . For LAB12, the quantitative data analyse was performed on normalized data according to the laboratory instructions.

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.

Box plots of the normalized data according to the instructions of the PT provider per reference serum samples and per participating laboratory were made using the statistical software R. The box plots for the laboratories participating in the PT IBRgB and PT IBRgE are shown in Figure 1 and Figure 2, respectively.

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than  $(P25 - 1.5(P75 - P25))$  and higher than  $(P75 + 1.5(P75 - P25))$  are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

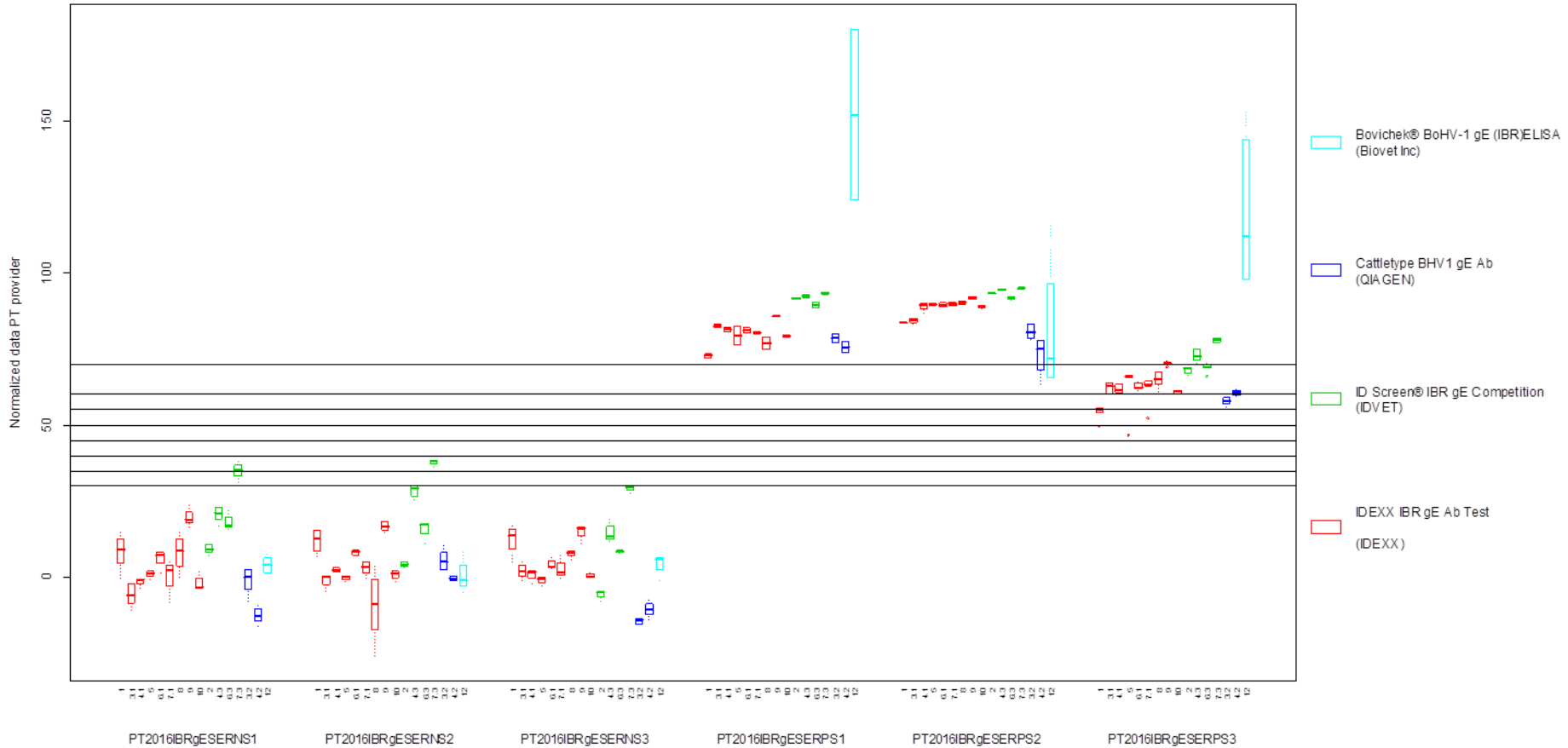


**PT2016IBRgBSER**


**Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory.** LAB4 used an in-house developed IBRgB antibody ELISA kit (Home Made ELISA (batch: 28/9/16)), whereas the other participants used IBRgB antibody ELISA kits from 4 different commercial kit producers: IDEXX (5 batches: F221 (LAB9), G281 (LAB5), G651 (LAB3.1), G671(LAB6.1, LAB6.2, LAB7, LAB8, LAB10) , G701 (LAB1)), IDVET (1 batch: 932 (LAB2)), Synbiotics Europe (1 batch: 16ZEA003 (LAB11)) and QIAGEN (1 batch: 254111122 (LAB3.2)). Hereby LAB1, LAB3.1, LAB6.2, LAB7, LAB8 and LAB10 performed the long incubation protocol and LAB5, LAB6.1 and LAB9 the short incubation protocol.

Cut-off values for the IDEXX IBR gB X3 Ab Test from IDEXX (45-55%, 65-75%), the ID Screen® IBR gB Competition from IDVET (45-55%), the SERELISA BHV-1 gB Ab Mono Blocking from Synbiotics Europe (50-55%), the Cattletype BHV1 gB from QIAGEN (45-55%) and the IBR Ab ELISA Home made (30-50%) are shown by horizontal lines.

**PT2016IBRgESER**



**Figure 2. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory.** The IBRgE participating laboratories used ELISA kits from 4 different producers : IDEXX (4 batches: KL403 (LAB1, LAB3.1, LAB10), ML601 (LAB4.1, LAB5, LAB9), CM965 (LAB6.1, LAB7.1), FM298 (LAB8)), QIAGEN (2 batches: 254130904 (LAB3.2), 254110207 (LAB4.2)), IDVET (2 batches: A70 (LAB2, LAB6.3), 934 (LAB4.3, LAB7.3)) and Biovet Inc (1 batch : 756110 (LAB12)).

Cut-off values for the IDEXX IBR gE Ab Test from IDEXX (30-40%, 60-70%), the Cattletype BHV1 gE Ab from QIAGEN (35-45%, 45-55%), the ID Screen® IBR gE Competition from IDVET (40-50%, 60-70%), and Bovichek® BoHV-1 gE (IBR)ELISA from Biovet Inc (30-50%) are shown by horizontal lines.