

## **PROFICIENCY TESTING 2018**

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

**SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS  
SCIENSANO**

**DATE BEGIN PT: 12 NOVEMBER 2018**

**DATE REPORT: 15 MARCH 2019**

## I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 '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 milk by ELISA.

## III. Materials and methods

### III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference milk 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 10 reference milk samples of bovine origin, either free from detectable IBRgB-specific antibodies ( $n = 5$ ; coded 'PT2018IBRgBSERNM1', 'PT2018IBRgBSERNM2', 'PT2018IBRgBSERNM3', 'PT2018IBRgBSERNM4' and 'PT2018IBRgBSERNM5') or containing detectable IBRgB-specific antibodies ( $n = 5$ ; coded 'PT2018IBRgBSERPM1', 'PT2018IBRgBSERPM2', 'PT2018IBRgBSERPM3', 'PT2018IBRgBSERPM4' and 'PT2018IBRgBSERPM5'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 2 aliquots of each reference milk sample (Table 4).

For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was available. The status of the reference milk samples was based on (i) the historical background of the herds and (ii) the results obtained during pre-verification using the ELISA ID Screen IBR milk indirect bicipule test from IDVET.

The reference milk samples PT2018IBRgBSERNM1 to PT2018IBRgBSERNM5 were tank milk collected from 5 distinct Belgian I4-certified farms (IBR-free without vaccination). The reference milk samples PT2018IBRgBSERPM1 to PT2018IBRgBSERPM5 were tank milk collected from 5 distinct Belgian I2 (PT2018IBRgBSERPM1, PT2018IBRgBSERPM2, PT2018IBRgBSERPM3, PT2018IBRgBSERPM4) or I2d (PT2018IBRgBSERPM5)-certified farms. Taken together, the reference milk samples PT2018IBRgBSERNM1 to PT2018IBRgBSERNM5 were considered as negative milk samples, the reference milk samples PT2018IBRgBSERPM1 to PT2018IBRgBSERPM5 as (strong) positive milk samples in IBRgB ELISA.

An homogeneity check was performed on 10 aliquots of each reference milk sample after aliquoting the different reference milk samples, using the IBR gE ELISA test from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Therefore only 2 aliquots of each reference milk sample were tested before the PT to confirm their stability and status (pre-verification) using the IBR milk indirect bicipule test from IDVET.

Consequently, all reference milk 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 milk. In addition, 1 aliquot of each reference milk sample was tested after the PT in order to confirm their stability and status (post-verification) using the IBR milk indirect bicipule test from IDVET.

### III.2.2. IBRgE reference samples

Replicates of 10 reference milk samples of bovine origin, either free from detectable IBRgE-specific antibodies (n = 5; coded 'PT2018IBRgESERNM1', 'PT2018IBRgESERNM2', 'PT2018IBRgESERNM3', 'PT2018IBRgESERNM4' and 'PT2018IBRgESERNM5') or containing detectable IBRgE-specific antibodies (n=5, coded 'PT2018IBRgESERPM1', 'PT2018IBRgESERPM2', 'PT2018IBRgESERPM3', 'PT2018IBRgESERPM4' and 'PT2018IBRgESERPM5'), were used. In total, 160 aliquots were distributed to 8 different participating laboratories. All participants received 20 aliquots: 2 aliquots of each reference milk sample (Table 5).

For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was available. The status of the reference milk samples was based on (i) the historical background of the herds and (ii) the results obtained during pre-verification using the Eradikit BoHV1 gE ELISA test from IN3 Diagnostic.

The reference milk samples PT2018IBRgESERNM1 to PT2018IBRgESERNM5 were tank milk collected from I3-certified herds. The reference milk samples PT2018IBRgESERPM1 to PT2018IBRgESERPM5 were tank milk collected from I2-certified herds.

Taken together, the reference milk samples PT2018IBRgESERNM1 to PT2018IBRgESERNM5 were considered as negative tank milk samples, the reference milk samples PT2018IBRgESERPM1 to PT2018IBRgESERPM5 as (strong) positive tank milk samples in IBRgE ELISA.

An homogeneity check was performed on 10 aliquots of each reference milk sample after aliquoting the different reference milk samples, using the IBR gE ELISA test from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Therefore only 3 aliquots of each reference milk sample were tested before the PT to confirm their stability and status (pre-verification) using the Eradikit BoHV1 gE ELISA test from IN3 Diagnostic. Consequently, all reference milk 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 milk. In addition, 1 aliquot of each reference milk sample was tested after the PT in order to confirm their stability and status (post-verification) using the Eradikit BoHV1 gE ELISA test from IN3 Diagnostic.

### 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

All laboratories participated in both the PT IBRgB and the PT IBRgE and hence received 40 aliquots of reference milk samples (20 for the PT IBRgB and 20 for the PT IBRgE). The lyophilized reference milk samples were sent to LAB1, LAB2,

LAB3, LAB4, LAB5, LAB6 and LAB7 by national or international courier on 12<sup>ve</sup> of November 2018. LAB1, LAB4, LAB6 and LAB7 acknowledged receipt of the samples on the same day, whereas the other laboratories (LAB2, LAB3 and LAB5) received the samples on 13<sup>th</sup> of November 2018. Regarding LAB8, the lyophilized reference milk samples were sent to him by national courier on 19<sup>th</sup> of November 2018 and he acknowledged receipt of the samples on the same day.

LAB4 and LAB8 informed us by reception of the samples that they did not have an ELISA kit to perform the test. Therefore they received more time to perform the analysis.

LAB1 and LAB7 reported problems during the test and did not have enough samples to repeat the analysis. We sent new IBRgB and IBRgE samples to LAB1 on 3<sup>rd</sup> of December 2018 and new IBRgE samples to LAB7 on 5<sup>th</sup> of December 2018. LAB1 and LAB7 acknowledged receipt of the samples on the same day as the day of sending.

LAB8 reported also problems with the reconstitution of the lyophilized samples. They received a new set of samples that they picked up on 7<sup>en</sup> of December 2018.

We informed the participants who had problems that the deadline to submit the results was postponed to 14<sup>th</sup> of December 2018.

Analyses were started between 19<sup>th</sup> of November and 11<sup>en</sup> of December 2018 (Table 1). LAB6 did not perform the ELISA IBRgB.

#### ***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 26<sup>th</sup> of November 2018 and 13<sup>th</sup> of December 2018 (Table 1). All participants respected the deadline which had been attributed to them according to the problems they met or not.

