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081-PT

PROFICIENCY TESTING 2019

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 START PT: 26 AUGUST 2019

DATE REPORT: 14 JANUARY 2020

THIS REPORT REPLACES AND CANCELS THE PREVIOUS REPORT PT2019IBRSER

Reason : error in the appendix (name of the participating laboratories)

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 'PT2019IBRgBSERNM1', 'PT2019IBRgBSERNM2' , 'PT2019IBRgBSERNM3' , 'PT2019IBRgBSERNM4' and 'PT2019IBRgBSERNM5') or containing detectable IBRgB-specific antibodies (n = 5; coded 'PT2019IBRgBSERPM1', 'PT2019IBRgBSERPM2', 'PT2019IBRgBSERPM3', 'PT2019IBRgBSERPM4' and 'PT2019IBRgBSERPM5'), were used. In total, 120 aliquots were distributed to 6 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 bicupule test from IDVET.

The reference milk samples PT2019IBRgBSERNM1 to PT2019IBRgBSERNM5 were tank milk collected from 5 distinct Belgian I4-certified farms (IBR-free without vaccination). The reference milk samples PT2019IBRgBSERPM1 to PT2019IBRgBSERPM5 were tank milk collected from 5 distinct Belgian I2 (PT2019IBRgBSERPM1, PT2019IBRgBSERPM2, PT2019IBRgBSERPM3, PT2019IBRgBSERPM4) or I2d (PT2019IBRgBSERPM5)-certified farms. Taken together, the reference milk samples PT2019IBRgBSERNM1 to PT2019IBRgBSERNM5 were considered as negative milk samples, the reference milk samples PT2019IBRgBSERPM1 to PT2019IBRgBSERPM5 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 bicupule 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 bicupule 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 'PT2019IBRgESERNM1', 'PT2019IBRgESERNM2', 'PT2019IBRgESERNM3', 'PT2019IBRgESERNM4' and 'PT2019IBRgESERNM5') or containing detectable IBRgE-specific antibodies (n=5, coded 'PT2019IBRgESERPM1', 'PT2019IBRgESERPM2' , 'PT2019IBRgESERPM3' , 'PT2019IBRgESERPM4' and 'PT2019IBRgESERPM5'), were used. In total, 120 aliquots were distributed to 6 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 PT2019IBRgESERNM1 to PT2019IBRgESERNM5 were tank milk collected from I3-certified herds. The reference milk samples PT2019IBRgESERPM1 to PT2019IBRgESERPM5 were tank milk collected from I2-certified herds.

Taken together, the reference milk samples PT2019IBRgESERNM1 to PT2019IBRgESERNM5 were considered as negative tank milk samples, the reference milk samples PT2019IBRgESERPM1 to PT2019IBRgESERPM5 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 the participants by national or international courier on 26th of August 2019. LAB2, LAB3, LAB5 and LAB6 acknowledged receipt of the samples on the same day, whereas LAB4 received the samples on 27th of August 2019 and LAB1 on 3th of September 2019.

Analyses were started between 2nd and 17th of September 2019 (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 10th and 27th of september 2019 (Table 1). All participants except LAB6 respected the deadline of 20th of September 2019 for submission of the results.

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	03/09/2019	16/09/2019	17/09/2019	18/09/2019
LAB2	26/08/2019	04/09/2019	04/09/2019	10/09/2019
LAB3	26/08/2019	12/09/2019	12/09/2019	16/09/2019
LAB4	27/08/2019	06/09/2019	13/09/2019	17/09/2019
LAB5	26/08/2019	05/09/2019 (short) 06/09/2019 (long)	04/09/2019	12/09/2019
LAB6	26/08/2019	02/09/2019	02/09/2019	**27/09/2019**

IV.3. Compliance with the procedure

Only LAB1, LAB2 and LAB5 provide a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

LAB5 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 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 2).

(ii) For the detection of **IBRgE-specific antibodies**, LAB2, LAB3, LAB5 and LAB6 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB1 and LAB4 misclassified 2 aliquots (90% 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	**2**	**3**	**4**	**5.1**	**5.2**	**6**
failure	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)

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**
failure	**2 (10)**	0 (0)	0 (0)	**2 (10)**	0 (0)	0 (0)
success	**18 (90)**	20 (100)	20 (100)	**18 (90)**	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 the participants could be observed since all participants correctly identified all reference milk samples.

