

PROFICIENCY TESTING 2018

***INFECTIOUS BOVINE RHINOTRACHEITIS (IBR)
DETECTION OF IBRGB- AND IBRGE-SPECIFIC ANTIBODIES IN SERUM BY
ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)***

**SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO**

DATE BEGIN PT: 3 SEPTEMBER 2018

DATE REPORT: 3 JANUARI 2019

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 'PT2018IBRgBSERNS1' and 'PT2018IBRgBSERNS2') or containing detectable IBRgB-specific antibodies ($n = 4$; coded 'PT2018IBRgBSERPS1', 'PT2018IBRgBSERPS2', 'PT2018IBRgBSERPS3' and 'PT2018IBRgBSERPS4'), were used. In total, 240 aliquots were distributed to 12 participating laboratories. All participants received 20 aliquots: 1 aliquot of the reference serum sample PT2018IBRgBSERPS1, 2 aliquots of the reference serum sample PT2018IBRgBSERPS4, 3 aliquots of the reference serum samples PT2018IBRgBSERPS3, 4 aliquots of the reference serum samples PT2018IBRgBSERPS2 and 5 aliquots of the reference serum samples PT2018IBRgBSERNS1 and PT2018IBRgBSERNS2. 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 IDEXX and a seroneutralisation assay (SN).

The reference serum samples PT2018IBRgBSERNS1 and PT2018IBRgBSERNS2 were obtained from two naïve animals from a Belgian I4-certified farm (IBR-free without vaccination). The reference serum sample PT2018IBRgBSERPS1 was collected at 9 days after experimental infection of one non-vaccinated animal. The reference serum samples PT2018IBRgBSERPS2 and PT2018IBRgBSERPS3 were obtained from two vaccinated but uninfected animals. The reference serum sample PT2018IBRgBSERPS4 was a 1/128 dilution of a sera obtained from a field infected animal. Taken together, the reference serum samples PT2018IBRgBSERNS1 and PT2018IBRgBSERNS2 were considered as negative sera, the reference serum samples PT2018IBRgBSERPS2 and PT2018IBRgBSERPS3 as (strong) positive sera and PT2018IBRgBSERPS1 and PT2018IBRgBSERPS4 as (weak) positive sera in IBRgB ELISA.

On previously years a homogeneity check was performed on 10 aliquots of each reference serum sample after aliquoting the different reference serum samples, using the HerdChek IBRgB and indirect antibody ELISA tests from IDEXX and a seroneutralisation assay (SN), hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Therefore only 3 aliquots of each reference serum sample were tested before the PT to confirm their stability and status (pre-verification) using the HerdChek IBRgB antibody ELISA test from IDEXX.

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, 1 aliquot

of each reference serum sample was 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 = 4; coded 'PT2018IBRgESERNS1', 'PT2018IBRgESERNS2', 'PT2018IBRgESERNS3' and 'PT2018IBRgESERNS4') or containing detectable IBRgE-specific antibodies (n=2, coded 'PT2018IBRgESERPS1' and 'PT2018IBRgESERPS2'), were used. In total, 200 aliquots were distributed to 10 different participating laboratories. All participants received 20 aliquots: 1 aliquot of the reference serum sample PT2018IBRgESERNS1, 3 aliquots of the reference serum samples PT2018IBRgESERNS2 and PT2018IBRgESERNS4, 4 aliquots of the reference serum sample PT2018IBRgESERPS1 and PT2018IBRgESERNS3 and 5 aliquots of the reference serum samples PT2018IBRgESERPS2. 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 PT2018IBRgESERNS1 and PT2018IBRgESERNS2 were the undiluted sera from two vaccinated but uninfected animals. The reference serum samples PT2018IBRgESERNS3 and PT2018IBRgESERNS4 were the undiluted sera from a naïve animal from a Belgian I4-certified farm (IBR-free without vaccination).

The reference serum sample PT2018IBRgESERPS1 was obtained 9 days after experimental infection of a non-vaccinated animal. The reference serum sample PT2018IBRgESERPS3 was a 1/8 dilution of a sera obtained from a field infected animal. For each reference serum sample, except for the reference serum sample PT2018IBRgESERPS1 (see below), the same qualitative result was obtained with all test methods used.

Taken together, the reference serum samples PT2018IBRgESERNS1, PT2018IBRgESERNS2, PT2018IBRgESERNS3 and PT2018IBRgESERNS4 were considered as negative sera, the reference serum sample PT2018IBRgESERPS1 as negative to (weak) positive sera and the reference serum sample PT2018IBRgESERPS2 as strong positive sera in IBRgE ELISA.

On previously years a homogeneity check was performed on 10 aliquots of each reference serum sample after aliquoting the different reference serum samples, 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. Therefore only 3 aliquots of each reference serum sample were tested before the PT to confirm their stability and status (pre-verification) using the HerdChek IBRgE antibody ELISA test from IDEXX. For reference serum sample PT2018IBRgESERPS1, the conservation has slightly altered the quality of the sample. On the annual testing, 2 positive and 1 doubtful qualitative results were obtained in the 3 repeats with the HerdChek IBRgE antibody ELISA test from IDEXX. This sample was collected at 9 days post infection and presented low gE antibody titers, the results accepted for this sample are positive, non-interpretable/doubtful (NI/DBS) or negative.

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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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 and LAB12 only participated in the PT IBRgB and 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 3th of September 2018. LAB1, LAB5, LAB6, LAB7 and LAB9 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 4th (LAB2, LAB3, LAB10, LAB11 and LAB12) and 5th (LAB4 and LAB8) of September 2018. LAB2 reported that he received on 4th of September 2018 only the IBRgE reference serum samples. The IBRgB reference serum samples were sent to him on 17th of September 2018 and received on 18th of September 2018.

Analyses were performed between 3th and 20th of September 2018 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 7th of September 2018 and 2nd of October 2018 (Table 1). All participants, except LAB2, LAB5 and LAB9 respected the deadline of 21st of September 2018 for submission of the results. LAB2 received more time to carry out the analyzes since the IBRgB reference serum samples only arrived on 18th of September 2018.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano.

Participating laboratory	Reference samples received	Start of analysis gB	Start of analysis gE	Submission of the results (Excel file)
LAB1	03/09/2018	05/09/2018 Long 06/09/2018 Short	06/09/2018	19/09/2018
LAB2	04/09/2018 gE samples 18/09/2018 gB samples	19/09/2018	20/09/2018	24/09/2018
LAB3	04/09/2018	12-13/09/2018	12-13/09/2018	18/09/2018
LAB4	05/09/2018	13/09/2018	12/09/2018 IDVET, IDEXX, QIAGEN	21/09/2018
LAB5	03/09/2018	13/09/2018	13/09/2018	26/09/2018
LAB6	03/09/2018	10/09/2018	14/09/2018	20/09/2018
LAB7	03/09/2018	07/09/2018	04/09/2018 IDVET 14/09/2018 IDEXX	18/09/2018
LAB8	05/09/2018	07/09/2018	07/09/2018	17/09/2018
LAB9	03/09/2018	03/09/2018	10/09/2018 IDEXX 13/09/2018 IDVET, QIAGEN	02/10/2018

LAB10	04/09/2018	11/09/2018	11/09/18	19/09/2018
LAB11	04/09/2018	11/09/2018	NA	13/09/2018
LAB12	04/09/2018	06/09/2018	NA	07/09/2018

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

LAB1 submitted 2 sets of results for the PT IBRgB whereas LAB7 submitted 2 sets and LAB4 and LAB9 submitted 3 sets of results for the 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 : LAB1 into LAB 1.1 (Producer 1 : Protocole 1) and LAB 1.2 (Producer 1 : Protocole 2);