**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.

<b>Participating laboratory</b>	<b>Reference samples received</b>	<b>Start of analysis gB</b>	<b>Start of analysis gE</b>	<b>Submission of the results (Excel file)</b>
<b>LAB1</b>	12/11/2018 + 03/12/2018	20/11/2018	06/12/2018	10/12/2018
<b>LAB2</b>	13/11/2018	22/11/2018	22/11/2018	26/11/2018
<b>LAB3</b>	13/11/2018	19-20/11/2018	20-21/11/2018	26/11/2018
<b>LAB4</b>	12/11/2018	20/11/2018	22/11/2018	27/11/2018
<b>LAB5</b>	13/11/2018	21/11/2018	28/11/2018	29/11/2018
<b>LAB6</b>	12/11/2018	NA	13/11/2018	26/11/2018
<b>LAB7</b>	12/11/2018 + 05/12/2018	28/11/2018	05/12/2018	13/12/2018
<b>LAB8</b>	19/11/2018 + 07/12/2018	10-11/12/2018	10-11/12/2018	12/12/2018

**Legend:** NA = not applicable

#### ***IV.3. Compliance with the procedure***

All participating laboratories, except LAB7, 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 for the same ELISA kit producer but different protocols (short and long incubation).

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

Qualitative data analysis showed that:

- (i) For the detection of **IBRgB-specific antibodies**, all participating laboratories except LAB3 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB3 misclassified 1 aliquot (95% of agreement) (Table 2).
- (ii) For the detection of **IBRgE-specific antibodies**, LAB1, LAB5, LAB6, LAB7 and LAB8 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB3 and LAB4 misclassified 1 aliquot (95% of agreement) and LAB2 misclassified 8 aliquots (60% of agreement) (Table 3).

**Table 2.** Agreement between the results obtained by the participating laboratories (LABNR) and the status of the **IBRgB** reference milk 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 milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1.1	1.2	2	3	4	5	7	8
<b>failure</b>	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)
<b>success</b>	20 (100)	20 (100)	20 (100)	19 (95)	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 milk 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 milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1	2	3	4	5	6	7	8
<b>failure</b>	0 (0)	8 (40)	1 (5)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)
<b>success</b>	20 (100)	12 (60)	19 (95)	19 (95)	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, LAB2, LAB4, LAB5, LAB7 and LAB8 could be observed since these participants correctly identified all reference milk samples. LAB1 obtained identical qualitative results using an ELISA kit from the same producer on 2 different protocols. In contrast, LAB3 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgBBSERNM5 (POS instead of NEG).
- (ii) For the detection of **IBRgE-specific antibodies**, no variability between LAB1, LAB5, LAB6, LAB7 and LAB8 could be observed since these participants correctly identified all reference milk samples. LAB3 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgESERPM5 (NEG instead of POS). LAB4 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgESERPM4 (NI instead of POS). LAB2 misclassified the 2 aliquots of the reference milk samples PT2018IBRgESERPM2, PT2018IBRgESERPM3, PT2018IBRgESERPM4 and PT2018IBRgESERPM5 (NEG instead of POS).