(ii) For the detection of **IBRgE-specific antibodies**, no variability between LAB2, LAB3, LAB5 and LAB6 could be observed since these participants correctly identified all reference milk samples. LAB1 misclassified the 2 aliquots of the reference milk sample PT2019IBRgESERPM5 (NEG instead of POS). LAB4 misclassified 1 out of 2 aliquots of the reference milk samples PT2019IBRgESERPM4 and PT2019IBRgESERPM5 (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	PT2019IBRgBSERNM4	NEG	NEG	1
2	1	2	PT2019IBRgBSERNM3	NEG	NEG	1
3	1	3	PT2019IBRgBSERPM1	POS	POS	1
4	1	4	PT2019IBRgBSERPM3	POS	POS	1
5	1	5	PT2019IBRgBSERNM1	NEG	NEG	1
6	1	6	PT2019IBRgBSERNM5	NEG	NEG	1
7	1	7	PT2019IBRgBSERNM2	NEG	NEG	1
8	1	8	PT2019IBRgBSERPM1	POS	POS	1
9	1	9	PT2019IBRgBSERNM4	NEG	NEG	1
10	1	10	PT2019IBRgBSERPM2	POS	POS	1
11	1	11	PT2019IBRgBSERNM3	NEG	NEG	1
12	1	12	PT2019IBRgBSERPM4	POS	POS	1
13	1	13	PT2019IBRgBSERPM5	POS	POS	1
14	1	14	PT2019IBRgBSERNM1	NEG	NEG	1
15	1	15	PT2019IBRgBSERNM5	NEG	NEG	1
16	1	16	PT2019IBRgBSERPM3	POS	POS	1
17	1	17	PT2019IBRgBSERNM2	NEG	NEG	1
18	1	18	PT2019IBRgBSERPM2	POS	POS	1
19	1	19	PT2019IBRgBSERPM5	POS	POS	1
20	1	20	PT2019IBRgBSERPM4	POS	POS	1
21	2	1	PT2019IBRgBSERNM3	NEG	NEG	1
22	2	2	PT2019IBRgBSERNM2	NEG	NEG	1
23	2	3	PT2019IBRgBSERNM4	NEG	NEG	1
24	2	4	PT2019IBRgBSERPM1	POS	POS	1
25	2	5	PT2019IBRgBSERNM5	NEG	NEG	1
26	2	6	PT2019IBRgBSERNM1	NEG	NEG	1
27	2	7	PT2019IBRgBSERPM1	POS	POS	1
28	2	8	PT2019IBRgBSERPM3	POS	POS	1
29	2	9	PT2019IBRgBSERNM2	NEG	NEG	1
30	2	10	PT2019IBRgBSERPM4	POS	POS	1
31	2	11	PT2019IBRgBSERPM2	POS	POS	1
32	2	12	PT2019IBRgBSERNM1	NEG	NEG	1
33	2	13	PT2019IBRgBSERPM4	POS	POS	1
34	2	14	PT2019IBRgBSERNM5	NEG	NEG	1
35	2	15	PT2019IBRgBSERPM5	POS	POS	1
36	2	16	PT2019IBRgBSERNM3	NEG	NEG	1
37	2	17	PT2019IBRgBSERPM5	POS	POS	1
38	2	18	PT2019IBRgBSERNM4	NEG	NEG	1
39	2	19	PT2019IBRgBSERPM2	POS	POS	1
40	2	20	PT2019IBRgBSERPM3	POS	POS	1
41	3	1	PT2019IBRgBSERNM4	NEG	NEG	1
42	3	2	PT2019IBRgBSERNM3	NEG	NEG	1
43	3	3	PT2019IBRgBSERPM1	POS	POS	1
44	3	4	PT2019IBRgBSERPM3	POS	POS	1
45	3	5	PT2019IBRgBSERNM1	NEG	NEG	1
46	3	6	PT2019IBRgBSERNM5	NEG	NEG	1
47	3	7	PT2019IBRgBSERNM2	NEG	NEG	1
48	3	8	PT2019IBRgBSERPM1	POS	POS	1
49	3	9	PT2019IBRgBSERNM4	NEG	NEG	1
50	3	10	PT2019IBRgBSERPM2	POS	POS	1
51	3	11	PT2019IBRgBSERNM3	NEG	NEG	1
52	3	12	PT2019IBRgBSERPM4	POS	POS	1