For PT IBRgE : LAB7 into LAB7.1 (Producer 2) and LAB7.2 (Producer 1), LAB4 into LAB4.1 (Producer 1), LAB4.2 (Producer 2) and LAB4.3 (Producer3) and LAB9 into LAB9.1 (Producer 1), LAB9.2 (Producer 2) and LAB9.3 (Producer3). 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 participating laboratories except LAB2 and LAB4 provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB2 misclassified 1 aliquot (95% of agreement) and LAB4 misclassified 3 aliquots (85% of agreement) (Table 2).
- (ii) For the detection of **IBRgE-specific antibodies**, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of IBRgB reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1.1	1.2	2	3	4	5	6
failure	0 (0)	0 (0)	1 (5)	0 (0)	3 (15)	0 (0)	0 (0)
success	20 (100)	20 (100)	19 (95)	20 (100)	17 (85)	20 (100)	20 (100)

	LABNR					
	7	8	9	10	11	12
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. 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	4.3	5	6	7.1	7.2	8	9.1	9.2	9.3	10
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)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

IV.4.2. Variability among participating laboratories

- (i) For the detection of **IBRgB-specific antibodies**, no variability between LAB1.1, LAB1.2, LAB3, LAB5, LAB6, LAB7, LAB8, LAB9, LAB10, LAB11 and LAB12 could be observed since these participants correctly identified all reference serum samples. LAB1 obtained identical qualitative results using an ELISA kit from the same producer on 2 different protocols. In contrast, LAB2 misclassified 1 out of 5 aliquots of the reference serum sample PT2018IBRgBSERNS2 (POS instead of NEG) and LAB4 misclassified the aliquot of the reference serum sample PT2018IBRgBSERPS1 (NEG instead of POS) and the 2 aliquots of the reference serum sample PT2018IBRgBSERPS4 (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. LAB7 obtained identical qualitative results using 2 ELISA kits from 2 different producers and LAB4 and LAB9 using 3 ELISA kits from 3 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 internal identification of the **IBRgB** reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the IBR reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1.1	1	PT2018IBRgBSERPS3	POS	POS	1
2	1.1	2	PT2018IBRgBSERNS1	NEG	NEG	1
3	1.1	3	PT2018IBRgBSERPS2	POS	POS	1
4	1.1	4	PT2018IBRgBSERPS4	POS	POS	1
5	1.1	5	PT2018IBRgBSERNS2	NEG	NEG	1
6	1.1	6	PT2018IBRgBSERPS1	POS	POS	1
7	1.1	7	PT2018IBRgBSERPS4	POS	POS	1
8	1.1	8	PT2018IBRgBSERNS1	NEG	NEG	1
9	1.1	9	PT2018IBRgBSERPS3	POS	POS	1
10	1.1	10	PT2018IBRgBSERNS2	NEG	NEG	1
11	1.1	11	PT2018IBRgBSERNS2	NEG	NEG	1
12	1.1	12	PT2018IBRgBSERPS2	POS	POS	1
13	1.1	13	PT2018IBRgBSERNS2	NEG	NEG	1
14	1.1	14	PT2018IBRgBSERNS1	NEG	NEG	1
15	1.1	15	PT2018IBRgBSERPS2	POS	POS	1
16	1.1	16	PT2018IBRgBSERNS1	NEG	NEG	1
17	1.1	17	PT2018IBRgBSERNS2	NEG	NEG	1
18	1.1	18	PT2018IBRgBSERPS3	POS	POS	1
19	1.1	19	PT2018IBRgBSERNS1	NEG	NEG	1
20	1.1	20	PT2018IBRgBSERPS2	POS	POS	1
21	1.2	1	PT2018IBRgBSERPS3	POS	POS	1
22	1.2	2	PT2018IBRgBSERNS1	NEG	NEG	1
23	1.2	3	PT2018IBRgBSERPS2	POS	POS	1
24	1.2	4	PT2018IBRgBSERPS4	POS	POS	1
25	1.2	5	PT2018IBRgBSERNS2	NEG	NEG	1
26	1.2	6	PT2018IBRgBSERPS1	POS	POS	1
27	1.2	7	PT2018IBRgBSERPS4	POS	POS	1
28	1.2	8	PT2018IBRgBSERNS1	NEG	NEG	1
29	1.2	9	PT2018IBRgBSERPS3	POS	POS	1
30	1.2	10	PT2018IBRgBSERNS2	NEG	NEG	1
31	1.2	11	PT2018IBRgBSERNS2	NEG	NEG	1
32	1.2	12	PT2018IBRgBSERPS2	POS	POS	1
33	1.2	13	PT2018IBRgBSERNS2	NEG	NEG	1
34	1.2	14	PT2018IBRgBSERNS1	NEG	NEG	1
35	1.2	15	PT2018IBRgBSERPS2	POS	POS	1
36	1.2	16	PT2018IBRgBSERNS1	NEG	NEG	1
37	1.2	17	PT2018IBRgBSERNS2	NEG	NEG	1
38	1.2	18	PT2018IBRgBSERPS3	POS	POS	1
39	1.2	19	PT2018IBRgBSERNS1	NEG	NEG	1
40	1.2	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	2	1	PT2018IBRgBSERPS2	POS	POS	1
42	2	2	PT2018IBRgBSERPS3	POS	POS	1
43	2	3	PT2018IBRgBSERNS1	NEG	NEG	1
44	2	4	PT2018IBRgBSERPS1	POS	POS	1
45	2	5	PT2018IBRgBSERNS1	NEG	NEG	1
46	2	6	PT2018IBRgBSERNS2	NEG	NEG	1
47	2	7	PT2018IBRgBSERPS3	POS	POS	1
48	2	8	PT2018IBRgBSERNS2	NEG	POS	0
49	2	9	PT2018IBRgBSERPS4	POS	POS	1
50	2	10	PT2018IBRgBSERPS2	POS	POS	1
51	2	11	PT2018IBRgBSERNS2	NEG	NEG	1
52	2	12	PT2018IBRgBSERPS3	POS	POS	1
53	2	13	PT2018IBRgBSERNS1	NEG	NEG	1
54	2	14	PT2018IBRgBSERNS2	NEG	NEG	1
55	2	15	PT2018IBRgBSERPS2	POS	POS	1
56	2	16	PT2018IBRgBSERPS4	POS	POS	1
57	2	17	PT2018IBRgBSERNS1	NEG	NEG	1
58	2	18	PT2018IBRgBSERNS2	NEG	NEG	1
59	2	19	PT2018IBRgBSERPS2	POS	POS	1
60	2	20	PT2018IBRgBSERNS1	NEG	NEG	1
61	3	1	PT2018IBRgBSERPS3	POS	POS	1
62	3	2	PT2018IBRgBSERNS1	NEG	NEG	1
63	3	3	PT2018IBRgBSERPS2	POS	POS	1
64	3	4	PT2018IBRgBSERPS4	POS	POS	1
65	3	5	PT2018IBRgBSERNS2	NEG	NEG	1
66	3	6	PT2018IBRgBSERPS1	POS	POS	1
67	3	7	PT2018IBRgBSERPS4	POS	POS	1
68	3	8	PT2018IBRgBSERNS1	NEG	NEG	1
69	3	9	PT2018IBRgBSERPS3	POS	POS	1
70	3	10	PT2018IBRgBSERNS2	NEG	NEG	1
71	3	11	PT2018IBRgBSERNS2	NEG	NEG	1
