CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE
GROESELENBERG 99 – B 1180 BRUSSELS (UKKEL)
TEL: +32 (0)2 379 04 11
FAX : + 32 (0)2 379 06 70
HTTP: // WWW.CODA-CERVA.BE

172-PT

PROFICIENCY TESTING 2017

BRUCELLOSIS (BRU)

Detection of BRU-specific antibodies in:

(i) bovine serum by the Serum Agglutination test of Wright with EDTA (SAW-EDTA) and/or the Rose Bengal Test (RBT) and/or Enzyme Linked Immunosorbent Assay (ELISA)

(ii) bovine milk by ELISA

CODA-CERVA-UCCLE

DATE BEGIN PT: 25 SEPTEMBER 2017

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I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of BRU-specific antibodies in (i) bovine serum by SAW-EDTA and/or RBT and/or ELISA, and/or (ii) bovine milk by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be tested by means of SAW-EDTA and/or RBT and/or a BRU antibody ELISA, whereas predefined reference milk samples must be tested by means of a BRU antibody ELISA. The procedures for these tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference serum samples

Replicates of 6 reference serum samples of bovine origin, either free from detectable BRU-specific antibodies (n=3; coded 'PT2017BRUSERNS1', 'PT2017BRUSERNS2' and 'PT2017BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2017BRUSERPS1', 'PT2017BRUSERPS2' and 'PT2017BRUSERPS3'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference serum samples PT2017BRUSERPS1, 3 aliquots of the reference serum samples PT2017BRUSERNS1 and PT2017BRUSERNS2 and 4 aliquots of the reference serum samples PT2017BRUSERNS3, PT2017BRUSERPS2 and PT2017BRUSERPS3. The identification numbers of the reference serum samples were randomized for all participants (Table 6, Table 7 and Table 8).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was determined based on (i) the historical background of the animals and/or (ii) the results obtained during pre-verification, hereby using SAW with and without EDTA, RBT, an in-house BRU antibody ELISA test and the complement fixation test.
The reference serum samples PT2017BRUSERNS2 and PT2017BRUSERNS3 were derived from BRU-free farms. The reference serum sample PT2017BRUSERNS1 was a sample taken at abattoir and obtained from a BRU-free farm, whereas the reference serum sample PT2017BRUSERPS2 and PT2017BRUSERPS3 were a 1/16 dilution of serum obtained from a BRU-positive farm during a BRU breakdown in December 2010 in Belgium (resp.serum 6456 and 1909). The reference serum sample PT2017BRUSERPS1 was a serum obtained from an animal that was experimentally infected with the *Brucella abortus* strain W99 (serum 1174). For each reference serum sample, the same qualitative result was obtained with all test methods used. Taken together, the reference serum samples PT2017BRUSERNS1, PT2017BRUSERNS2 and PT2017BRUSERNS3 were considered as negative sera, and the reference serum samples PT2017BRUSERPS1, PT2017BRUSERPS2 and PT2017BRUSERPS3 as positive sera for BRU-specific antibodies.

After aliquoting and lyophilisation of the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using SAW-EDTA, RBT and an in-house BRU antibody ELISA test, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample with each test method used. Before the PT, only 3 aliquots of each reference serum sample were tested to confirm their stability and status (pre-verification) using SAW-EDTA, RBT and an in-house BRU antibody ELISA test. 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 BRU-specific antibodies in bovine serum. In addition, one aliquot of each reference serum sample was tested after the PT in order to confirm their stability and status (post-verification) using SAW-EDTA, RBT and an in-house BRU antibody ELISA test.

III.2.2. Reference milk samples

Replicates of 6 reference milk samples of bovine origin, either free from detectable BRU-specific antibodies (n=2; coded 'PT2017BRUSERNM1' and 'PT2017BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2017BRUSERPM1', 'PT2017BRUSERPM2', 'PT2017BRUSERPM3' and 'PT2017BRUSERPM4'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference milk samples PT2017BRUSERNM2 and PT2017BRUSERPM4, 3 aliquots of the reference milk sample PT2017BRUSERPM1, 4 aliquots of the reference milk samples PT2017BRUSERNM1 and PT2017BRUSERPM3 and 5 aliquots of the reference milk sample PT2017BRUSERPM2. The identification numbers of the reference milk samples were randomized for all participants (Table 9).