53	3	13	PT2019IBRgBSERPM5	POS	POS	1
54	3	14	PT2019IBRgBSERNM1	NEG	NEG	1
55	3	15	PT2019IBRgBSERNM5	NEG	NEG	1
56	3	16	PT2019IBRgBSERPM3	POS	POS	1
57	3	17	PT2019IBRgBSERNM2	NEG	NEG	1
58	3	18	PT2019IBRgBSERPM2	POS	POS	1
59	3	19	PT2019IBRgBSERPM5	POS	POS	1
60	3	20	PT2019IBRgBSERPM4	POS	POS	1
61	4	1	PT2019IBRgBSERNM3	NEG	NEG	1
62	4	2	PT2019IBRgBSERNM2	NEG	NEG	1
63	4	3	PT2019IBRgBSERNM4	NEG	NEG	1
64	4	4	PT2019IBRgBSERPM1	POS	POS	1
65	4	5	PT2019IBRgBSERNM5	NEG	NEG	1
66	4	6	PT2019IBRgBSERNM1	NEG	NEG	1
67	4	7	PT2019IBRgBSERPM1	POS	POS	1
68	4	8	PT2019IBRgBSERPM3	POS	POS	1
69	4	9	PT2019IBRgBSERNM2	NEG	NEG	1
70	4	10	PT2019IBRgBSERPM4	POS	POS	1
71	4	11	PT2019IBRgBSERPM2	POS	POS	1
72	4	12	PT2019IBRgBSERNM1	NEG	NEG	1
73	4	13	PT2019IBRgBSERPM4	POS	POS	1
74	4	14	PT2019IBRgBSERNM5	NEG	NEG	1
75	4	15	PT2019IBRgBSERPM5	POS	POS	1
76	4	16	PT2019IBRgBSERNM3	NEG	NEG	1
77	4	17	PT2019IBRgBSERPM5	POS	POS	1
78	4	18	PT2019IBRgBSERNM4	NEG	NEG	1
79	4	19	PT2019IBRgBSERPM2	POS	POS	1
80	4	20	PT2019IBRgBSERPM3	POS	POS	1
81	5.1	1	PT2019IBRgBSERNM4	NEG	NEG	1
82	5.1	2	PT2019IBRgBSERNM3	NEG	NEG	1
83	5.1	3	PT2019IBRgBSERPM1	POS	POS	1
84	5.1	4	PT2019IBRgBSERPM3	POS	POS	1
85	5.1	5	PT2019IBRgBSERNM1	NEG	NEG	1
86	5.1	6	PT2019IBRgBSERNM5	NEG	NEG	1
87	5.1	7	PT2019IBRgBSERNM2	NEG	NEG	1
88	5.1	8	PT2019IBRgBSERPM1	POS	POS	1
89	5.1	9	PT2019IBRgBSERNM4	NEG	NEG	1
90	5.1	10	PT2019IBRgBSERPM2	POS	POS	1
91	5.1	11	PT2019IBRgBSERNM3	NEG	NEG	1
92	5.1	12	PT2019IBRgBSERPM4	POS	POS	1
93	5.1	13	PT2019IBRgBSERPM5	POS	POS	1
94	5.1	14	PT2019IBRgBSERNM1	NEG	NEG	1
95	5.1	15	PT2019IBRgBSERNM5	NEG	NEG	1
96	5.1	16	PT2019IBRgBSERPM3	POS	POS	1
97	5.1	17	PT2019IBRgBSERNM2	NEG	NEG	1
98	5.1	18	PT2019IBRgBSERPM2	POS	POS	1
99	5.1	19	PT2019IBRgBSERPM5	POS	POS	1
100	5.1	20	PT2019IBRgBSERPM4	POS	POS	1
101	5.2	1	PT2019IBRgBSERNM4	NEG	NEG	1
102	5.2	2	PT2019IBRgBSERNM3	NEG	NEG	1
103	5.2	3	PT2019IBRgBSERPM1	POS	POS	1
104	5.2	4	PT2019IBRgBSERPM3	POS	POS	1
105	5.2	5	PT2019IBRgBSERNM1	NEG	NEG	1
106	5.2	6	PT2019IBRgBSERNM5	NEG	NEG	1
107	5.2	7	PT2019IBRgBSERNM2	NEG	NEG	1
108	5.2	8	PT2019IBRgBSERPM1	POS	POS	1
109	5.2	9	PT2019IBRgBSERNM4	NEG	NEG	1