72	3	12	PT2018IBRgBSERPS2	POS	POS	1
73	3	13	PT2018IBRgBSERNS2	NEG	NEG	1
74	3	14	PT2018IBRgBSERNS1	NEG	NEG	1
75	3	15	PT2018IBRgBSERPS2	POS	POS	1
76	3	16	PT2018IBRgBSERNS1	NEG	NEG	1
77	3	17	PT2018IBRgBSERNS2	NEG	NEG	1
78	3	18	PT2018IBRgBSERPS3	POS	POS	1
79	3	19	PT2018IBRgBSERNS1	NEG	NEG	1
80	3	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4	1	PT2018IBRgBSERPS2	POS	POS	1
82	4	2	PT2018IBRgBSERPS3	POS	POS	1
83	4	3	PT2018IBRgBSERNS1	NEG	NEG	1
84	4	4	PT2018IBRgBSERPS1	POS	NEG	0
85	4	5	PT2018IBRgBSERNS1	NEG	NEG	1
86	4	6	PT2018IBRgBSERNS2	NEG	NEG	1
87	4	7	PT2018IBRgBSERPS3	POS	POS	1
88	4	8	PT2018IBRgBSERNS2	NEG	NEG	1
89	4	9	PT2018IBRgBSERPS4	POS	NEG	0
90	4	10	PT2018IBRgBSERPS2	POS	POS	1
91	4	11	PT2018IBRgBSERNS2	NEG	NEG	1
92	4	12	PT2018IBRgBSERPS3	POS	POS	1
93	4	13	PT2018IBRgBSERNS1	NEG	NEG	1
94	4	14	PT2018IBRgBSERNS2	NEG	NEG	1
95	4	15	PT2018IBRgBSERPS2	POS	POS	1
96	4	16	PT2018IBRgBSERPS4	POS	NEG	0
97	4	17	PT2018IBRgBSERNS1	NEG	NEG	1
98	4	18	PT2018IBRgBSERNS2	NEG	NEG	1
99	4	19	PT2018IBRgBSERPS2	POS	POS	1
100	4	20	PT2018IBRgBSERNS1	NEG	NEG	1
101	5	1	PT2018IBRgBSERPS3	POS	POS	1
102	5	2	PT2018IBRgBSERNS1	NEG	NEG	1
103	5	3	PT2018IBRgBSERPS2	POS	POS	1
104	5	4	PT2018IBRgBSERPS4	POS	POS	1
105	5	5	PT2018IBRgBSERNS2	NEG	NEG	1
106	5	6	PT2018IBRgBSERPS1	POS	POS	1
107	5	7	PT2018IBRgBSERPS4	POS	POS	1
108	5	8	PT2018IBRgBSERNS1	NEG	NEG	1
109	5	9	PT2018IBRgBSERPS3	POS	POS	1
110	5	10	PT2018IBRgBSERNS2	NEG	NEG	1
111	5	11	PT2018IBRgBSERNS2	NEG	NEG	1
112	5	12	PT2018IBRgBSERPS2	POS	POS	1
113	5	13	PT2018IBRgBSERNS2	NEG	NEG	1
114	5	14	PT2018IBRgBSERNS1	NEG	NEG	1
115	5	15	PT2018IBRgBSERPS2	POS	POS	1
116	5	16	PT2018IBRgBSERNS1	NEG	NEG	1
117	5	17	PT2018IBRgBSERNS2	NEG	NEG	1
118	5	18	PT2018IBRgBSERPS3	POS	POS	1
119	5	19	PT2018IBRgBSERNS1	NEG	NEG	1
120	5	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	6	1	PT2018IBRgBSERPS2	POS	POS	1
122	6	2	PT2018IBRgBSERPS3	POS	POS	1
123	6	3	PT2018IBRgBSERNS1	NEG	NEG	1
124	6	4	PT2018IBRgBSERPS1	POS	POS	1
125	6	5	PT2018IBRgBSERNS1	NEG	NEG	1
126	6	6	PT2018IBRgBSERNS2	NEG	NEG	1
127	6	7	PT2018IBRgBSERPS3	POS	POS	1
128	6	8	PT2018IBRgBSERNS2	NEG	NEG	1
129	6	9	PT2018IBRgBSERPS4	POS	POS	1
130	6	10	PT2018IBRgBSERPS2	POS	POS	1
131	6	11	PT2018IBRgBSERNS2	NEG	NEG	1
132	6	12	PT2018IBRgBSERPS3	POS	POS	1
133	6	13	PT2018IBRgBSERNS1	NEG	NEG	1
134	6	14	PT2018IBRgBSERNS2	NEG	NEG	1
135	6	15	PT2018IBRgBSERPS2	POS	POS	1
136	6	16	PT2018IBRgBSERPS4	POS	POS	1
137	6	17	PT2018IBRgBSERNS1	NEG	NEG	1
138	6	18	PT2018IBRgBSERNS2	NEG	NEG	1
139	6	19	PT2018IBRgBSERPS2	POS	POS	1
140	6	20	PT2018IBRgBSERNS1	NEG	NEG	1
141	7	1	PT2018IBRgBSERPS3	POS	POS	1
142	7	2	PT2018IBRgBSERNS1	NEG	NEG	1
143	7	3	PT2018IBRgBSERPS2	POS	POS	1
144	7	4	PT2018IBRgBSERPS4	POS	POS	1
145	7	5	PT2018IBRgBSERNS2	NEG	NEG	1
146	7	6	PT2018IBRgBSERPS1	POS	POS	1
147	7	7	PT2018IBRgBSERPS4	POS	POS	1
148	7	8	PT2018IBRgBSERNS1	NEG	NEG	1
149	7	9	PT2018IBRgBSERPS3	POS	POS	1
150	7	10	PT2018IBRgBSERNS2	NEG	NEG	1
151	7	11	PT2018IBRgBSERNS2	NEG	NEG	1
152	7	12	PT2018IBRgBSERPS2	POS	POS	1
153	7	13	PT2018IBRgBSERNS2	NEG	NEG	1
154	7	14	PT2018IBRgBSERNS1	NEG	NEG	1
155	7	15	PT2018IBRgBSERPS2	POS	POS	1
156	7	16	PT2018IBRgBSERNS1	NEG	NEG	1
157	7	17	PT2018IBRgBSERNS2	NEG	NEG	1
158	7	18	PT2018IBRgBSERPS3	POS	POS	1
159	7	19	PT2018IBRgBSERNS1	NEG	NEG	1
160	7	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	8	1	PT2018IBRgBSERPS2	POS	POS	1
162	8	2	PT2018IBRgBSERPS3	POS	POS	1
163	8	3	PT2018IBRgBSERNS1	NEG	NEG	1
164	8	4	PT2018IBRgBSERPS1	POS	POS	1
165	8	5	PT2018IBRgBSERNS1	NEG	NEG	1
166	8	6	PT2018IBRgBSERNS2	NEG	NEG	1
167	8	7	PT2018IBRgBSERPS3	POS	POS	1
168	8	8	PT2018IBRgBSERNS2	NEG	NEG	1
169	8	9	PT2018IBRgBSERPS4	POS	POS	1
170	8	10	PT2018IBRgBSERPS2	POS	POS	1
171	8	11	PT2018IBRgBSERNS2	NEG	NEG	1
172	8	12	PT2018IBRgBSERPS3	POS	POS	1
173	8	13	PT2018IBRgBSERNS1	NEG	NEG	1
174	8	14	PT2018IBRgBSERNS2	NEG	NEG	1
175	8	15	PT2018IBRgBSERPS2	POS	POS	1
176	8	16	PT2018IBRgBSERPS4	POS	POS	1
177	8	17	PT2018IBRgBSERNS1	NEG	NEG	1
178	8	18	PT2018IBRgBSERNS2	NEG	NEG	1
179	8	19	PT2018IBRgBSERPS2	POS	POS	1
180	8	20	PT2018IBRgBSERNS1	NEG	NEG	1
181	9	1	PT2018IBRgBSERPS3	POS	POS	1
182	9	2	PT2018IBRgBSERNS1	NEG	NEG	1
183	9	3	PT2018IBRgBSERPS2	POS	POS	1
184	9	4	PT2018IBRgBSERPS4	POS	POS	1
185	9	5	PT2018IBRgBSERNS2	NEG	NEG	1
186	9	6	PT2018IBRgBSERPS1	POS	POS	1
187	9	7	PT2018IBRgBSERPS4	POS	POS	1
188	9	8	PT2018IBRgBSERNS1	NEG	NEG	1
189	9	9	PT2018IBRgBSERPS3	POS	POS	1
190	9	10	PT2018IBRgBSERNS2	NEG	NEG	1
191	9	11	PT2018IBRgBSERNS2	NEG	NEG	1
192	9	12	PT2018IBRgBSERPS2	POS	POS	1
193	9	13	PT2018IBRgBSERNS2	NEG	NEG	1
194	9	14	PT2018IBRgBSERNS1	NEG	NEG	1
195	9	15	PT2018IBRgBSERPS2	POS	POS	1
196	9	16	PT2018IBRgBSERNS1	NEG	NEG	1
197	9	17	PT2018IBRgBSERNS2	NEG	NEG	1
198	9	18	PT2018IBRgBSERPS3	POS	POS	1
199	9	19	PT2018IBRgBSERNS1	NEG	NEG	1
200	9	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	10	1	PT2018IBRgBSERPS2	POS	POS	1