For each participating laboratory, the obtained results and the assigned statuses for the reference milk 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 milk samples (SAMPLE), the external identification of the reference milk 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	PT2018IBRgBSERPM3	POS	POS	1
2	1.1	2	PT2018IBRgBSERPM2	POS	POS	1
3	1.1	3	PT2018IBRgBSERNM2	NEG	NEG	1
4	1.1	4	PT2018IBRgBSERNM1	NEG	NEG	1
5	1.1	5	PT2018IBRgBSERPM4	POS	POS	1
6	1.1	6	PT2018IBRgBSERPM1	POS	POS	1
7	1.1	7	PT2018IBRgBSERNM3	NEG	NEG	1
8	1.1	8	PT2018IBRgBSERPM3	POS	POS	1
9	1.1	9	PT2018IBRgBSERPM5	POS	POS	1
10	1.1	10	PT2018IBRgBSERPM2	POS	POS	1
11	1.1	11	PT2018IBRgBSERNM3	NEG	NEG	1
12	1.1	12	PT2018IBRgBSERNM4	NEG	NEG	1
13	1.1	13	PT2018IBRgBSERPM1	POS	POS	1
14	1.1	14	PT2018IBRgBSERNM4	NEG	NEG	1
15	1.1	15	PT2018IBRgBSERNM5	NEG	NEG	1
16	1.1	16	PT2018IBRgBSERPM4	POS	POS	1
17	1.1	17	PT2018IBRgBSERPM5	POS	POS	1
18	1.1	18	PT2018IBRgBSERNM1	NEG	NEG	1
19	1.1	19	PT2018IBRgBSERNM2	NEG	NEG	1
20	1.1	20	PT2018IBRgBSERNM5	NEG	NEG	1
21	1.2	1	PT2018IBRgBSERPM3	POS	POS	1
22	1.2	2	PT2018IBRgBSERPM2	POS	POS	1
23	1.2	3	PT2018IBRgBSERNM2	NEG	NEG	1
24	1.2	4	PT2018IBRgBSERNM1	NEG	NEG	1
25	1.2	5	PT2018IBRgBSERPM4	POS	POS	1
26	1.2	6	PT2018IBRgBSERPM1	POS	POS	1
27	1.2	7	PT2018IBRgBSERNM3	NEG	NEG	1
28	1.2	8	PT2018IBRgBSERPM3	POS	POS	1
29	1.2	9	PT2018IBRgBSERPM5	POS	POS	1
30	1.2	10	PT2018IBRgBSERPM2	POS	POS	1
31	1.2	11	PT2018IBRgBSERNM3	NEG	NEG	1
32	1.2	12	PT2018IBRgBSERNM4	NEG	NEG	1
33	1.2	13	PT2018IBRgBSERPM1	POS	POS	1
34	1.2	14	PT2018IBRgBSERNM4	NEG	NEG	1
35	1.2	15	PT2018IBRgBSERNM5	NEG	NEG	1
36	1.2	16	PT2018IBRgBSERPM4	POS	POS	1
37	1.2	17	PT2018IBRgBSERPM5	POS	POS	1
38	1.2	18	PT2018IBRgBSERNM1	NEG	NEG	1
39	1.2	19	PT2018IBRgBSERNM2	NEG	NEG	1
40	1.2	20	PT2018IBRgBSERNM5	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	2	1	PT2018IBRgBSERPM5	POS	POS	1
42	2	2	PT2018IBRgBSERPM4	POS	POS	1
43	2	3	PT2018IBRgBSERPM2	POS	POS	1
44	2	4	PT2018IBRgBSERPM3	POS	POS	1
45	2	5	PT2018IBRgBSERPM1	POS	POS	1
46	2	6	PT2018IBRgBSERNM2	NEG	NEG	1
47	2	7	PT2018IBRgBSERNM4	NEG	NEG	1
48	2	8	PT2018IBRgBSERNM1	NEG	NEG	1
49	2	9	PT2018IBRgBSERNM3	NEG	NEG	1
50	2	10	PT2018IBRgBSERPM5	POS	POS	1
51	2	11	PT2018IBRgBSERPM1	POS	POS	1
52	2	12	PT2018IBRgBSERNM5	NEG	NEG	1
53	2	13	PT2018IBRgBSERPM2	POS	POS	1
54	2	14	PT2018IBRgBSERNM5	NEG	NEG	1
55	2	15	PT2018IBRgBSERNM1	NEG	NEG	1
56	2	16	PT2018IBRgBSERPM3	POS	POS	1
57	2	17	PT2018IBRgBSERNM2	NEG	NEG	1
58	2	18	PT2018IBRgBSERPM4	POS	POS	1
59	2	19	PT2018IBRgBSERNM3	NEG	NEG	1
60	2	20	PT2018IBRgBSERNM4	NEG	NEG	1
61	3	1	PT2018IBRgBSERPM3	POS	POS	1
62	3	2	PT2018IBRgBSERPM2	POS	POS	1
63	3	3	PT2018IBRgBSERNM2	NEG	NEG	1
64	3	4	PT2018IBRgBSERNM1	NEG	NEG	1
65	3	5	PT2018IBRgBSERPM4	POS	POS	1
66	3	6	PT2018IBRgBSERPM1	POS	POS	1
67	3	7	PT2018IBRgBSERNM3	NEG	NEG	1
68	3	8	PT2018IBRgBSERPM3	POS	POS	1
69	3	9	PT2018IBRgBSERPM5	POS	POS	1
70	3	10	PT2018IBRgBSERPM2	POS	POS	1
71	3	11	PT2018IBRgBSERNM3	NEG	NEG	1
72	3	12	PT2018IBRgBSERNM4	NEG	NEG	1
73	3	13	PT2018IBRgBSERPM1	POS	POS	1
74	3	14	PT2018IBRgBSERNM4	NEG	NEG	1
75	3	15	PT2018IBRgBSERNM5	<b>NEG</b>	<b>POS</b>	<b>0</b>
76	3	16	PT2018IBRgBSERPM4	POS	POS	1
77	3	17	PT2018IBRgBSERPM5	POS	POS	1
78	3	18	PT2018IBRgBSERNM1	NEG	NEG	1
79	3	19	PT2018IBRgBSERNM2	NEG	NEG	1
80	3	20	PT2018IBRgBSERNM5	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	4	1	PT2018IBRgBSERPM5	POS	POS	1
82	4	2	PT2018IBRgBSERPM4	POS	POS	1
83	4	3	PT2018IBRgBSERPM2	POS	POS	1
84	4	4	PT2018IBRgBSERPM3	POS	POS	1
85	4	5	PT2018IBRgBSERPM1	POS	POS	1
86	4	6	PT2018IBRgBSERNM2	NEG	NEG	1
87	4	7	PT2018IBRgBSERNM4	NEG	NEG	1
88	4	8	PT2018IBRgBSERNM1	NEG	NEG	1
89	4	9	PT2018IBRgBSERNM3	NEG	NEG	1
90	4	10	PT2018IBRgBSERPM5	POS	POS	1
91	4	11	PT2018IBRgBSERPM1	POS	POS	1
92	4	12	PT2018IBRgBSERNM5	NEG	NEG	1
93	4	13	PT2018IBRgBSERPM2	POS	POS	1
94	4	14	PT2018IBRgBSERNM5	NEG	NEG	1
95	4	15	PT2018IBRgBSERNM1	NEG	NEG	1
96	4	16	PT2018IBRgBSERPM3	POS	POS	1
97	4	17	PT2018IBRgBSERNM2	NEG	NEG	1
98	4	18	PT2018IBRgBSERPM4	POS	POS	1
99	4	19	PT2018IBRgBSERNM3	NEG	NEG	1
100	4	20	PT2018IBRgBSERNM4	NEG	NEG	1
101	5	1	PT2018IBRgBSERPM3	POS	POS	1
102	5	2	PT2018IBRgBSERPM2	POS	POS	1
103	5	3	PT2018IBRgBSERNM2	NEG	NEG	1
104	5	4	PT2018IBRgBSERNM1	NEG	NEG	1
105	5	5	PT2018IBRgBSERPM4	POS	POS	1
106	5	6	PT2018IBRgBSERPM1	POS	POS	1
107	5	7	PT2018IBRgBSERNM3	NEG	NEG	1
108	5	8	PT2018IBRgBSERPM3	POS	POS	1
109	5	9	PT2018IBRgBSERPM5	POS	POS	1
110	5	10	PT2018IBRgBSERPM2	POS	POS	1
111	5	11	PT2018IBRgBSERNM3	NEG	NEG	1
112	5	12	PT2018IBRgBSERNM4	NEG	NEG	1
113	5	13	PT2018IBRgBSERPM1	POS	POS	1
114	5	14	PT2018IBRgBSERNM4	NEG	NEG	1
115	5	15	PT2018IBRgBSERNM5	NEG	NEG	1
116	5	16	PT2018IBRgBSERPM4	POS	POS	1
117	5	17	PT2018IBRgBSERPM5	POS	POS	1
118	5	18	PT2018IBRgBSERNM1	NEG	NEG	1
119	5	19	PT2018IBRgBSERNM2	NEG	NEG	1
120	5	20	PT2018IBRgBSERNM5	NEG	NEG	1