110	5.2	10	PT2019IBRgBSERPM2	POS	POS	1
111	5.2	11	PT2019IBRgBSERNM3	NEG	NEG	1
112	5.2	12	PT2019IBRgBSERPM4	POS	POS	1
113	5.2	13	PT2019IBRgBSERPM5	POS	POS	1
114	5.2	14	PT2019IBRgBSERNM1	NEG	NEG	1
115	5.2	15	PT2019IBRgBSERNM5	NEG	NEG	1
116	5.2	16	PT2019IBRgBSERPM3	POS	POS	1
117	5.2	17	PT2019IBRgBSERNM2	NEG	NEG	1
118	5.2	18	PT2019IBRgBSERPM2	POS	POS	1
119	5.2	19	PT2019IBRgBSERPM5	POS	POS	1
120	5.2	20	PT2019IBRgBSERPM4	POS	POS	1
121	6	1	PT2019IBRgBSERNM3	NEG	NEG	1
122	6	2	PT2019IBRgBSERNM2	NEG	NEG	1
123	6	3	PT2019IBRgBSERNM4	NEG	NEG	1
124	6	4	PT2019IBRgBSERPM1	POS	POS	1
125	6	5	PT2019IBRgBSERNM5	NEG	NEG	1
126	6	6	PT2019IBRgBSERNM1	NEG	NEG	1
127	6	7	PT2019IBRgBSERPM1	POS	POS	1
128	6	8	PT2019IBRgBSERPM3	POS	POS	1
129	6	9	PT2019IBRgBSERNM2	NEG	NEG	1
130	6	10	PT2019IBRgBSERPM4	POS	POS	1
131	6	11	PT2019IBRgBSERPM2	POS	POS	1
132	6	12	PT2019IBRgBSERNM1	NEG	NEG	1
133	6	13	PT2019IBRgBSERPM4	POS	POS	1
134	6	14	PT2019IBRgBSERNM5	NEG	NEG	1
135	6	15	PT2019IBRgBSERPM5	POS	POS	1
136	6	16	PT2019IBRgBSERNM3	NEG	NEG	1
137	6	17	PT2019IBRgBSERPM5	POS	POS	1
138	6	18	PT2019IBRgBSERNM4	NEG	NEG	1
139	6	19	PT2019IBRgBSERPM2	POS	POS	1
140	6	20	PT2019IBRgBSERPM3	POS	POS	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.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019IBRgESERNM3	NEG	NEG	1
2	1	2	PT2019IBRgESERNM1	NEG	NEG	1
3	1	3	PT2019IBRgESERPM2	POS	POS	1
4	1	4	PT2019IBRgESERNM4	NEG	NEG	1
5	1	5	PT2019IBRgESERPM3	POS	POS	1
6	1	6	PT2019IBRgESERPM1	POS	POS	1
7	1	7	PT2019IBRgESERNM2	NEG	NEG	1
8	1	8	PT2019IBRgESERNM5	NEG	NEG	1
9	1	9	PT2019IBRgESERPM4	POS	POS	1
10	1	10	PT2019IBRgESERNM3	NEG	NEG	1
11	1	11	PT2019IBRgESERPM5	**POS**	**NEG**	**0**
12	1	12	PT2019IBRgESERNM1	NEG	NEG	1
13	1	13	PT2019IBRgESERPM5	**POS**	**NEG**	**0**
14	1	14	PT2019IBRgESERPM4	POS	POS	1
15	1	15	PT2019IBRgESERPM2	POS	POS	1
16	1	16	PT2019IBRgESERNM4	NEG	NEG	1
17	1	17	PT2019IBRgESERNM5	NEG	NEG	1
18	1	18	PT2019IBRgESERNM2	NEG	NEG	1
19	1	19	PT2019IBRgESERPM3	POS	POS	1
20	1	20	PT2019IBRgESERPM1	POS	POS	1
21	2	1	PT2019IBRgESERNM2	NEG	NEG	1
22	2	2	PT2019IBRgESERNM4	NEG	NEG	1
23	2	3	PT2019IBRgESERNM3	NEG	NEG	1
24	2	4	PT2019IBRgESERPM3	POS	POS	1
25	2	5	PT2019IBRgESERNM1	NEG	NEG	1
26	2	6	PT2019IBRgESERPM2	POS	POS	1
27	2	7	PT2019IBRgESERPM4	POS	POS	1
28	2	8	PT2019IBRgESERNM4	NEG	NEG	1
29	2	9	PT2019IBRgESERNM5	NEG	NEG	1
30	2	10	PT2019IBRgESERPM5	POS	POS	1
31	2	11	PT2019IBRgESERNM1	NEG	NEG	1
32	2	12	PT2019IBRgESERPM1	POS	POS	1
33	2	13	PT2019IBRgESERPM4	POS	POS	1
34	2	14	PT2019IBRgESERPM5	POS	POS	1
35	2	15	PT2019IBRgESERPM1	POS	POS	1
36	2	16	PT2019IBRgESERPM2	POS	POS	1
37	2	17	PT2019IBRgESERNM2	NEG	NEG	1
38	2	18	PT2019IBRgESERPM3	POS	POS	1
39	2	19	PT2019IBRgESERNM3	NEG	NEG	1
40	2	20	PT2019IBRgESERNM5	NEG	NEG	1
41	3	1	PT2019IBRgESERNM3	NEG	NEG	1
42	3	2	PT2019IBRgESERNM1	NEG	NEG	1
43	3	3	PT2019IBRgESERPM2	POS	POS	1
44	3	4	PT2019IBRgESERNM4	NEG	NEG	1
45	3	5	PT2019IBRgESERPM3	POS	POS	1
46	3	6	PT2019IBRgESERPM1	POS	POS	1
47	3	7	PT2019IBRgESERNM2	NEG	NEG	1
48	3	8	PT2019IBRgESERNM5	NEG	NEG	1
49	3	9	PT2019IBRgESERPM4	POS	POS	1
50	3	10	PT2019IBRgESERNM3	NEG	NEG	1
51	3	11	PT2019IBRgESERPM5	POS	POS	1
52	3	12	PT2019IBRgESERNM1	NEG	NEG	1