202	10	2	PT2018IBRgBSERPS3	POS	POS	1
203	10	3	PT2018IBRgBSERNS1	NEG	NEG	1
204	10	4	PT2018IBRgBSERPS1	POS	POS	1
205	10	5	PT2018IBRgBSERNS1	NEG	NEG	1
206	10	6	PT2018IBRgBSERNS2	NEG	NEG	1
207	10	7	PT2018IBRgBSERPS3	POS	POS	1
208	10	8	PT2018IBRgBSERNS2	NEG	NEG	1
209	10	9	PT2018IBRgBSERPS4	POS	POS	1
210	10	10	PT2018IBRgBSERPS2	POS	POS	1
211	10	11	PT2018IBRgBSERNS2	NEG	NEG	1
212	10	12	PT2018IBRgBSERPS3	POS	POS	1
213	10	13	PT2018IBRgBSERNS1	NEG	NEG	1
214	10	14	PT2018IBRgBSERNS2	NEG	NEG	1
215	10	15	PT2018IBRgBSERPS2	POS	POS	1
216	10	16	PT2018IBRgBSERPS4	POS	POS	1
217	10	17	PT2018IBRgBSERNS1	NEG	NEG	1
218	10	18	PT2018IBRgBSERNS2	NEG	NEG	1
219	10	19	PT2018IBRgBSERPS2	POS	POS	1
220	10	20	PT2018IBRgBSERNS1	NEG	NEG	1
221	11	1	PT2018IBRgBSERPS3	POS	POS	1
222	11	2	PT2018IBRgBSERNS1	NEG	NEG	1
223	11	3	PT2018IBRgBSERPS2	POS	POS	1
224	11	4	PT2018IBRgBSERPS4	POS	POS	1
225	11	5	PT2018IBRgBSERNS2	NEG	NEG	1
226	11	6	PT2018IBRgBSERPS1	POS	POS	1
227	11	7	PT2018IBRgBSERPS4	POS	POS	1
228	11	8	PT2018IBRgBSERNS1	NEG	NEG	1
229	11	9	PT2018IBRgBSERPS3	POS	POS	1
230	11	10	PT2018IBRgBSERNS2	NEG	NEG	1
231	11	11	PT2018IBRgBSERNS2	NEG	NEG	1
232	11	12	PT2018IBRgBSERPS2	POS	POS	1
233	11	13	PT2018IBRgBSERNS2	NEG	NEG	1
234	11	14	PT2018IBRgBSERNS1	NEG	NEG	1
235	11	15	PT2018IBRgBSERPS2	POS	POS	1
236	11	16	PT2018IBRgBSERNS1	NEG	NEG	1
237	11	17	PT2018IBRgBSERNS2	NEG	NEG	1
238	11	18	PT2018IBRgBSERPS3	POS	POS	1
239	11	19	PT2018IBRgBSERNS1	NEG	NEG	1
240	11	20	PT2018IBRgBSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
241	12	1	PT2018IBRgBSERPS2	POS	POS	1
242	12	2	PT2018IBRgBSERPS3	POS	POS	1
243	12	3	PT2018IBRgBSERNS1	NEG	NEG	1
244	12	4	PT2018IBRgBSERPS1	POS	POS	1
245	12	5	PT2018IBRgBSERNS1	NEG	NEG	1
246	12	6	PT2018IBRgBSERNS2	NEG	NEG	1
247	12	7	PT2018IBRgBSERPS3	POS	POS	1
248	12	8	PT2018IBRgBSERNS2	NEG	NEG	1
249	12	9	PT2018IBRgBSERPS4	POS	POS	1
250	12	10	PT2018IBRgBSERPS2	POS	POS	1
251	12	11	PT2018IBRgBSERNS2	NEG	NEG	1
252	12	12	PT2018IBRgBSERPS3	POS	POS	1
253	12	13	PT2018IBRgBSERNS1	NEG	NEG	1
254	12	14	PT2018IBRgBSERNS2	NEG	NEG	1
255	12	15	PT2018IBRgBSERPS2	POS	POS	1
256	12	16	PT2018IBRgBSERPS4	POS	POS	1
257	12	17	PT2018IBRgBSERNS1	NEG	NEG	1
258	12	18	PT2018IBRgBSERNS2	NEG	NEG	1
259	12	19	PT2018IBRgBSERPS2	POS	POS	1
260	12	20	PT2018IBRgBSERNS1	NEG	NEG	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the **IBRgE** reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the IBR reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive, DBS : doubtful; NI (non-interpretable).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2018IBRgESERNS2	NEG	NEG	1
2	1	2	PT2018IBRgESERPS1	DBS	POS	1
3	1	3	PT2018IBRgESERNS1	NEG	NEG	1
4	1	4	PT2018IBRgESERNS3	NEG	NEG	1
5	1	5	PT2018IBRgESERPS2	POS	POS	1
6	1	6	PT2018IBRgESERPS2	POS	POS	1
7	1	7	PT2018IBRgESERNS2	NEG	NEG	1
8	1	8	PT2018IBRgESERPS1	DBS	POS	1
9	1	9	PT2018IBRgESERNS2	NEG	NEG	1
10	1	10	PT2018IBRgESERPS2	POS	POS	1
11	1	11	PT2018IBRgESERNS4	NEG	NEG	1
12	1	12	PT2018IBRgESERPS1	DBS	POS	1
13	1	13	PT2018IBRgESERNS4	NEG	NEG	1
14	1	14	PT2018IBRgESERPS2	POS	POS	1
15	1	15	PT2018IBRgESERNS3	NEG	NEG	1
16	1	16	PT2018IBRgESERNS4	NEG	NEG	1
17	1	17	PT2018IBRgESERPS1	DBS	POS	1
18	1	18	PT2018IBRgESERNS3	NEG	NEG	1
19	1	19	PT2018IBRgESERPS2	POS	POS	1
20	1	20	PT2018IBRgESERNS3	NEG	NEG	1
21	2	1	PT2018IBRgESERPS2	POS	POS	1
22	2	2	PT2018IBRgESERNS2	NEG	NEG	1
23	2	3	PT2018IBRgESERPS1	DBS	NEG	1
24	2	4	PT2018IBRgESERNS1	NEG	NEG	1
25	2	5	PT2018IBRgESERNS2	NEG	NEG	1
26	2	6	PT2018IBRgESERNS3	NEG	NEG	1
27	2	7	PT2018IBRgESERPS1	DBS	NEG	1
28	2	8	PT2018IBRgESERNS3	NEG	NEG	1
29	2	9	PT2018IBRgESERNS4	NEG	NEG	1
30	2	10	PT2018IBRgESERPS2	POS	POS	1
31	2	11	PT2018IBRgESERNS3	NEG	NEG	1
32	2	12	PT2018IBRgESERPS2	POS	POS	1
33	2	13	PT2018IBRgESERNS2	NEG	NEG	1
34	2	14	PT2018IBRgESERPS1	DBS	NEG	1
35	2	15	PT2018IBRgESERPS2	POS	POS	1
36	2	16	PT2018IBRgESERNS3	NEG	NEG	1
37	2	17	PT2018IBRgESERNS4	NEG	NEG	1
38	2	18	PT2018IBRgESERPS1	DBS	NEG	1
39	2	19	PT2018IBRgESERPS2	POS	POS	1
40	2	20	PT2018IBRgESERNS4	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018IBRgESERNS2	NEG	NEG	1
42	3	2	PT2018IBRgESERPS1	DBS	NEG	1
43	3	3	PT2018IBRgESERNS1	NEG	NEG	1
44	3	4	PT2018IBRgESERNS3	NEG	NEG	1
45	3	5	PT2018IBRgESERPS2	POS	POS	1
46	3	6	PT2018IBRgESERPS2	POS	POS	1
47	3	7	PT2018IBRgESERNS2	NEG	NEG	1
48	3	8	PT2018IBRgESERPS1	DBS	NEG	1
49	3	9	PT2018IBRgESERNS2	NEG	NEG	1
50	3	10	PT2018IBRgESERPS2	POS	POS	1
51	3	11	PT2018IBRgESERNS4	NEG	NEG	1
52	3	12	PT2018IBRgESERPS1	DBS	NEG	1
53	3	13	PT2018IBRgESERNS4	NEG	NEG	1
54	3	14	PT2018IBRgESERPS2	POS	POS	1
55	3	15	PT2018IBRgESERNS3	NEG	NEG	1
56	3	16	PT2018IBRgESERNS4	NEG	NEG	1
57	3	17	PT2018IBRgESERPS1	DBS	NEG	1
58	3	18	PT2018IBRgESERNS3	NEG	NEG	1
59	3	19	PT2018IBRgESERPS2	POS	POS	1
60	3	20	PT2018IBRgESERNS3	NEG	NEG	1
61	4.1	1	PT2018IBRgESERPS2	POS	POS	1
62	4.1	2	PT2018IBRgESERNS2	NEG	NEG	1
63	4.1	3	PT2018IBRgESERPS1	DBS	NI	1