For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference milk samples was determined based on (i) the historical background of the samples and (ii) the results obtained during pre-verification, hereby using the BRU antibody ELISA test kit from IDEXX Montpellier SAS. The reference milk samples PT2017BRUSERNM1 and PT2017BRUSERNM2 were derived from 2 different batches of commercial whole milk, whereas the other reference milk samples were commercial whole milk samples spiked with serum containing BRU-specific antibodies. More specifically, PT2017BRUSERPM1 was spiked with serum 3467 in a 1/12800 dilution and PT2017BRUSERPM2 was spiked with serum 3667 in a 1/1000 dilution, respectively, whereas PT2017BRUSERPM3 was spiked with serum 3467 in a 1/6400 and PT2017BRUSERPM4 was spiked with serum 1275 in a 1/150 dilution. Serums 3467, 3667 and 1275 were obtained from animals that were experimentally infected with the *Brucella abortus* strain W99. Taken together, the reference samples PT2017BRUSERNM1 and PT2017BRUSERNM2 were considered as negative milk samples, and the reference samples PT2017BRUSERPM1, PT2017BRUSERPM2, PT2017BRUSERPM3 and PT2017BRUSERPM4 as variably positive milk samples in BRU antibody ELISA.

After aliquoting the different reference milk samples, a homogeneity check was performed on 10 aliquots of each reference milk sample using the BRU antibody ELISA test kit from IDEXX Montpellier SAS, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Before the PT, only 3 aliquots of each reference milk sample were tested to confirm their stability and status (pre-verification) using the BRU antibody ELISA test kit from IDEXX Montpellier SAS. 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 BRU-specific antibodies in bovine milk. In addition, one aliquot of each reference milk sample was tested after the PT in order to confirm their stability and status (post-verification) using the BRU antibody ELISA test kit from IDEXX Montpellier SAS.

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Reference serum samples

III.3.1.1. Classification of results

Results provided by the participating laboratories are categorized as *success* or *failure* as follows:

- For SAW-EDTA: *success* = the reported result equals the assigned titre ± 1; *failure* = the reported result does not equal the assigned titre ± 1. According the PT-provider instructions the following possibilities were forseen: NEG, 25 (NEG), 30, 50 and ≥ 100.
- For RBT and ELISA: *success* = the reported result matches with the assigned status; *failure* = the reported result does not match with the assigned status.

III.3.1.2. Level of agreement

For each serological test performed, the level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference serum samples.

III.3.1.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference serum samples is 100% for SAW-EDTA and at least 90% for RBT and ELISA.

III.3.2. Reference milk samples

III.3.2.1. 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.3.2.2. 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 milk samples used for this PT.

III.3.2.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference milk samples is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at CODA-CERVA-Uccle.

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

LAB1, LAB2 and LAB5 participated in both the PT serum and the PT milk and hence received 40 aliquots: 20 aliquots of reference serum samples and 20 aliquots of reference milk samples. In contrast, LAB3, LAB4 and LAB6 only participated in the PT serum, whereas LAB7 only participated in the PT milk. These 4 participating laboratories hence received either 20 aliquots of reference serum samples or 20 aliquots of reference milk samples. An overview of the different serological tests performed by the laboratories participating to the PT serum and milk can be found in Table 1. The reference serum samples were sent lyophilized, whereas the reference milk samples were sent frozen (dry ice) to each of the participating laboratories by national courier or international courier on 25th of September 2017 (200 aliquots in total). All participants acknowledged receipt of the samples on the same day except LAB6 which acknowledged receipt of the serum samples on 27th of September 2017 and LAB4 on 28th of September 2017.
Analyses were performed between 26th of September and 6th of October 2017 for serum and between 26th of September and 2nd of October 2017 for milk (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 4th of October and 24th of October 2017 (Table 1). Hereby, all laboratories except LAB1 respected the deadline of the 13th of October 2017 for submission of the results.

LAB4 did not submit results for the test SAW-EDTA.