53	3	13	PT2019IBRgESERPM5	POS	POS	1
54	3	14	PT2019IBRgESERPM4	POS	POS	1
55	3	15	PT2019IBRgESERPM2	POS	POS	1
56	3	16	PT2019IBRgESERNM4	NEG	NEG	1
57	3	17	PT2019IBRgESERNM5	NEG	NEG	1
58	3	18	PT2019IBRgESERNM2	NEG	NEG	1
59	3	19	PT2019IBRgESERPM3	POS	POS	1
60	3	20	PT2019IBRgESERPM1	POS	POS	1
61	4	1	PT2019IBRgESERNM2	NEG	NEG	1
62	4	2	PT2019IBRgESERNM4	NEG	NEG	1
63	4	3	PT2019IBRgESERNM3	NEG	NEG	1
64	4	4	PT2019IBRgESERPM3	POS	POS	1
65	4	5	PT2019IBRgESERNM1	NEG	NEG	1
66	4	6	PT2019IBRgESERPM2	POS	POS	1
67	4	7	PT2019IBRgESERPM4	POS	POS	1
68	4	8	PT2019IBRgESERNM4	NEG	NEG	1
69	4	9	PT2019IBRgESERNM5	NEG	NEG	1
70	4	10	PT2019IBRgESERPM5	**POS**	**NEG**	**0**
71	4	11	PT2019IBRgESERNM1	NEG	NEG	1
72	4	12	PT2019IBRgESERPM1	POS	POS	1
73	4	13	PT2019IBRgESERPM4	**POS**	**NEG**	**0**
74	4	14	PT2019IBRgESERPM5	POS	POS	1
75	4	15	PT2019IBRgESERPM1	POS	POS	1
76	4	16	PT2019IBRgESERPM2	POS	POS	1
77	4	17	PT2019IBRgESERNM2	NEG	NEG	1
78	4	18	PT2019IBRgESERPM3	POS	POS	1
79	4	19	PT2019IBRgESERNM3	NEG	NEG	1
80	4	20	PT2019IBRgESERNM5	NEG	NEG	1
81	5	1	PT2019IBRgESERNM3	NEG	NEG	1
82	5	2	PT2019IBRgESERNM1	NEG	NEG	1
83	5	3	PT2019IBRgESERPM2	POS	POS	1
84	5	4	PT2019IBRgESERNM4	NEG	NEG	1
85	5	5	PT2019IBRgESERPM3	POS	POS	1
86	5	6	PT2019IBRgESERPM1	POS	POS	1
87	5	7	PT2019IBRgESERNM2	NEG	NEG	1
88	5	8	PT2019IBRgESERNM5	NEG	NEG	1
89	5	9	PT2019IBRgESERPM4	POS	POS	1
90	5	10	PT2019IBRgESERNM3	NEG	NEG	1
91	5	11	PT2019IBRgESERPM5	POS	POS	1
92	5	12	PT2019IBRgESERNM1	NEG	NEG	1
93	5	13	PT2019IBRgESERPM5	POS	POS	1
94	5	14	PT2019IBRgESERPM4	POS	POS	1
95	5	15	PT2019IBRgESERPM2	POS	POS	1
96	5	16	PT2019IBRgESERNM4	NEG	NEG	1
97	5	17	PT2019IBRgESERNM5	NEG	NEG	1
98	5	18	PT2019IBRgESERNM2	NEG	NEG	1
99	5	19	PT2019IBRgESERPM3	POS	POS	1
100	5	20	PT2019IBRgESERPM1	POS	POS	1
101	6	1	PT2019IBRgESERNM2	NEG	NEG	1
102	6	2	PT2019IBRgESERNM4	NEG	NEG	1
103	6	3	PT2019IBRgESERNM3	NEG	NEG	1
104	6	4	PT2019IBRgESERPM3	POS	POS	1
105	6	5	PT2019IBRgESERNM1	NEG	NEG	1
106	6	6	PT2019IBRgESERPM2	POS	POS	1
107	6	7	PT2019IBRgESERPM4	POS	POS	1
108	6	8	PT2019IBRgESERNM4	NEG	NEG	1
109	6	9	PT2019IBRgESERNM5	NEG	NEG	1

110	6	10	PT2019IBRgESERPM5	POS	POS	1
111	6	11	PT2019IBRgESERNM1	NEG	NEG	1
112	6	12	PT2019IBRgESERPM1	POS	POS	1
113	6	13	PT2019IBRgESERPM4	POS	POS	1
114	6	14	PT2019IBRgESERPM5	POS	POS	1
115	6	15	PT2019IBRgESERPM1	POS	POS	1
116	6	16	PT2019IBRgESERPM2	POS	POS	1
117	6	17	PT2019IBRgESERNM2	NEG	NEG	1
118	6	18	PT2019IBRgESERPM3	POS	POS	1
119	6	19	PT2019IBRgESERNM3	NEG	NEG	1
120	6	20	PT2019IBRgESERNM5	NEG	NEG	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 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 2 and Table 4).
The IBRgB participating laboratories, except LAB1, used IBRgB antibody ELISA kits from 3 different commercial kit producers : IDVet (1 batch: C59), Idexx (1 batch: P701), Indical (2 batches: F20180041, CT270045). LAB1 used a home made ELISA (batch: CGB.9Q08).
LAB5 performed long (LAB5.1) and short (LAB5.2) incubation with the same ELISA kit.

For the detection of IBRgE-specific antibodies in reference milk samples LAB2, LAB3, LAB5 and LAB6 provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). LAB1 misclassified the 2 aliquots of the reference milk sample PT2019IBRgESERPM5 (90% of agreement). LAB4 misclassified 1 out of 2 aliquots of the reference milk sample PT2019IBRgESERPM4 and 1 out of 2 aliquots of the reference milk sample PT2019IBRgESERPM5 (90% of agreement) (Table 3 and Table 5).
The IBRgE participating laboratories, except LAB1, used ELISA kits from 2 different producers: IN3 Diagnostics (2 batches: 738120, 739070) and IDEXX (1 batch : Q141). LAB1 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 and IBRgE achieved a satisfactory performance for the detection of IBRgB-specific and IBRgE-specific antibodies in bovine milk samples.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

Comité du Lait ASBL (Battice, Belgium)

HIPRA Scientific SLU (Girona, Spain)

IDEXX Technologies GmbH (Bäch, Switzerland)

Lavetan NV (Turnhout, Belgium)

MCC-Vlaanderen (Lier, Belgium)

Sciensano (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 (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=minimum and P75=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