64	4.1	4	PT2018IBRgESERNS1	NEG	NEG	1
65	4.1	5	PT2018IBRgESERNS2	NEG	NEG	1
66	4.1	6	PT2018IBRgESERNS3	NEG	NEG	1
67	4.1	7	PT2018IBRgESERPS1	DBS	NI	1
68	4.1	8	PT2018IBRgESERNS3	NEG	NEG	1
69	4.1	9	PT2018IBRgESERNS4	NEG	NEG	1
70	4.1	10	PT2018IBRgESERPS2	POS	POS	1
71	4.1	11	PT2018IBRgESERNS3	NEG	NEG	1
72	4.1	12	PT2018IBRgESERPS2	POS	POS	1
73	4.1	13	PT2018IBRgESERNS2	NEG	NEG	1
74	4.1	14	PT2018IBRgESERPS1	DBS	NI	1
75	4.1	15	PT2018IBRgESERPS2	POS	POS	1
76	4.1	16	PT2018IBRgESERNS3	NEG	NEG	1
77	4.1	17	PT2018IBRgESERNS4	NEG	NEG	1
78	4.1	18	PT2018IBRgESERPS1	DBS	NI	1
79	4.1	19	PT2018IBRgESERPS2	POS	POS	1
80	4.1	20	PT2018IBRgESERNS4	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4.2	1	PT2018IBRgESERPS2	POS	POS	1
82	4.2	2	PT2018IBRgESERNS2	NEG	NEG	1
83	4.2	3	PT2018IBRgESERPS1	DBS	NEG	1
84	4.2	4	PT2018IBRgESERNS1	NEG	NEG	1
85	4.2	5	PT2018IBRgESERNS2	NEG	NEG	1
86	4.2	6	PT2018IBRgESERNS3	NEG	NEG	1
87	4.2	7	PT2018IBRgESERPS1	DBS	NEG	1
88	4.2	8	PT2018IBRgESERNS3	NEG	NEG	1
89	4.2	9	PT2018IBRgESERNS4	NEG	NEG	1
90	4.2	10	PT2018IBRgESERPS2	POS	POS	1
91	4.2	11	PT2018IBRgESERNS3	NEG	NEG	1
92	4.2	12	PT2018IBRgESERPS2	POS	POS	1
93	4.2	13	PT2018IBRgESERNS2	NEG	NEG	1
94	4.2	14	PT2018IBRgESERPS1	DBS	NEG	1
95	4.2	15	PT2018IBRgESERPS2	POS	POS	1
96	4.2	16	PT2018IBRgESERNS3	NEG	NEG	1
97	4.2	17	PT2018IBRgESERNS4	NEG	NEG	1
98	4.2	18	PT2018IBRgESERPS1	DBS	NEG	1
99	4.2	19	PT2018IBRgESERPS2	POS	POS	1
100	4.2	20	PT2018IBRgESERNS4	NEG	NEG	1
101	4.3	1	PT2018IBRgESERPS2	POS	POS	1
102	4.3	2	PT2018IBRgESERNS2	NEG	NEG	1
103	4.3	3	PT2018IBRgESERPS1	DBS	NEG	1
104	4.3	4	PT2018IBRgESERNS1	NEG	NEG	1
105	4.3	5	PT2018IBRgESERNS2	NEG	NEG	1
106	4.3	6	PT2018IBRgESERNS3	NEG	NEG	1
107	4.3	7	PT2018IBRgESERPS1	DBS	NEG	1
108	4.3	8	PT2018IBRgESERNS3	NEG	NEG	1
109	4.3	9	PT2018IBRgESERNS4	NEG	NEG	1
110	4.3	10	PT2018IBRgESERPS2	POS	POS	1
111	4.3	11	PT2018IBRgESERNS3	NEG	NEG	1
112	4.3	12	PT2018IBRgESERPS2	POS	POS	1
113	4.3	13	PT2018IBRgESERNS2	NEG	NEG	1
114	4.3	14	PT2018IBRgESERPS1	DBS	NEG	1
115	4.3	15	PT2018IBRgESERPS2	POS	POS	1
116	4.3	16	PT2018IBRgESERNS3	NEG	NEG	1
117	4.3	17	PT2018IBRgESERNS4	NEG	NEG	1
118	4.3	18	PT2018IBRgESERPS1	DBS	NEG	1
119	4.3	19	PT2018IBRgESERPS2	POS	POS	1
120	4.3	20	PT2018IBRgESERNS4	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	5	1	PT2018IBRgESERNS2	NEG	NEG	1
122	5	2	PT2018IBRgESERPS1	DBS	POS	1
123	5	3	PT2018IBRgESERNS1	NEG	NEG	1
124	5	4	PT2018IBRgESERNS3	NEG	NEG	1
125	5	5	PT2018IBRgESERPS2	POS	POS	1
126	5	6	PT2018IBRgESERPS2	POS	POS	1
127	5	7	PT2018IBRgESERNS2	NEG	NEG	1
128	5	8	PT2018IBRgESERPS1	DBS	POS	1
129	5	9	PT2018IBRgESERNS2	NEG	NEG	1
130	5	10	PT2018IBRgESERPS2	POS	POS	1
131	5	11	PT2018IBRgESERNS4	NEG	NEG	1
132	5	12	PT2018IBRgESERPS1	DBS	POS	1
133	5	13	PT2018IBRgESERNS4	NEG	NEG	1
134	5	14	PT2018IBRgESERPS2	POS	POS	1
135	5	15	PT2018IBRgESERNS3	NEG	NEG	1
136	5	16	PT2018IBRgESERNS4	NEG	NEG	1
137	5	17	PT2018IBRgESERPS1	DBS	POS	1
138	5	18	PT2018IBRgESERNS3	NEG	NEG	1
139	5	19	PT2018IBRgESERPS2	POS	POS	1
140	5	20	PT2018IBRgESERNS3	NEG	NEG	1
141	6	1	PT2018IBRgESERPS2	POS	POS	1
142	6	2	PT2018IBRgESERNS2	NEG	NEG	1
143	6	3	PT2018IBRgESERPS1	DBS	POS	1
144	6	4	PT2018IBRgESERNS1	NEG	NEG	1
145	6	5	PT2018IBRgESERNS2	NEG	NEG	1
146	6	6	PT2018IBRgESERNS3	NEG	NEG	1
147	6	7	PT2018IBRgESERPS1	DBS	POS	1
148	6	8	PT2018IBRgESERNS3	NEG	NEG	1
149	6	9	PT2018IBRgESERNS4	NEG	NEG	1
150	6	10	PT2018IBRgESERPS2	POS	POS	1
151	6	11	PT2018IBRgESERNS3	NEG	NEG	1
152	6	12	PT2018IBRgESERPS2	POS	POS	1
153	6	13	PT2018IBRgESERNS2	NEG	NEG	1
154	6	14	PT2018IBRgESERPS1	DBS	POS	1
155	6	15	PT2018IBRgESERPS2	POS	POS	1
156	6	16	PT2018IBRgESERNS3	NEG	NEG	1
157	6	17	PT2018IBRgESERNS4	NEG	NEG	1
158	6	18	PT2018IBRgESERPS1	DBS	POS	1
159	6	19	PT2018IBRgESERPS2	POS	POS	1
160	6	20	PT2018IBRgESERNS4	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	7.1	1	PT2018IBRgESERNS2	NEG	NEG	1
162	7.1	2	PT2018IBRgESERPS1	DBS	NEG	1
163	7.1	3	PT2018IBRgESERNS1	NEG	NEG	1
164	7.1	4	PT2018IBRgESERNS3	NEG	NEG	1
165	7.1	5	PT2018IBRgESERPS2	POS	POS	1
166	7.1	6	PT2018IBRgESERPS2	POS	POS	1
167	7.1	7	PT2018IBRgESERNS2	NEG	NEG	1
168	7.1	8	PT2018IBRgESERPS1	DBS	NEG	1
169	7.1	9	PT2018IBRgESERNS2	NEG	NEG	1
170	7.1	10	PT2018IBRgESERPS2	POS	POS	1
171	7.1	11	PT2018IBRgESERNS4	NEG	NEG	1
172	7.1	12	PT2018IBRgESERPS1	DBS	NEG	1
173	7.1	13	PT2018IBRgESERNS4	NEG	NEG	1
174	7.1	14	PT2018IBRgESERPS2	POS	POS	1
175	7.1	15	PT2018IBRgESERNS3	NEG	NEG	1
176	7.1	16	PT2018IBRgESERNS4	NEG	NEG	1
177	7.1	17	PT2018IBRgESERPS1	DBS	NEG	1
178	7.1	18	PT2018IBRgESERNS3	NEG	NEG	1
179	7.1	19	PT2018IBRgESERPS2	POS	POS	1
180	7.1	20	PT2018IBRgESERNS3	NEG	NEG	1
181	7.2	1	PT2018IBRgESERNS2	NEG	NEG	1
182	7.2	2	PT2018IBRgESERPS1	DBS	POS	1
183	7.2	3	PT2018IBRgESERNS1	NEG	NEG	1
184	7.2	4	PT2018IBRgESERNS3	NEG	NEG	1
185	7.2	5	PT2018IBRgESERPS2	POS	POS	1
186	7.2	6	PT2018IBRgESERPS2	POS	POS	1
187	7.2	7	PT2018IBRgESERNS2	NEG	NEG	1
188	7.2	8	PT2018IBRgESERPS1	DBS	POS	1