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

Laboratory	Reference samples received	Start of analysis serum			Start of analysis milk	Submission of the results (Excel file)
		SAW-EDTA	ELISA	RBT		
LAB1	25/09/2017	27-28/09/2017	28/09/2017	28/09/2017	27/09/2017	19/10/2017 (serum) 24/10/2017 (milk)
LAB2	25/09/2017	26/09/2017	28/09/2017	26/09/2017	27/09/2017	11/10/2017
LAB3	25/09/2017	28/09/2017	28/09/2017	28/09/2017	NA	12/10/2017
LAB4	28/09/2017	/	04/10/2017	02/10/2017	NA	11/10/2017
LAB5	25/09/2017	NA	NA	26/09/2017	26/09/2017	04/10/2017
LAB6	27/09/2017	NA	06/10/2017	NA	NA	06/10/2017
LAB7	25/09/2017	NA	NA	NA	02/10/2017	10/10/2017

Legend: NA = not applicable

IV.3. Compliance with the procedure

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

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

IV.4.1.1. Reference serum samples

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence achieved 100% of agreement for the serological tests performed: SAW-EDTA (Table 2) and/or RBT (Table 3) and/or ELISA (Table 4).

Table 2. SAW-EDTA: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)

Table 3. RBT: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**2**	**3**	**4**	**5**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

Table 4. ELISA: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**2**	**3**	**4**	**6**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

IV.4.1.2. Reference milk samples

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples and hence achieved 100% of agreement (Table 5).

Table 5. ELISA: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the BRU reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	**2**	**5**	**7**
failure	0 (0)	0 (0)	0 (0)	0 (0)
success	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

IV.4.2.1. Reference serum samples

No variability between LAB1, LAB2, LAB3, LAB4, LAB5 and LAB6 could be observed since these participants correctly identified all reference serum samples with each serological test performed.

For all 3 serological tests included in the PT serum, the obtained results and the assigned statuses for the reference serum samples are shown per participating laboratory in Table 6 (SAW-EDTA), Table 7 (RBT) and Table 8 (ELISA).

IV.4.2.2. Reference milk samples

No variability between LAB1, LAB2, LAB5 and LAB7 could be observed since these participants correctly identified all reference milk samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference milk samples are shown in Table 9.

Table 6. SAW-EDTA: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BRUSERNS3	NEG	NEG	1
2	1	2	PT2017BRUSERPS3	>=100	>=100	1
3	1	3	PT2017BRUSERNS2	NEG	NEG	1
4	1	4	PT2017BRUSERPS2	50	50	1
5	1	5	PT2017BRUSERNS3	NEG	NEG	1
6	1	6	PT2017BRUSERNS1	NEG	NEG	1
7	1	7	PT2017BRUSERNS2	NEG	NEG	1
8	1	8	PT2017BRUSERPS1	>=100	>=100	1
9	1	9	PT2017BRUSERPS3	>=100	>=100	1
10	1	10	PT2017BRUSERNS3	NEG	NEG	1
11	1	11	PT2017BRUSERPS2	50	50	1
12	1	12	PT2017BRUSERNS1	NEG	NEG	1
13	1	13	PT2017BRUSERPS3	>=100	>=100	1
14	1	14	PT2017BRUSERNS2	NEG	NEG	1
15	1	15	PT2017BRUSERPS1	>=100	>=100	1
16	1	16	PT2017BRUSERPS2	50	50	1
17	1	17	PT2017BRUSERPS3	>=100	>=100	1
18	1	18	PT2017BRUSERNS1	NEG	NEG	1
19	1	19	PT2017BRUSERNS3	NEG	NEG	1
20	1	20	PT2017BRUSERPS2	50	50	1