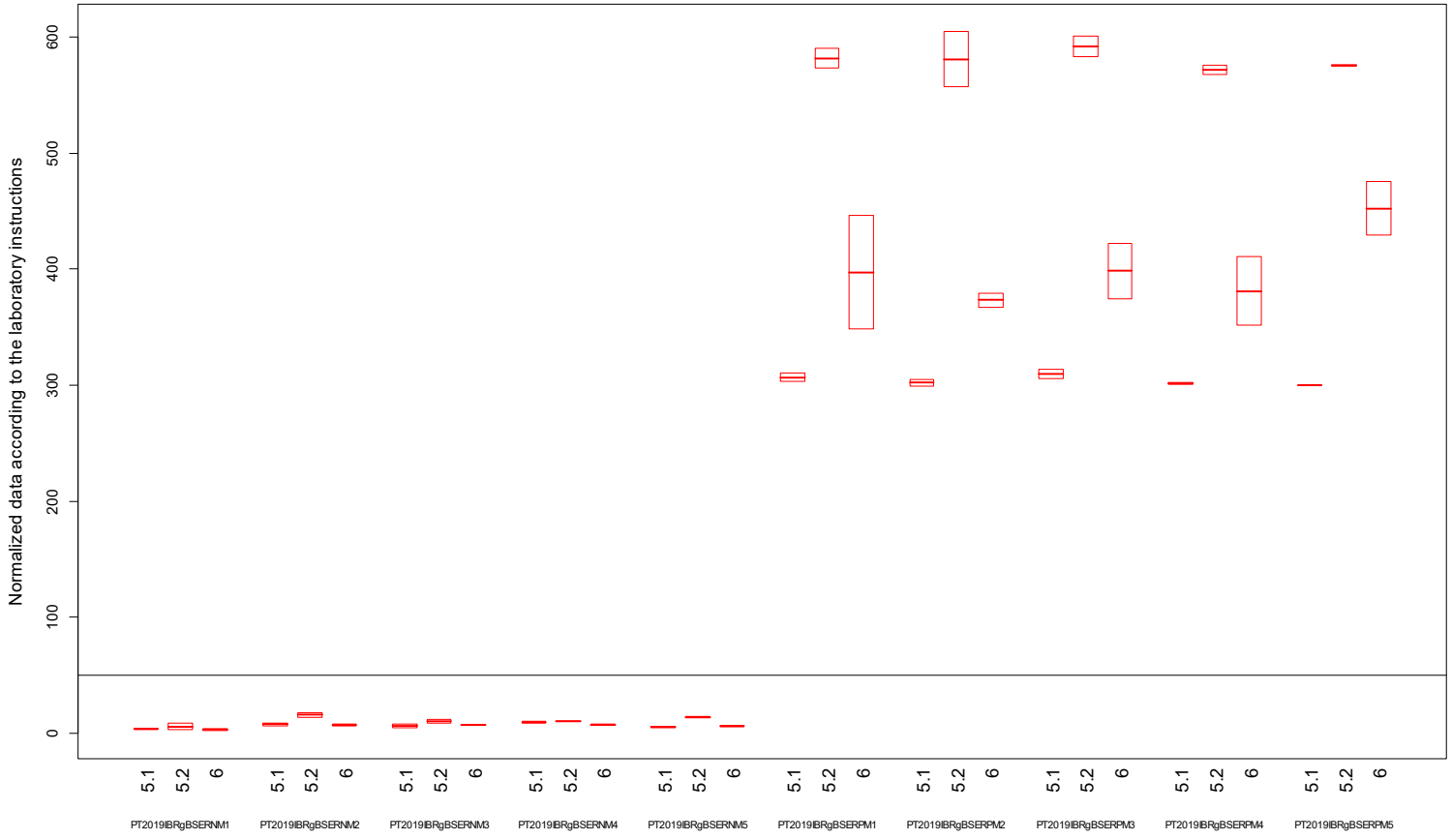

Figure 1. Box plots showing the normalized data according the laboratory instructions per reference milk and per participating laboratory.
Cut-off values 50 is shown by a horizontal line.