189	7.2	9	PT2018IBRgESERNS2	NEG	NEG	1
190	7.2	10	PT2018IBRgESERPS2	POS	POS	1
191	7.2	11	PT2018IBRgESERNS4	NEG	NEG	1
192	7.2	12	PT2018IBRgESERPS1	DBS	POS	1
193	7.2	13	PT2018IBRgESERNS4	NEG	NEG	1
194	7.2	14	PT2018IBRgESERPS2	POS	POS	1
195	7.2	15	PT2018IBRgESERNS3	NEG	NEG	1
196	7.2	16	PT2018IBRgESERNS4	NEG	NEG	1
197	7.2	17	PT2018IBRgESERPS1	DBS	POS	1
198	7.2	18	PT2018IBRgESERNS3	NEG	NEG	1
199	7.2	19	PT2018IBRgESERPS2	POS	POS	1
200	7.2	20	PT2018IBRgESERNS3	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
201	8	1	PT2018IBRgESERPS2	POS	POS	1
202	8	2	PT2018IBRgESERNS2	NEG	NEG	1
203	8	3	PT2018IBRgESERPS1	DBS	NI	1
204	8	4	PT2018IBRgESERNS1	NEG	NEG	1
205	8	5	PT2018IBRgESERNS2	NEG	NEG	1
206	8	6	PT2018IBRgESERNS3	NEG	NEG	1
207	8	7	PT2018IBRgESERPS1	DBS	NI	1
208	8	8	PT2018IBRgESERNS3	NEG	NEG	1
209	8	9	PT2018IBRgESERNS4	NEG	NEG	1
210	8	10	PT2018IBRgESERPS2	POS	POS	1
211	8	11	PT2018IBRgESERNS3	NEG	NEG	1
212	8	12	PT2018IBRgESERPS2	POS	POS	1
213	8	13	PT2018IBRgESERNS2	NEG	NEG	1
214	8	14	PT2018IBRgESERPS1	DBS	NI	1
215	8	15	PT2018IBRgESERPS2	POS	POS	1
216	8	16	PT2018IBRgESERNS3	NEG	NEG	1
217	8	17	PT2018IBRgESERNS4	NEG	NEG	1
218	8	18	PT2018IBRgESERPS1	DBS	NI	1
219	8	19	PT2018IBRgESERPS2	POS	POS	1
220	8	20	PT2018IBRgESERNS4	NEG	NEG	1
221	9.1	1	PT2018IBRgESERNS2	NEG	NEG	1
222	9.1	2	PT2018IBRgESERPS1	DBS	NI	1
223	9.1	3	PT2018IBRgESERNS1	NEG	NEG	1
224	9.1	4	PT2018IBRgESERNS3	NEG	NEG	1
225	9.1	5	PT2018IBRgESERPS2	POS	POS	1
226	9.1	6	PT2018IBRgESERPS2	POS	POS	1
227	9.1	7	PT2018IBRgESERNS2	NEG	NEG	1
228	9.1	8	PT2018IBRgESERPS1	DBS	NI	1
229	9.1	9	PT2018IBRgESERNS2	NEG	NEG	1
230	9.1	10	PT2018IBRgESERPS2	POS	POS	1
231	9.1	11	PT2018IBRgESERNS4	NEG	NEG	1
232	9.1	12	PT2018IBRgESERPS1	DBS	NI	1
233	9.1	13	PT2018IBRgESERNS4	NEG	NEG	1
234	9.1	14	PT2018IBRgESERPS2	POS	POS	1
235	9.1	15	PT2018IBRgESERNS3	NEG	NEG	1
236	9.1	16	PT2018IBRgESERNS4	NEG	NEG	1
237	9.1	17	PT2018IBRgESERPS1	DBS	NI	1
238	9.1	18	PT2018IBRgESERNS3	NEG	NEG	1
239	9.1	19	PT2018IBRgESERPS2	POS	POS	1
240	9.1	20	PT2018IBRgESERNS4	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
241	9.2	1	PT2018IBRgESERNS2	NEG	NEG	1
242	9.2	2	PT2018IBRgESERPS1	DBS	NEG	1
243	9.2	3	PT2018IBRgESERNS1	NEG	NEG	1
244	9.2	4	PT2018IBRgESERNS3	NEG	NEG	1
245	9.2	5	PT2018IBRgESERPS2	POS	POS	1
246	9.2	6	PT2018IBRgESERPS2	POS	POS	1
247	9.2	7	PT2018IBRgESERNS2	NEG	NEG	1
248	9.2	8	PT2018IBRgESERPS1	DBS	NEG	1
249	9.2	9	PT2018IBRgESERNS2	NEG	NEG	1
250	9.2	10	PT2018IBRgESERPS2	POS	POS	1
251	9.2	11	PT2018IBRgESERNS4	NEG	NEG	1
252	9.2	12	PT2018IBRgESERPS1	DBS	NEG	1
253	9.2	13	PT2018IBRgESERNS4	NEG	NEG	1
254	9.2	14	PT2018IBRgESERPS2	POS	POS	1
255	9.2	15	PT2018IBRgESERNS3	NEG	NEG	1
256	9.2	16	PT2018IBRgESERNS4	NEG	NEG	1
257	9.2	17	PT2018IBRgESERPS1	DBS	NEG	1
258	9.2	18	PT2018IBRgESERNS3	NEG	NEG	1
259	9.2	19	PT2018IBRgESERPS2	POS	POS	1
260	9.2	20	PT2018IBRgESERNS4	NEG	NEG	1
261	9.3	1	PT2018IBRgESERNS2	NEG	NEG	1
262	9.3	2	PT2018IBRgESERPS1	DBS	NEG	1
263	9.3	3	PT2018IBRgESERNS1	NEG	NEG	1
264	9.3	4	PT2018IBRgESERNS3	NEG	NEG	1
265	9.3	5	PT2018IBRgESERPS2	POS	POS	1
266	9.3	6	PT2018IBRgESERPS2	POS	POS	1
267	9.3	7	PT2018IBRgESERNS2	NEG	NEG	1
268	9.3	8	PT2018IBRgESERPS1	DBS	NEG	1
269	9.3	9	PT2018IBRgESERNS2	NEG	NEG	1
270	9.3	10	PT2018IBRgESERPS2	POS	POS	1
271	9.3	11	PT2018IBRgESERNS4	NEG	NEG	1
272	9.3	12	PT2018IBRgESERPS1	DBS	NEG	1
273	9.3	13	PT2018IBRgESERNS4	NEG	NEG	1
274	9.3	14	PT2018IBRgESERPS2	POS	POS	1
275	9.3	15	PT2018IBRgESERNS3	NEG	NEG	1
276	9.3	16	PT2018IBRgESERNS4	NEG	NEG	1
277	9.3	17	PT2018IBRgESERPS1	DBS	NEG	1
278	9.3	18	PT2018IBRgESERNS3	NEG	NEG	1
279	9.3	19	PT2018IBRgESERPS2	POS	POS	1
280	9.3	20	PT2018IBRgESERNS3	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
281	10	1	PT2018IBRgESERPS2	POS	POS	1
282	10	2	PT2018IBRgESERNS2	NEG	NEG	1
283	10	3	PT2018IBRgESERPS1	DBS	NI	1
284	10	4	PT2018IBRgESERNS1	NEG	NEG	1
285	10	5	PT2018IBRgESERNS2	NEG	NEG	1
286	10	6	PT2018IBRgESERNS3	NEG	NEG	1
287	10	7	PT2018IBRgESERPS1	DBS	POS	1
288	10	8	PT2018IBRgESERNS3	NEG	NEG	1
289	10	9	PT2018IBRgESERNS4	NEG	NEG	1
290	10	10	PT2018IBRgESERPS2	POS	POS	1
291	10	11	PT2018IBRgESERNS3	NEG	NEG	1
292	10	12	PT2018IBRgESERPS2	POS	POS	1
293	10	13	PT2018IBRgESERNS2	NEG	NEG	1
294	10	14	PT2018IBRgESERPS1	DBS	NI	1
295	10	15	PT2018IBRgESERPS2	POS	POS	1
296	10	16	PT2018IBRgESERNS3	NEG	NEG	1
297	10	17	PT2018IBRgESERNS4	NEG	NEG	1
298	10	18	PT2018IBRgESERPS1	DBS	NI	1
299	10	19	PT2018IBRgESERPS2	POS	POS	1
300	10	20	PT2018IBRgESERNS4	NEG	NEG	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 except LAB2 and LAB4 provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB2 misclassified 1 out of 5 aliquots of the reference serum sample PT2018IBRgESERNS2 (95% of agreement) and LAB4 misclassified the aliquot of the reference serum sample PT2018IBRgESERPS1 and the 2 aliquots of the reference serum sample PT2018IBRgESERPS4 (85% of agreement). (Table 2 and Table 4).