(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2018IBRgBSERPM3	POS	POS	1
122	7	2	PT2018IBRgBSERPM2	POS	POS	1
123	7	3	PT2018IBRgBSERNM2	NEG	NEG	1
124	7	4	PT2018IBRgBSERNM1	NEG	NEG	1
125	7	5	PT2018IBRgBSERPM4	POS	POS	1
126	7	6	PT2018IBRgBSERPM1	POS	POS	1
127	7	7	PT2018IBRgBSERNM3	NEG	NEG	1
128	7	8	PT2018IBRgBSERPM3	POS	POS	1
129	7	9	PT2018IBRgBSERPM5	POS	POS	1
130	7	10	PT2018IBRgBSERPM2	POS	POS	1
131	7	11	PT2018IBRgBSERNM3	NEG	NEG	1
132	7	12	PT2018IBRgBSERNM4	NEG	NEG	1
133	7	13	PT2018IBRgBSERPM1	POS	POS	1
134	7	14	PT2018IBRgBSERNM4	NEG	NEG	1
135	7	15	PT2018IBRgBSERNM5	NEG	NEG	1
136	7	16	PT2018IBRgBSERPM4	POS	POS	1
137	7	17	PT2018IBRgBSERPM5	POS	POS	1
138	7	18	PT2018IBRgBSERNM1	NEG	NEG	1
139	7	19	PT2018IBRgBSERNM2	NEG	NEG	1
140	7	20	PT2018IBRgBSERNM5	NEG	NEG	1
141	8	1	PT2018IBRgBSERPM5	POS	POS	1
142	8	2	PT2018IBRgBSERPM4	POS	POS	1
143	8	3	PT2018IBRgBSERPM2	POS	POS	1
144	8	4	PT2018IBRgBSERPM3	POS	POS	1
145	8	5	PT2018IBRgBSERPM1	POS	POS	1
146	8	6	PT2018IBRgBSERNM2	NEG	NEG	1
147	8	7	PT2018IBRgBSERNM4	NEG	NEG	1
148	8	8	PT2018IBRgBSERNM1	NEG	NEG	1
149	8	9	PT2018IBRgBSERNM3	NEG	NEG	1
150	8	10	PT2018IBRgBSERPM5	POS	POS	1
151	8	11	PT2018IBRgBSERPM1	POS	POS	1
152	8	12	PT2018IBRgBSERNM5	NEG	NEG	1
153	8	13	PT2018IBRgBSERPM2	POS	POS	1
154	8	14	PT2018IBRgBSERNM5	NEG	NEG	1
155	8	15	PT2018IBRgBSERNM1	NEG	NEG	1
156	8	16	PT2018IBRgBSERPM3	POS	POS	1
157	8	17	PT2018IBRgBSERNM2	NEG	NEG	1
158	8	18	PT2018IBRgBSERPM4	POS	POS	1
159	8	19	PT2018IBRgBSERNM3	NEG	NEG	1
160	8	20	PT2018IBRgBSERNM4	NEG	NEG	1