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

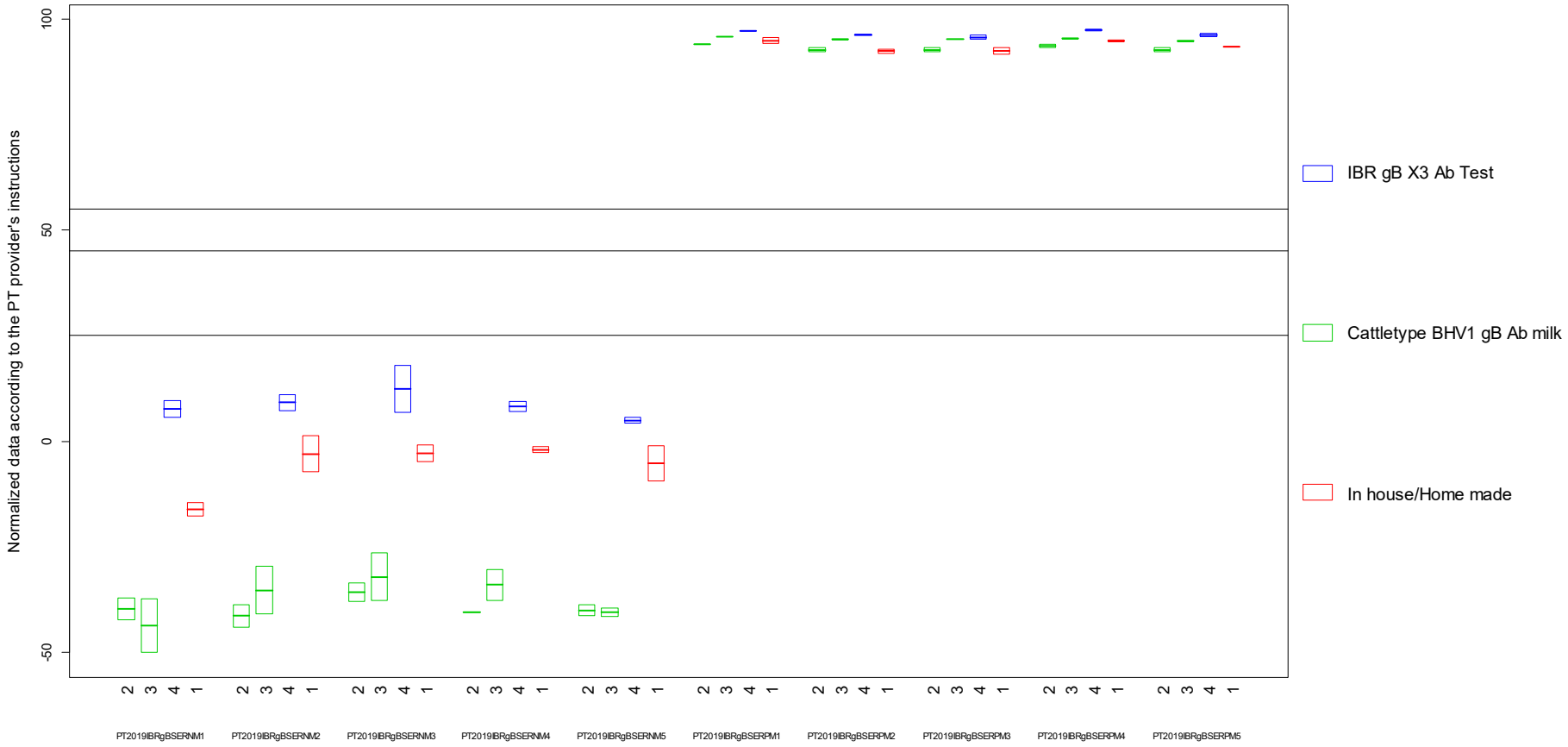

Figure 2. Box plots showing the normalized data according the PT provider per reference milk and per participating laboratory. The laboratories, except LAB1, used IBRgB antibody ELISA kits from 2 different commercial kit producers : Indical and Idexx. LAB1 used a home made ELISA. Cut-off values (Indical and Idexx 45-55, Home made 25) are shown by horizontal lines.