The IBRgB participating laboratories, except LAB3 and LAB4, used IBRgB antibody ELISA kits from 4 different commercial kit producers : IDEXX (2 batches: L451 and K511), Biosellal (1 batch: 257111020), ZOETIS (1 batch: 17ZEAS006) and ThermoFisher/Prionics (1 batch : H170681L). LAB3 and LAB4 used a home made ELISA (batch: CGB5Q05 and 23/6/18 respectively).

LAB1.1, LAB1.2, LAB5, LAB6, LAB7, LAB8, LAB9 and LAB12 used the IBRgB ELISA kit from IDEXX. Hereby LAB1.2, LAB5, LAB7 and LAB9 performed the long incubation protocol and LAB1.1, LAB6, LAB8 and LAB12 the short incubation protocol. In addition, LAB1.1, LAB1.2, LAB6, LAB7, LAB8, LAB9 and LAB12 used the same batch L451.

For the detection of IBRgE-specific antibodies in reference serum samples, considering that the reference serum sample PT2018IBRgESERPS1 could have the following three results : POS, NEG and NI, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). (Table 3 and Table 5).

The IBRgE participating laboratories used ELISA kits from 5 different producers: IDEXX (6 batches : K091, K131, K481, L131, L281 and L421), Qiagen (1 batch: 257110609), IDVET (3 batches: B15, B92 and D17), Hipra (1 batch CGE.6C90) and Biosellal (1 batch EIGE-01A).

LAB1, LAB4.1, LAB5, LAB6, LAB7.2, LAB8, LAB9.1 and LAB10 used the IBRgE ELISA kit from IDEXX. In addition, LAB1, and LAB6 used the batch K131, LAB4.1 the batch K091, LAB5 the batch L281, LAB7.2 and LAB8 the batch L131, LAB9.1 the batch L421 and LAB10 the batch K481 .

LAB4.2, LAB7.1 and LAB9.2 used the IBRgE ELISA kit from IDVET. In addition, LAB4.2 used the batch B92, LAB7.1 used the batch D17 and LAB9.2 used the batch B15.

LAB4.3 and LAB9.3 used the IBRgE ELISA kit from Qiagen. The same batch 257110609.

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 the IBR reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, 11 out 12 participants to the PT IBRgB achieved a satisfactory performance for the detection of IBRgB-specific antibodies in bovine serum samples. LAB4 did not achieve a satisfactory performance for the detection of IBRgB-specific antibodies in bovine serum samples.

For the PT IBRgE, all the participants achieved a satisfactory performance for the detection of IBRgE-specific antibodies in bovine serum samples.

Coordinator proficiency tests

Katia Knapen

Appendix

Name 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)

Biosellal (Dardilly, France)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

HIPRA Scientific SLU (Girona, Spain)

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)

Sciensano (Ukkel, Belgium)

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

ZOETIS France (Lyon, France)

Wageningen Boveterinary Research (WBVR) (Lelystad, The Netherlands)

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 (box plots).