**Table 5.** The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the **IBRgE** reference milk samples (SAMPLE), the external identification of the reference milk 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	PT2018IBRgESERNM3	NEG	NEG	1
2	1	2	PT2018IBRgESERPM1	POS	POS	1
3	1	3	PT2018IBRgESERPM3	POS	POS	1
4	1	4	PT2018IBRgESERNM2	NEG	NEG	1
5	1	5	PT2018IBRgESERPM2	POS	POS	1
6	1	6	PT2018IBRgESERNM4	NEG	NEG	1
7	1	7	PT2018IBRgESERNM1	NEG	NEG	1
8	1	8	PT2018IBRgESERPM1	POS	POS	1
9	1	9	PT2018IBRgESERNM3	NEG	NEG	1
10	1	10	PT2018IBRgESERNM5	NEG	NEG	1
11	1	11	PT2018IBRgESERPM4	POS	POS	1
12	1	12	PT2018IBRgESERNM1	NEG	NEG	1
13	1	13	PT2018IBRgESERNM4	NEG	NEG	1
14	1	14	PT2018IBRgESERPM3	POS	POS	1
15	1	15	PT2018IBRgESERPM2	POS	POS	1
16	1	16	PT2018IBRgESERNM5	NEG	NEG	1
17	1	17	PT2018IBRgESERPM5	POS	POS	1
18	1	18	PT2018IBRgESERNM2	NEG	NEG	1
19	1	19	PT2018IBRgESERPM4	POS	POS	1
20	1	20	PT2018IBRgESERPM5	POS	POS	1
21	2	1	PT2018IBRgESERPM2	<b>POS</b>	<b>NEG</b>	<b>0</b>
22	2	2	PT2018IBRgESERNM3	NEG	NEG	1
23	2	3	PT2018IBRgESERNM2	NEG	NEG	1
24	2	4	PT2018IBRgESERNM4	NEG	NEG	1
25	2	5	PT2018IBRgESERNM5	NEG	NEG	1
26	2	6	PT2018IBRgESERNM2	NEG	NEG	1
27	2	7	PT2018IBRgESERPM2	<b>POS</b>	<b>NEG</b>	<b>0</b>
28	2	8	PT2018IBRgESERPM3	<b>POS</b>	<b>NEG</b>	<b>0</b>
29	2	9	PT2018IBRgESERNM1	NEG	NEG	1
30	2	10	PT2018IBRgESERPM4	<b>POS</b>	<b>NEG</b>	<b>0</b>
31	2	11	PT2018IBRgESERNM4	NEG	NEG	1
32	2	12	PT2018IBRgESERPM5	<b>POS</b>	<b>NEG</b>	<b>0</b>
33	2	13	PT2018IBRgESERNM1	NEG	NEG	1
34	2	14	PT2018IBRgESERPM1	POS	POS	1
35	2	15	PT2018IBRgESERPM5	<b>POS</b>	<b>NEG</b>	<b>0</b>
36	2	16	PT2018IBRgESERPM3	<b>POS</b>	<b>NEG</b>	<b>0</b>
37	2	17	PT2018IBRgESERPM4	<b>POS</b>	<b>NEG</b>	<b>0</b>
38	2	18	PT2018IBRgESERNM3	NEG	NEG	1
39	2	19	PT2018IBRgESERNM5	NEG	NEG	1
40	2	20	PT2018IBRgESERPM1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018IBRgESERNM3	NEG	NEG	1
42	3	2	PT2018IBRgESERPM1	POS	POS	1
43	3	3	PT2018IBRgESERPM3	POS	POS	1
44	3	4	PT2018IBRgESERNM2	NEG	NEG	1
45	3	5	PT2018IBRgESERPM2	POS	POS	1
46	3	6	PT2018IBRgESERNM4	NEG	NEG	1
47	3	7	PT2018IBRgESERNM1	NEG	NEG	1
48	3	8	PT2018IBRgESERPM1	POS	POS	1
49	3	9	PT2018IBRgESERNM3	NEG	NEG	1
50	3	10	PT2018IBRgESERNM5	NEG	NEG	1
51	3	11	PT2018IBRgESERPM4	POS	POS	1
52	3	12	PT2018IBRgESERNM1	NEG	NEG	1
53	3	13	PT2018IBRgESERNM4	NEG	NEG	1
54	3	14	PT2018IBRgESERPM3	POS	POS	1
55	3	15	PT2018IBRgESERPM2	POS	POS	1
56	3	16	PT2018IBRgESERNM5	NEG	NEG	1
57	3	17	PT2018IBRgESERPM5	POS	POS	1
58	3	18	PT2018IBRgESERNM2	NEG	NEG	1
59	3	19	PT2018IBRgESERPM4	POS	POS	1
60	3	20	PT2018IBRgESERPM5	<b>POS</b>	<b>NEG</b>	<b>0</b>
61	4	1	PT2018IBRgESERPM2	POS	POS	1
62	4	2	PT2018IBRgESERNM3	NEG	NEG	1
63	4	3	PT2018IBRgESERNM2	NEG	NEG	1
64	4	4	PT2018IBRgESERNM4	NEG	NEG	1
65	4	5	PT2018IBRgESERNM5	NEG	NEG	1
66	4	6	PT2018IBRgESERNM2	NEG	NEG	1
67	4	7	PT2018IBRgESERPM2	POS	POS	1
68	4	8	PT2018IBRgESERPM3	POS	POS	1
69	4	9	PT2018IBRgESERNM1	NEG	NEG	1
70	4	10	PT2018IBRgESERPM4	POS	POS	1
71	4	11	PT2018IBRgESERNM4	NEG	NEG	1
72	4	12	PT2018IBRgESERPM5	POS	POS	1
73	4	13	PT2018IBRgESERNM1	NEG	NEG	1
74	4	14	PT2018IBRgESERPM1	POS	POS	1
75	4	15	PT2018IBRgESERPM5	POS	POS	1
76	4	16	PT2018IBRgESERPM3	POS	POS	1
77	4	17	PT2018IBRgESERPM4	<b>POS</b>	<b>NI</b>	<b>0</b>
78	4	18	PT2018IBRgESERNM3	NEG	NEG	1
79	4	19	PT2018IBRgESERNM5	NEG	NEG	1
80	4	20	PT2018IBRgESERPM1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2018IBRgESERNM3	NEG	NEG	1
82	5	2	PT2018IBRgESERPM1	POS	POS	1
83	5	3	PT2018IBRgESERPM3	POS	POS	1
84	5	4	PT2018IBRgESERNM2	NEG	NEG	1
85	5	5	PT2018IBRgESERPM2	POS	POS	1
86	5	6	PT2018IBRgESERNM4	NEG	NEG	1
87	5	7	PT2018IBRgESERNM1	NEG	NEG	1
88	5	8	PT2018IBRgESERPM1	POS	POS	1
89	5	9	PT2018IBRgESERNM3	NEG	NEG	1
90	5	10	PT2018IBRgESERNM5	NEG	NEG	1
91	5	11	PT2018IBRgESERPM4	POS	POS	1
92	5	12	PT2018IBRgESERNM1	NEG	NEG	1
93	5	13	PT2018IBRgESERNM4	NEG	NEG	1
94	5	14	PT2018IBRgESERPM3	POS	POS	1
95	5	15	PT2018IBRgESERPM2	POS	POS	1
96	5	16	PT2018IBRgESERNM5	NEG	NEG	1
97	5	17	PT2018IBRgESERPM5	POS	POS	1
98	5	18	PT2018IBRgESERNM2	NEG	NEG	1
99	5	19	PT2018IBRgESERPM4	POS	POS	1
100	5	20	PT2018IBRgESERPM5	POS	POS	1
101	6	1	PT2018IBRgESERPM2	POS	POS	1
102	6	2	PT2018IBRgESERNM3	NEG	NEG	1
103	6	3	PT2018IBRgESERNM2	NEG	NEG	1
104	6	4	PT2018IBRgESERNM4	NEG	NEG	1
105	6	5	PT2018IBRgESERNM5	NEG	NEG	1
106	6	6	PT2018IBRgESERNM2	NEG	NEG	1
107	6	7	PT2018IBRgESERPM2	POS	POS	1
108	6	8	PT2018IBRgESERPM3	POS	POS	1
109	6	9	PT2018IBRgESERNM1	NEG	NEG	1
110	6	10	PT2018IBRgESERPM4	POS	POS	1
111	6	11	PT2018IBRgESERNM4	NEG	NEG	1
112	6	12	PT2018IBRgESERPM5	POS	POS	1
113	6	13	PT2018IBRgESERNM1	NEG	NEG	1
114	6	14	PT2018IBRgESERPM1	POS	POS	1
115	6	15	PT2018IBRgESERPM5	POS	POS	1
116	6	16	PT2018IBRgESERPM3	POS	POS	1
117	6	17	PT2018IBRgESERPM4	POS	POS	1
118	6	18	PT2018IBRgESERNM3	NEG	NEG	1
119	6	19	PT2018IBRgESERNM5	NEG	NEG	1
120	6	20	PT2018IBRgESERPM1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2018IBRgESERNM3	NEG	NEG	1
122	7	2	PT2018IBRgESERPM1	POS	POS	1
123	7	3	PT2018IBRgESERPM3	POS	POS	1
124	7	4	PT2018IBRgESERNM2	NEG	NEG	1
125	7	5	PT2018IBRgESERPM2	POS	POS	1
126	7	6	PT2018IBRgESERNM4	NEG	NEG	1
127	7	7	PT2018IBRgESERNM1	NEG	NEG	1
128	7	8	PT2018IBRgESERPM1	POS	POS	1
129	7	9	PT2018IBRgESERNM3	NEG	NEG	1
130	7	10	PT2018IBRgESERNM5	NEG	NEG	1
131	7	11	PT2018IBRgESERPM4	POS	POS	1
132	7	12	PT2018IBRgESERNM1	NEG	NEG	1
133	7	13	PT2018IBRgESERNM4	NEG	NEG	1
134	7	14	PT2018IBRgESERPM3	POS	POS	1
135	7	15	PT2018IBRgESERPM2	POS	POS	1
136	7	16	PT2018IBRgESERNM5	NEG	NEG	1
137	7	17	PT2018IBRgESERPM5	POS	POS	1
138	7	18	PT2018IBRgESERNM2	NEG	NEG	1
139	7	19	PT2018IBRgESERPM4	POS	POS	1
140	7	20	PT2018IBRgESERPM5	POS	POS	1
141	8	1	PT2018IBRgESERPM2	POS	POS	1
142	8	2	PT2018IBRgESERNM3	NEG	NEG	1
143	8	3	PT2018IBRgESERNM2	NEG	NEG	1
144	8	4	PT2018IBRgESERNM4	NEG	NEG	1
145	8	5	PT2018IBRgESERNM5	NEG	NEG	1
146	8	6	PT2018IBRgESERNM2	NEG	NEG	1
147	8	7	PT2018IBRgESERPM2	POS	POS	1
148	8	8	PT2018IBRgESERPM3	POS	POS	1
149	8	9	PT2018IBRgESERNM1	NEG	NEG	1
150	8	10	PT2018IBRgESERPM4	POS	POS	1
151	8	11	PT2018IBRgESERNM4	NEG	NEG	1
152	8	12	PT2018IBRgESERPM5	POS	POS	1
153	8	13	PT2018IBRgESERNM1	NEG	NEG	1
154	8	14	PT2018IBRgESERPM1	POS	POS	1
155	8	15	PT2018IBRgESERPM5	POS	POS	1
156	8	16	PT2018IBRgESERPM3	POS	POS	1
157	8	17	PT2018IBRgESERPM4	POS	POS	1
158	8	18	PT2018IBRgESERNM3	NEG	NEG	1
159	8	19	PT2018IBRgESERNM5	NEG	NEG	1
160	8	20	PT2018IBRgESERPM1	POS	POS	1

## V. Discussion

The purpose of this PT was to assess performances of the participating laboratories when analyzing reference milk 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 milk samples, all participating laboratories except LAB3 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB3 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgBSERNM5 (95% of agreement). (Table 2 and Table 4).