(Table 6 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2017BRUSERNS2	NEG	NEG	1
22	2	2	PT2017BRUSERPS1	>=100	>=100	1
23	2	3	PT2017BRUSERNS3	NEG	NEG	1
24	2	4	PT2017BRUSERNS1	NEG	NEG	1
25	2	5	PT2017BRUSERPS2	50	50	1
26	2	6	PT2017BRUSERNS2	NEG	NEG	1
27	2	7	PT2017BRUSERPS2	50	50	1
28	2	8	PT2017BRUSERPS3	>=100	>=100	1
29	2	9	PT2017BRUSERNS1	NEG	NEG	1
30	2	10	PT2017BRUSERPS3	>=100	>=100	1
31	2	11	PT2017BRUSERPS1	>=100	>=100	1
32	2	12	PT2017BRUSERNS3	NEG	NEG	1
33	2	13	PT2017BRUSERNS2	NEG	NEG	1
34	2	14	PT2017BRUSERPS2	50	50	1
35	2	15	PT2017BRUSERPS3	>=100	>=100	1
36	2	16	PT2017BRUSERNS3	NEG	NEG	1
37	2	17	PT2017BRUSERPS3	>=100	>=100	1
38	2	18	PT2017BRUSERPS2	50	50	1
39	2	19	PT2017BRUSERNS1	NEG	NEG	1
40	2	20	PT2017BRUSERNS3	NEG	NEG	1
41	3	1	PT2017BRUSERNS3	NEG	NEG	1
42	3	2	PT2017BRUSERPS3	>=100	>=100	1
43	3	3	PT2017BRUSERNS2	NEG	NEG	1
44	3	4	PT2017BRUSERPS2	50	50	1
45	3	5	PT2017BRUSERNS3	NEG	NEG	1
46	3	6	PT2017BRUSERNS1	NEG	NEG	1
47	3	7	PT2017BRUSERNS2	NEG	NEG	1
48	3	8	PT2017BRUSERPS1	>=100	50	1
49	3	9	PT2017BRUSERPS3	>=100	>=100	1
50	3	10	PT2017BRUSERNS3	NEG	NEG	1
51	3	11	PT2017BRUSERPS2	50	50	1
52	3	12	PT2017BRUSERNS1	NEG	NEG	1
53	3	13	PT2017BRUSERPS3	>=100	>=100	1
54	3	14	PT2017BRUSERNS2	NEG	NEG	1
55	3	15	PT2017BRUSERPS1	>=100	50	1
56	3	16	PT2017BRUSERPS2	50	50	1
57	3	17	PT2017BRUSERPS3	>=100	>=100	1
58	3	18	PT2017BRUSERNS1	NEG	NEG	1
59	3	19	PT2017BRUSERNS3	NEG	NEG	1
60	3	20	PT2017BRUSERPS2	50	50	1