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

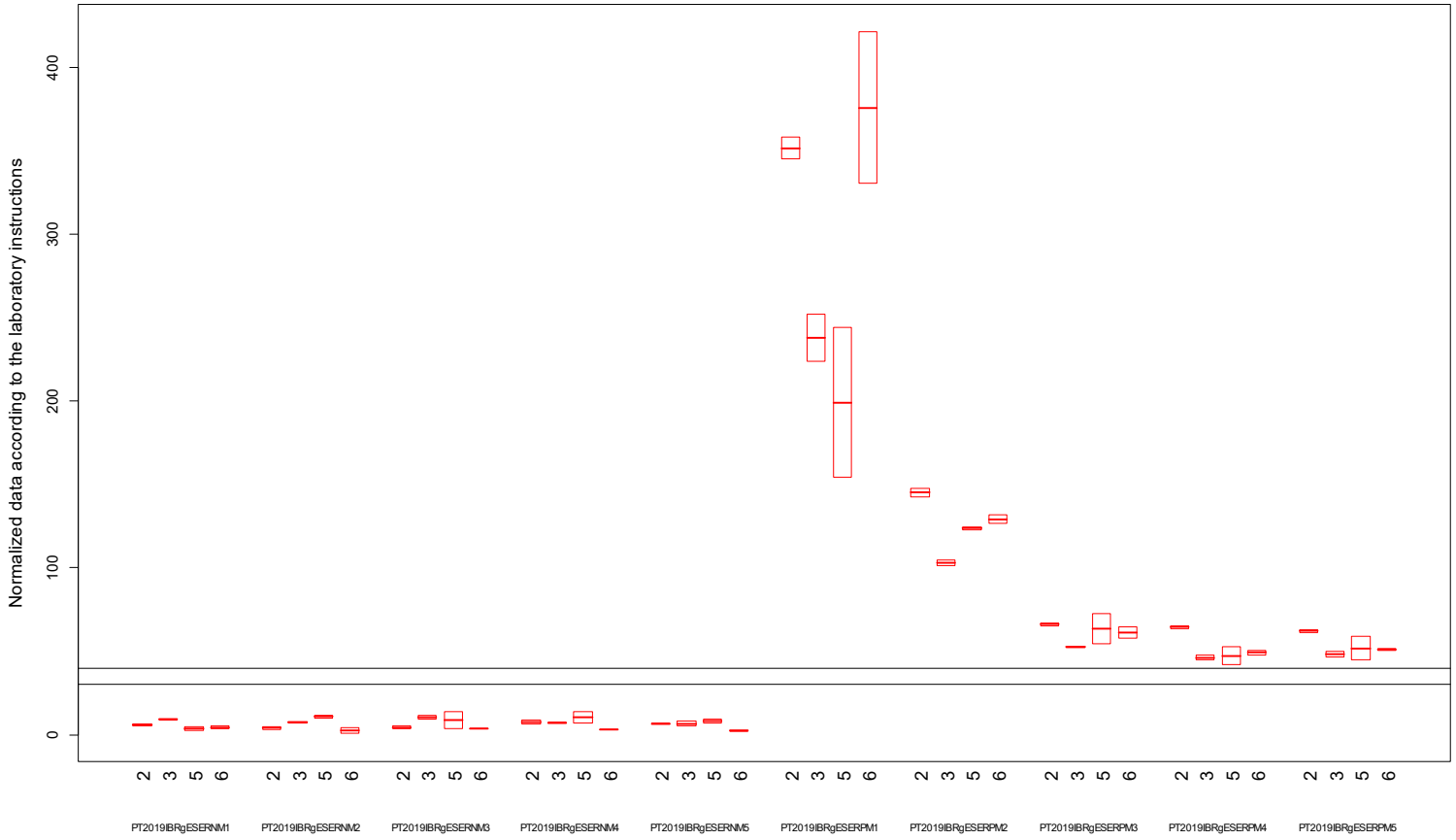

Figure 3. Box plots showing the normalized data according the laboratory instructions per reference milk and per participating laboratory.
Cut-off values 30-40 are shown by horizontal lines.

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

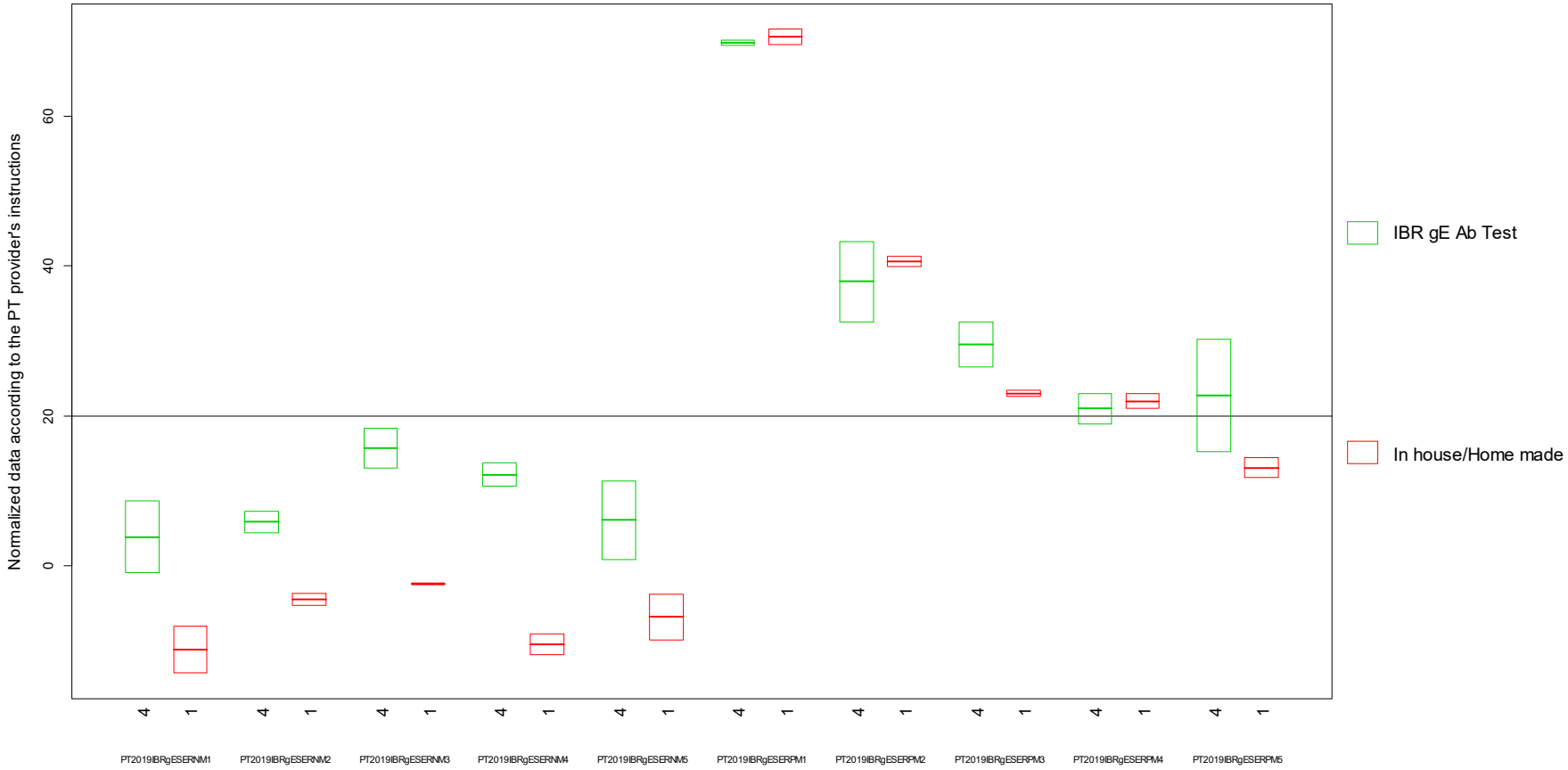

Figure 4. Box plots showing the normalized data according the PT provider per reference milk and per participating laboratory. LAB4 used the IDEXX ELISA IBR gE Ab test and LAB1 used a home made ELISA. Cut-off value 20 of the home made ELISA is shown by a horizontal line.