The IBRgB participating laboratories, except LAB3, used IBRgB antibody ELISA kits from 4 different commercial kit producers : IDVet (2 batches: C59 and C74), Biosellal (1 batch: F201800014), Qiagen/Indical (1 batch: F201800013) and a producer not identified by the participant (1 batch : H170601L). LAB3 used a home made ELISA (batch: CGB.5Q05). LAB1 performed long (LAB1.1) and short (LAB1.2) incubation with the same ELISA kit.

For the detection of IBRgE-specific antibodies in reference milk samples LAB1, LAB5, LAB6, LAB7 and LAB8 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB3 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgESERPM5 (95% of agreement). LAB4 misclassified 1 out of 2 aliquots of the reference milk sample PT2018IBRgESERPM4 (95% of agreement). LAB2 misclassified the 2 aliquots of the reference milk samples PT2018IBRgESERPM2, PT2018IBRgESERPM3, PT2018IBRgESERPM4 and PT2018IBRgESERPM5 (60% of agreement) (Table 3 and Table 5).

The IBRgE participating laboratories, except LAB3, used ELISA kits from 3 different producers: IN3 Diagnostics (1 batch : 738010), IDEXX (1 batch : K481) and Biosellal (1 batch : 260110105). LAB3 used a home made ELISA.

## 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 milk samples assigned by the IBR reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.).

Consequently, all the participants to the PT IBRgB achieved a satisfactory performance for the detection of IBRgB-specific antibodies in bovine milk samples.

For the PT IBRgE, all the participants, except LAB2, achieved a satisfactory performance for the detection of IBRgE-specific antibodies in bovine milk samples.

Coordinator proficiency tests

Katia Knapen

## Appendix

### Name of the participating laboratories

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

Biosellal (Dardilly, France)

Comité du Lait ASBL (Battice, Belgium)

HIPRA Scientific SLU (Girona, Spain)

Lavetan NV (Turnhout, Belgium)

MCC-Vlaanderen (Lier, Belgium)

Sciensano (Ukkel, Belgium)

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.

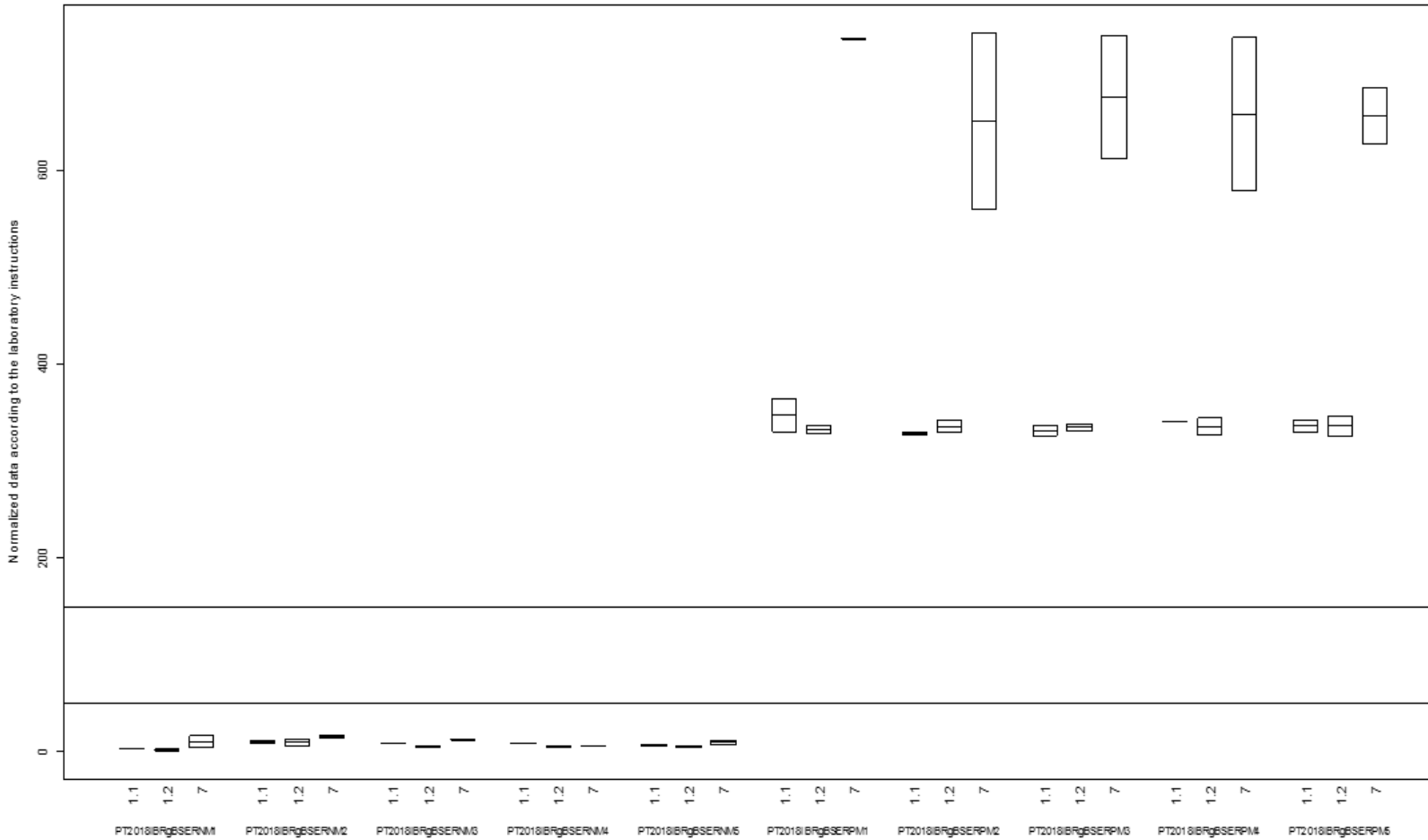
The box plot for the laboratories participating in the PT IBRgB is shown in Figure 1 and Figure 2 and the box plot for the laboratories participating in the PT IBRgE is shown in Figure 3 and Figure 4.

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 two different ways. For participants who used the IDVet ELISA kit the quantitative data analyse was performed on the normalized data according to the laboratory instructions per reference milk sample and per participating laboratory (Figure 1). For the other laboratories the quantitative data analyse was performed on the normalized data according to the instructions of the PT provider per reference milk sample and per participating laboratory (Figure 2).