Table 7. RBT: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BRUSERNS3	NEG	NEG	1
2	1	2	PT2017BRUSERPS3	POS	POS	1
3	1	3	PT2017BRUSERNS2	NEG	NEG	1
4	1	4	PT2017BRUSERPS2	POS	POS	1
5	1	5	PT2017BRUSERNS3	NEG	NEG	1
6	1	6	PT2017BRUSERNS1	NEG	NEG	1
7	1	7	PT2017BRUSERNS2	NEG	NEG	1
8	1	8	PT2017BRUSERPS1	POS	POS	1
9	1	9	PT2017BRUSERPS3	POS	POS	1
10	1	10	PT2017BRUSERNS3	NEG	NEG	1
11	1	11	PT2017BRUSERPS2	POS	POS	1
12	1	12	PT2017BRUSERNS1	NEG	NEG	1
13	1	13	PT2017BRUSERPS3	POS	POS	1
14	1	14	PT2017BRUSERNS2	NEG	NEG	1
15	1	15	PT2017BRUSERPS1	POS	POS	1
16	1	16	PT2017BRUSERPS2	POS	POS	1
17	1	17	PT2017BRUSERPS3	POS	POS	1
18	1	18	PT2017BRUSERNS1	NEG	NEG	1
19	1	19	PT2017BRUSERNS3	NEG	NEG	1
20	1	20	PT2017BRUSERPS2	POS	POS	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2017BRUSERNS2	NEG	NEG	1
22	2	2	PT2017BRUSERPS1	POS	POS	1
23	2	3	PT2017BRUSERNS3	NEG	NEG	1
24	2	4	PT2017BRUSERNS1	NEG	NEG	1
25	2	5	PT2017BRUSERPS2	POS	POS	1
26	2	6	PT2017BRUSERNS2	NEG	NEG	1
27	2	7	PT2017BRUSERPS2	POS	POS	1
28	2	8	PT2017BRUSERPS3	POS	POS	1
29	2	9	PT2017BRUSERNS1	NEG	NEG	1
30	2	10	PT2017BRUSERPS3	POS	POS	1
31	2	11	PT2017BRUSERPS1	POS	POS	1
32	2	12	PT2017BRUSERNS3	NEG	NEG	1
33	2	13	PT2017BRUSERNS2	NEG	NEG	1
34	2	14	PT2017BRUSERPS2	POS	POS	1
35	2	15	PT2017BRUSERPS3	POS	POS	1
36	2	16	PT2017BRUSERNS3	NEG	NEG	1
37	2	17	PT2017BRUSERPS3	POS	POS	1
38	2	18	PT2017BRUSERPS2	POS	POS	1
39	2	19	PT2017BRUSERNS1	NEG	NEG	1
40	2	20	PT2017BRUSERNS3	NEG	NEG	1
41	3	1	PT2017BRUSERNS3	NEG	NEG	1
42	3	2	PT2017BRUSERPS3	POS	POS	1
43	3	3	PT2017BRUSERNS2	NEG	NEG	1
44	3	4	PT2017BRUSERPS2	POS	POS	1
45	3	5	PT2017BRUSERNS3	NEG	NEG	1
46	3	6	PT2017BRUSERNS1	NEG	NEG	1
47	3	7	PT2017BRUSERNS2	NEG	NEG	1
48	3	8	PT2017BRUSERPS1	POS	POS	1
49	3	9	PT2017BRUSERPS3	POS	POS	1