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 $P25 = \text{minimum}$ and $P75 = \text{maximum}$ when the number data is 2.

The box plot for the laboratories participating in the PT IBRgB is shown in Figures 1 and the box plot for the laboratories participating in the PT IBRgE is shown in Figure 2.

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.

The quantitative data analyse of the PT IBRgB was performed on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory except for LAB4 for which the data could not be processed. (Figure 1). The samples PT2018IBRgBSESRPS1 is not included in the figure because there was only one aliquot in the panel for this sample.

The quantitative data analyse of the PT IBRgE was performed on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory (Figure 2). The samples PT2018IBRgESERN1 is not included in the figure because there was only one aliquot in the panel for this sample.

Detection of IBRgB-specific antibodies in serum by ELISA

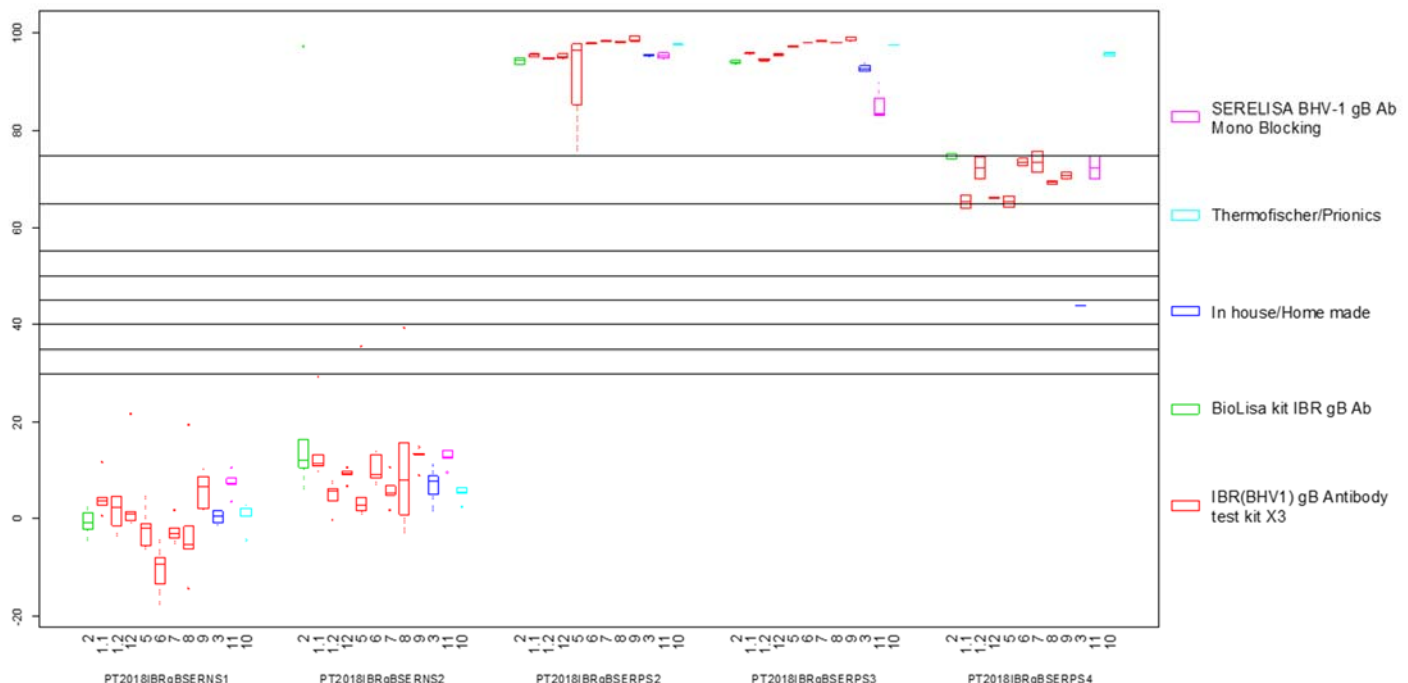


Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory except for LAB4. The participating laboratories, except LAB3, used IBRgB antibody ELISA kits from 4 different commercial kit producers : IDEXX, Biosellal, ZOETIS and Thermofischer/Prionics. LAB3 used an home made ELISA. Cut-off values (IDEXX 45-55 or 65-75, Biosellal 45-55, ZOETIS 50-55, Thermofischer/Prionics 40 and Home made 30-35) are shown by horizontal lines.

Detection of IBRgE-specific antibodies in serum by ELISA

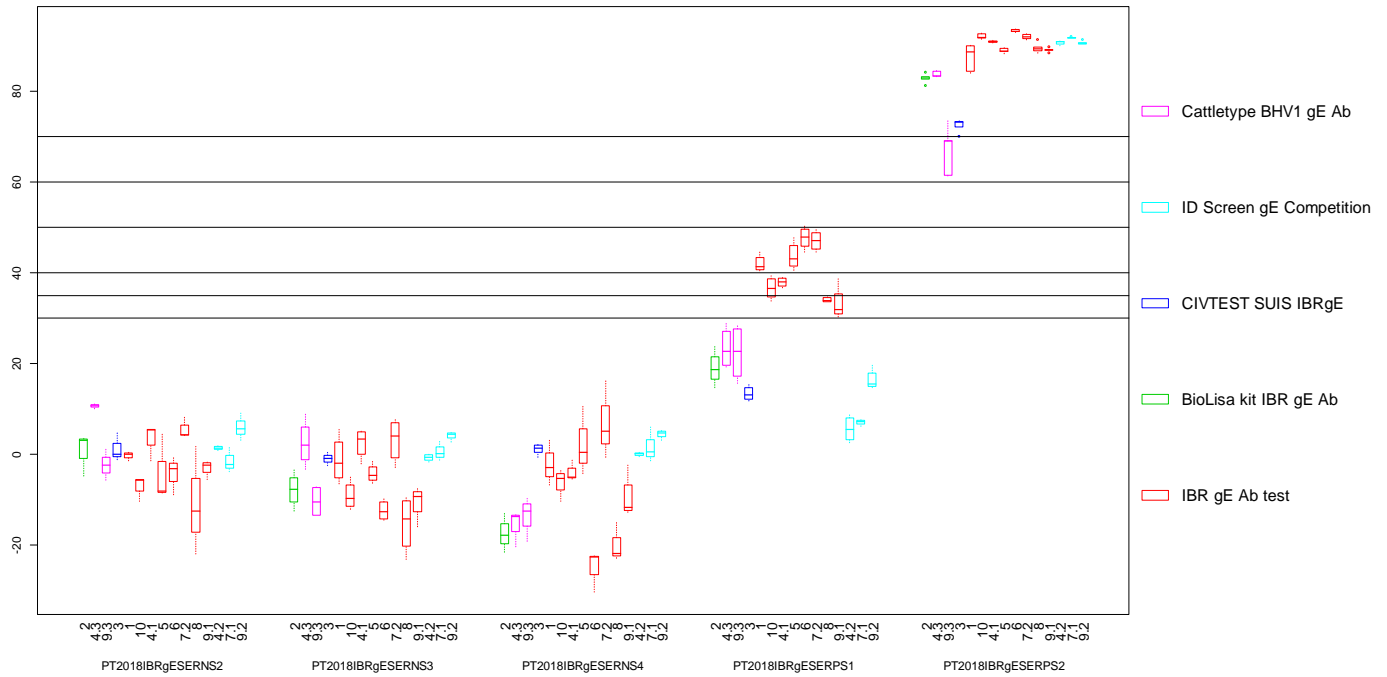


Figure 3. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory. The IBRgE participating laboratories used ELISA kits from 5 different producers: IDEXX, Qiagen, IDVET, Hipra and Biosellal. Cut-off value (Idexx 30-40 or 60-70, Qiagen 40-50, IDVET 30-40, Hipra 30-35 and Biosellal 35) are shown by a horizontal line.