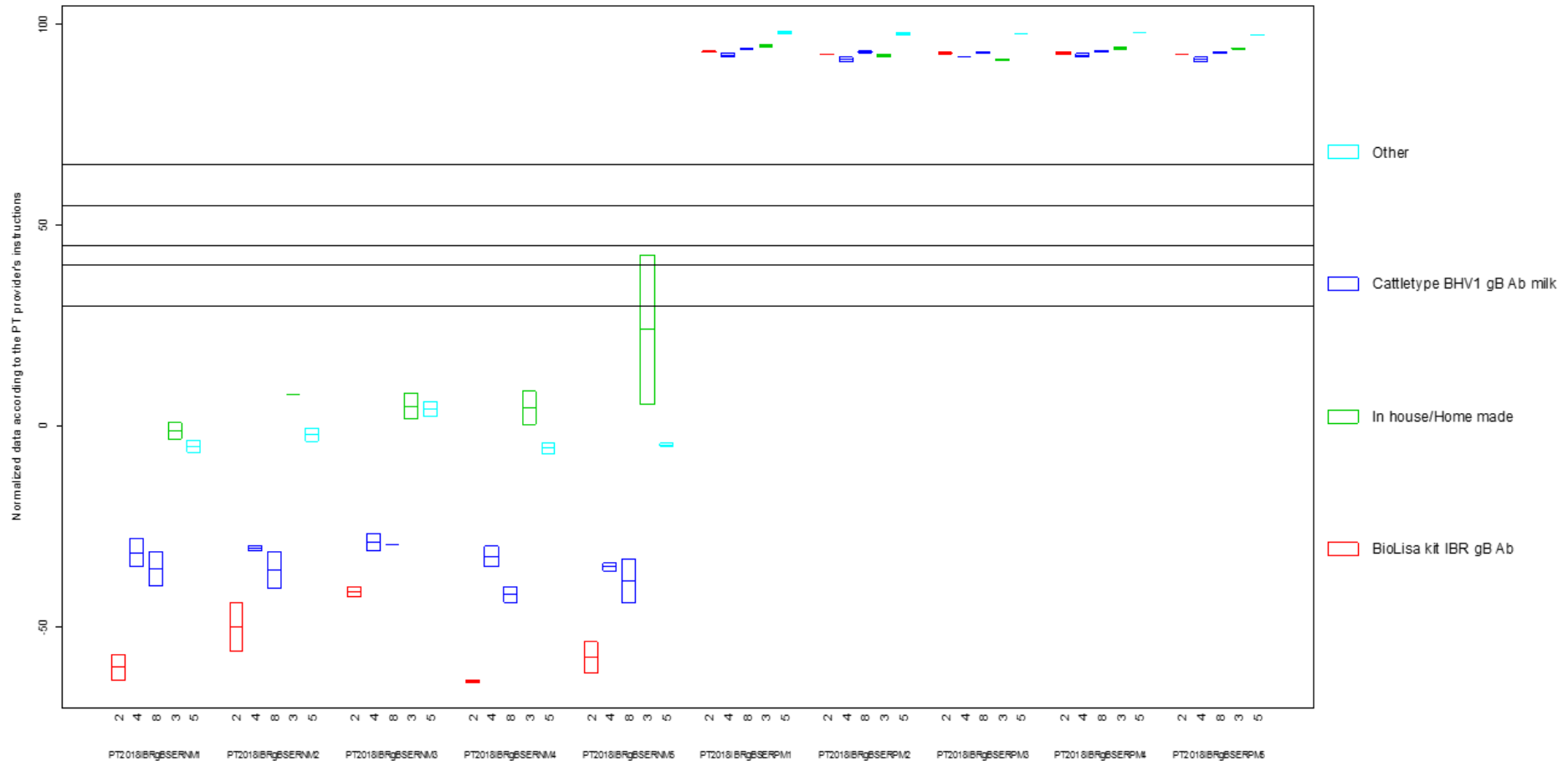
The quantitative data analyse of the PT IBRgE was performed on two different ways. For participants who used the IN3 Diagnostics ELISA kit the quantitative data analyse was performed on the normalized data according to the laboratory instructions per reference milk sample and per participating laboratory (Figure 3). For the other laboratories the quantitative data analyse was performed on the normalized data according to the instructions of the PT provider per reference milk sample and per participating laboratory (Figure 4).

Detection of IBRgB-specific antibodies in milk by the IDVet ELISA



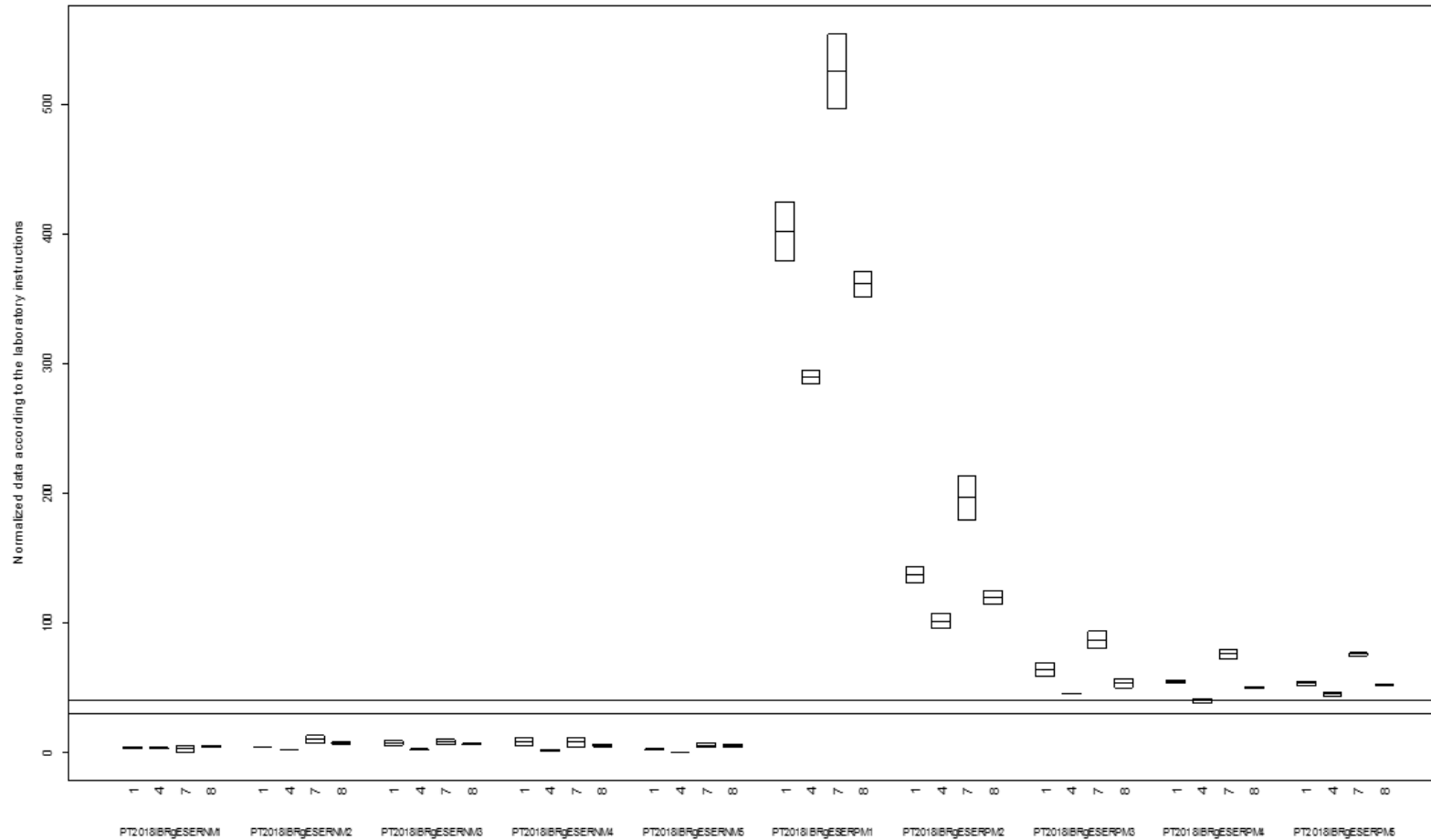
**Figure 1. Box plots showing the normalized data according the laboratory instructions per reference milk and per participating laboratory.** Cut-off values 50 or 150 are shown by horizontal lines.