50	3	10	PT2017BRUSERNS3	NEG	NEG	1
51	3	11	PT2017BRUSERPS2	POS	POS	1
52	3	12	PT2017BRUSERNS1	NEG	NEG	1
53	3	13	PT2017BRUSERPS3	POS	POS	1
54	3	14	PT2017BRUSERNS2	NEG	NEG	1
55	3	15	PT2017BRUSERPS1	POS	POS	1
56	3	16	PT2017BRUSERPS2	POS	POS	1
57	3	17	PT2017BRUSERPS3	POS	POS	1
58	3	18	PT2017BRUSERNS1	NEG	NEG	1
59	3	19	PT2017BRUSERNS3	NEG	NEG	1
60	3	20	PT2017BRUSERPS2	POS	POS	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2017BRUSERNS2	NEG	NEG	1
62	4	2	PT2017BRUSERPS1	POS	POS	1
63	4	3	PT2017BRUSERNS3	NEG	NEG	1
64	4	4	PT2017BRUSERNS1	NEG	NEG	1
65	4	5	PT2017BRUSERPS2	POS	POS	1
66	4	6	PT2017BRUSERNS2	NEG	NEG	1
67	4	7	PT2017BRUSERPS2	POS	POS	1
68	4	8	PT2017BRUSERPS3	POS	POS	1
69	4	9	PT2017BRUSERNS1	NEG	NEG	1
70	4	10	PT2017BRUSERPS3	POS	POS	1
71	4	11	PT2017BRUSERPS1	POS	POS	1
72	4	12	PT2017BRUSERNS3	NEG	NEG	1
73	4	13	PT2017BRUSERNS2	NEG	NEG	1
74	4	14	PT2017BRUSERPS2	POS	POS	1
75	4	15	PT2017BRUSERPS3	POS	POS	1
76	4	16	PT2017BRUSERNS3	NEG	NEG	1
77	4	17	PT2017BRUSERPS3	POS	POS	1
78	4	18	PT2017BRUSERPS2	POS	POS	1
79	4	19	PT2017BRUSERNS1	NEG	NEG	1
80	4	20	PT2017BRUSERNS3	NEG	NEG	1
81	5	1	PT2017BRUSERNS3	NEG	NEG	1
82	5	2	PT2017BRUSERPS3	POS	POS	1
83	5	3	PT2017BRUSERNS2	NEG	NEG	1
84	5	4	PT2017BRUSERPS2	POS	POS	1
85	5	5	PT2017BRUSERNS3	NEG	NEG	1
86	5	6	PT2017BRUSERNS1	NEG	NEG	1
87	5	7	PT2017BRUSERNS2	NEG	NEG	1
88	5	8	PT2017BRUSERPS1	POS	POS	1
89	5	9	PT2017BRUSERPS3	POS	POS	1
90	5	10	PT2017BRUSERNS3	NEG	NEG	1
91	5	11	PT2017BRUSERPS2	POS	POS	1
92	5	12	PT2017BRUSERNS1	NEG	NEG	1
93	5	13	PT2017BRUSERPS3	POS	POS	1
94	5	14	PT2017BRUSERNS2	NEG	NEG	1
95	5	15	PT2017BRUSERPS1	POS	POS	1
96	5	16	PT2017BRUSERPS2	POS	POS	1
97	5	17	PT2017BRUSERPS3	POS	POS	1
98	5	18	PT2017BRUSERNS1	NEG	NEG	1
99	5	19	PT2017BRUSERNS3	NEG	NEG	1
100	5	20	PT2017BRUSERPS2	POS	POS	1

Table 8. ELISA-SERUM: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BRUSERNS3	NEG	NEG	1
2	1	2	PT2017BRUSERPS3	POS	POS	1
3	1	3	PT2017BRUSERNS2	NEG	NEG	1
4	1	4	PT2017BRUSERPS2	POS	POS	1
5	1	5	PT2017BRUSERNS3	NEG	NEG	1
6	1	6	PT2017BRUSERNS1	NEG	NEG	1
7	1	7	PT2017BRUSERNS2	NEG	NEG	1
8	1	8	PT2017BRUSERPS1	POS	POS	1
9	1	9	PT2017BRUSERPS3	POS	POS	1
10	1	10	PT2017BRUSERNS3	NEG	NEG	1
11	1	11	PT2017BRUSERPS2	POS	POS	1
12	1	12	PT2017BRUSERNS1	NEG	NEG	1
13	1	13	PT2017BRUSERPS3	POS	POS	1
14	1	14	PT2017BRUSERNS2	NEG	NEG	1
15	1	15	PT2017BRUSERPS1	POS	POS	1
16	1	16	PT2017BRUSERPS2	POS	POS	1
17	1	17	PT2017BRUSERPS3	POS	POS	1
18	1	18	PT2017BRUSERNS1	NEG	NEG	1
19	1	19	PT2017BRUSERNS3	NEG	NEG	1
20	1	20	PT2017BRUSERPS2	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2017BRUSERNS2	NEG	NEG	1
22	2	2	PT2017BRUSERPS1	POS	POS	1
23	2	3	PT2017BRUSERNS3	NEG	NEG	1
24	2	4	PT2017BRUSERNS1	NEG	NEG	1
25	2	5	PT2017BRUSERPS2	POS	POS	1
26	2	6	PT2017BRUSERNS2	NEG	NEG	1
27	2	7	PT2017BRUSERPS2	POS	POS	1
28	2	8	PT2017BRUSERPS3	POS	POS	1
29	2	9	PT2017BRUSERNS1	NEG	NEG	1
30	2	10	PT2017BRUSERPS3	POS	POS	1
31	2	11	PT2017BRUSERPS1	POS	POS	1
32	2	12	PT2017BRUSERNS3	NEG	NEG	1
33	2	13	PT2017BRUSERNS2	NEG	NEG	1
34	2	14	PT2017BRUSERPS2	POS	POS	1
35	2	15	PT2017BRUSERPS3	POS	POS	1
36	2	16	PT2017BRUSERNS3	NEG	NEG	1
37	2	17	PT2017BRUSERPS3	POS	POS	1
38	2	18	PT2017BRUSERPS2	POS	POS	1
39	2	19	PT2017BRUSERNS1	NEG	NEG	1
40	2	20	PT2017BRUSERNS3	NEG	NEG	1
41	3	1	PT2017BRUSERNS3	NEG	NEG	1
42	3	2	PT2017BRUSERPS3	POS	POS	1
43	3	3	PT2017BRUSERNS2	NEG	NEG	1
44	3	4	PT2017BRUSERPS2	POS	POS	1
45	3	5	PT2017BRUSERNS3	NEG	NEG	1
46	3	6	PT2017BRUSERNS1	NEG	NEG	1
47	3	7	PT2017BRUSERNS2	NEG	NEG	1
48	3	8	PT2017BRUSERPS1	POS	POS	1
49	3	9	PT2017BRUSERPS3	POS	POS	1
50	3	10	PT2017BRUSERNS3	NEG	NEG	1
51	3	11	PT2017BRUSERPS2	POS	POS	1
52	3	12	PT2017BRUSERNS1	NEG	NEG	1
53	3	13	PT2017BRUSERPS3	POS	POS	1
54	3	14	PT2017BRUSERNS2	NEG	NEG	1
55	3	15	PT2017BRUSERPS1	POS	POS	1
56	3	16	PT2017BRUSERPS2	POS	POS	1
57	3	17	PT2017BRUSERPS3	POS	POS	1
58	3	18	PT2017BRUSERNS1	NEG	NEG	1
59	3	19	PT2017BRUSERNS3	NEG	NEG	1
60	3	20	PT2017BRUSERPS2	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2017BRUSERNS2	NEG	NEG	1
62	4	2	PT2017BRUSERPS1	POS	POS	1
63	4	3	PT2017BRUSERNS3	NEG	NEG	1
64	4	4	PT2017BRUSERNS1	NEG	NEG	1
65	4	5	PT2017BRUSERPS2	POS	POS	1
66	4	6	PT2017BRUSERNS2	NEG	NEG	1
67	4	7	PT2017BRUSERPS2	POS	POS	1
68	4	8	PT2017BRUSERPS3	POS	POS	1
69	4	9	PT2017BRUSERNS1	NEG	NEG	1
70	4	10	PT2017BRUSERPS3	POS	POS	1
71	4	11	PT2017BRUSERPS1	POS	POS	1
72	4	12	PT2017BRUSERNS3	NEG	NEG	1
73	4	13	PT2017BRUSERNS2	NEG	NEG	1
74	4	14	PT2017BRUSERPS2	POS	POS	1
75	4	15	PT2017BRUSERPS3	POS	POS	1
76	4	16	PT2017BRUSERNS3	NEG	NEG	1
77	4	17	PT2017BRUSERPS3	POS	POS	1
78	4	18	PT2017BRUSERPS2	POS	POS	1
79	4	19	PT2017BRUSERNS1	NEG	NEG	1
80	4	20	PT2017BRUSERNS3	NEG	NEG	1
81	6	1	PT2017BRUSERNS2	NEG	NEG	1
82	6	2	PT2017BRUSERPS1	POS	POS	1
83	6	3	PT2017BRUSERNS3	NEG	NEG	1
84	6	4	PT2017BRUSERNS1	NEG	NEG	1
85	6	5	PT2017BRUSERPS2	POS	POS	1
86	6	6	PT2017BRUSERNS2	NEG	NEG	1
87	6	7	PT2017BRUSERPS2	POS	POS	1
88	6	8	PT2017BRUSERPS3	POS	POS	1
89	6	9	PT2017BRUSERNS1	NEG	NEG	1
90	6	10	PT2017BRUSERPS3	POS	POS	1
91	6	11	PT2017BRUSERPS1	POS	POS	1
92	6	12	PT2017BRUSERNS3	NEG	NEG	1
93	6	13	PT2017BRUSERNS2	NEG	NEG	1
94	6	14	PT2017BRUSERPS2	POS	POS	1
95	6	15	PT2017BRUSERPS3	POS	POS	1
96	6	16	PT2017BRUSERNS3	NEG	NEG	1
97	6	17	PT2017BRUSERPS3	POS	POS	1
98	6	18	PT2017BRUSERPS2	POS	POS	1
99	6	19	PT2017BRUSERNS1	NEG	NEG	1
100	6	20	PT2017BRUSERNS3	NEG	NEG	1