### Detection of IBRgB-specific antibodies in milk by the other ELISA's



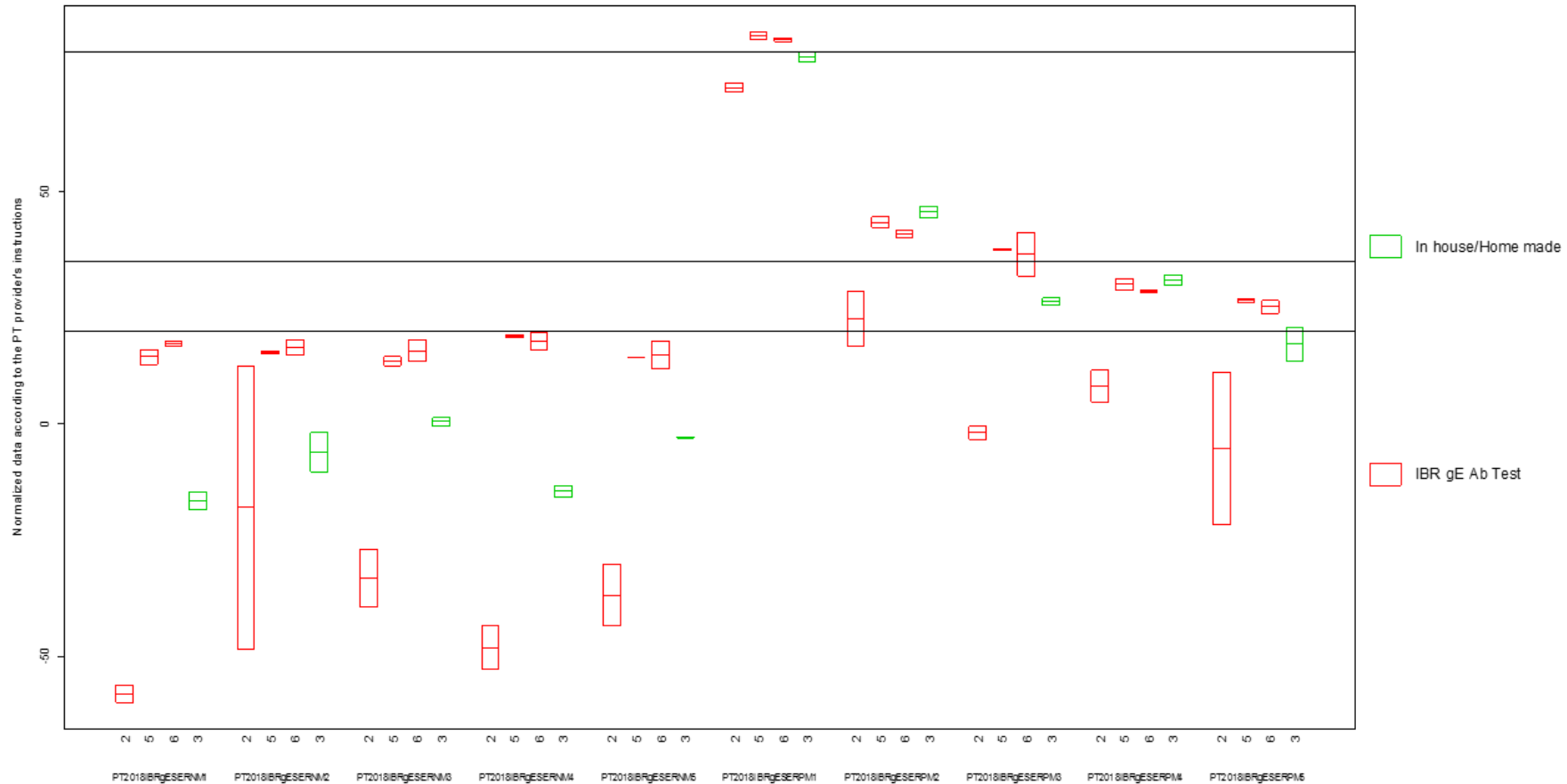
**Figure 2. Box plots showing the normalized data according the PT provider per reference milk and per participating laboratory.** The laboratories, except LAB3, used IBRgB antibody ELISA kits from 3 different commercial kit producers : Biosellal, Qiagen/Indical and an other producer not identified by the participant. LAB3 used a home made ELISA. Cut-off values (Biosellal 55-65, Qiagen/Indical 45-55, other producer 40, Home made 30) are shown by horizontal lines.

Detection of IBRgE-specific antibodies in milk by IN3 Diagnostics ELISA



**Figure 3. Box plots showing the normalized data according the laboratory instructions per reference milk and per participating laboratory. Cut-off values 30-40 are shown by horizontal lines.**

### Detection of IBRgE-specific antibodies in milk by the other ELISA's



**Figure 4. Box plots showing the normalized data according the PT provider per reference milk and per participating laboratory.** The laboratories, except LAB3, used ELISA kits from 2 different producers: IDEXX (LAB5 and LAB6) and Biosellal (LAB2). LAB3 used a home made ELISA. Cut-off values (Home made 20, Biosellal 35, IDEXX 80) are shown by horizontal lines.