Table 9. ELISA-MILK: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference milk samples (SAMPLE), the external identification of the reference milk samples (LABPOSIT), and the status assigned by the BRU reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BRUSERPM2	POS	POS	1
2	1	2	PT2017BRUSERNM1	NEG	NEG	1
3	1	3	PT2017BRUSERPM1	POS	POS	1
4	1	4	PT2017BRUSERNM1	NEG	NEG	1
5	1	5	PT2017BRUSERPM2	POS	POS	1
6	1	6	PT2017BRUSERPM3	POS	POS	1
7	1	7	PT2017BRUSERNM2	NEG	NEG	1
8	1	8	PT2017BRUSERPM4	POS	POS	1
9	1	9	PT2017BRUSERPM3	POS	POS	1
10	1	10	PT2017BRUSERPM1	POS	POS	1
11	1	11	PT2017BRUSERNM2	NEG	NEG	1
12	1	12	PT2017BRUSERNM1	NEG	NEG	1
13	1	13	PT2017BRUSERPM2	POS	POS	1
14	1	14	PT2017BRUSERPM1	POS	POS	1
15	1	15	PT2017BRUSERPM3	POS	POS	1
16	1	16	PT2017BRUSERPM2	POS	POS	1
17	1	17	PT2017BRUSERPM4	POS	POS	1
18	1	18	PT2017BRUSERNM1	NEG	NEG	1
19	1	19	PT2017BRUSERPM2	POS	POS	1
20	1	20	PT2017BRUSERPM3	POS	POS	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2017BRUSERNM2	NEG	NEG	1
22	2	2	PT2017BRUSERPM1	POS	POS	1
23	2	3	PT2017BRUSERPM2	POS	POS	1
24	2	4	PT2017BRUSERPM2	POS	POS	1
25	2	5	PT2017BRUSERNM1	NEG	NEG	1
26	2	6	PT2017BRUSERNM2	NEG	NEG	1
27	2	7	PT2017BRUSERPM3	POS	POS	1
28	2	8	PT2017BRUSERNM1	NEG	NEG	1
29	2	9	PT2017BRUSERPM2	POS	POS	1
30	2	10	PT2017BRUSERPM3	POS	POS	1
31	2	11	PT2017BRUSERNM1	NEG	NEG	1
32	2	12	PT2017BRUSERPM2	POS	POS	1
33	2	13	PT2017BRUSERPM1	POS	POS	1
34	2	14	PT2017BRUSERPM3	POS	POS	1
35	2	15	PT2017BRUSERPM4	POS	POS	1
36	2	16	PT2017BRUSERPM1	POS	POS	1
37	2	17	PT2017BRUSERNM1	NEG	NEG	1
38	2	18	PT2017BRUSERPM2	POS	POS	1
39	2	19	PT2017BRUSERPM3	POS	POS	1
40	2	20	PT2017BRUSERPM4	POS	POS	1
41	5	1	PT2017BRUSERPM2	POS	POS	1
42	5	2	PT2017BRUSERNM1	NEG	NEG	1
43	5	3	PT2017BRUSERPM1	POS	POS	1
44	5	4	PT2017BRUSERNM1	NEG	NEG	1
45	5	5	PT2017BRUSERPM2	POS	POS	1
46	5	6	PT2017BRUSERPM3	POS	POS	1
47	5	7	PT2017BRUSERNM2	NEG	NEG	1
48	5	8	PT2017BRUSERPM4	POS	POS	1
49	5	9	PT2017BRUSERPM3	POS	POS	1
50	5	10	PT2017BRUSERPM1	POS	POS	1
51	5	11	PT2017BRUSERNM2	NEG	NEG	1
52	5	12	PT2017BRUSERNM1	NEG	NEG	1
53	5	13	PT2017BRUSERPM2	POS	POS	1
54	5	14	PT2017BRUSERPM1	POS	POS	1
55	5	15	PT2017BRUSERPM3	POS	POS	1
56	5	16	PT2017BRUSERPM2	POS	POS	1
57	5	17	PT2017BRUSERPM4	POS	POS	1
58	5	18	PT2017BRUSERNM1	NEG	NEG	1
59	5	19	PT2017BRUSERPM2	POS	POS	1
60	5	20	PT2017BRUSERPM3	POS	POS	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	7	1	PT2017BRUSERNM2	NEG	NEG	1
62	7	2	PT2017BRUSERPM1	POS	POS	1
63	7	3	PT2017BRUSERPM2	POS	POS	1
64	7	4	PT2017BRUSERPM2	POS	POS	1
65	7	5	PT2017BRUSERNM1	NEG	NEG	1
66	7	6	PT2017BRUSERNM2	NEG	NEG	1
67	7	7	PT2017BRUSERPM3	POS	POS	1
68	7	8	PT2017BRUSERNM1	NEG	NEG	1
69	7	9	PT2017BRUSERPM2	POS	POS	1
70	7	10	PT2017BRUSERPM3	POS	POS	1
71	7	11	PT2017BRUSERNM1	NEG	NEG	1
72	7	12	PT2017BRUSERPM2	POS	POS	1
73	7	13	PT2017BRUSERPM1	POS	POS	1
74	7	14	PT2017BRUSERPM3	POS	POS	1
75	7	15	PT2017BRUSERPM4	POS	POS	1
76	7	16	PT2017BRUSERPM1	POS	POS	1
77	7	17	PT2017BRUSERNM1	NEG	NEG	1
78	7	18	PT2017BRUSERPM2	POS	POS	1
79	7	19	PT2017BRUSERPM3	POS	POS	1
80	7	20	PT2017BRUSERPM4	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing (i) reference serum samples of bovine origin for the detection of BRU-specific antibodies by SAW-EDTA and/or RBT and/or ELISA, and/or (ii) reference milk samples of bovine origin for the detection of BRU-specific antibodies by ELISA.

For the detection of BRU-specific antibodies in reference serum samples, all participating laboratories provided for all serological tests performed qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement).
The 3 participating laboratories that performed SAW-EDTA used a SAW antigen from the same producer, namely Zoetis (Synbiotics) (batch : 15SAW14). The 5 participating laboratories that performed RBT used a RBT antigen from 3 different producers, namely Zoetis (Synbiotics) (2 batches: 16ZBAB003 and 14BGT73), Institut Pourquier (batch: 406-100) and IDEXX (2 batches: 400-100 and 404-100). From the 5 participating laboratories that performed ELISA, LAB1 used an home made developed BRU antibody ELISA kit, whereas LAB2, LAB3, LAB4 and LAB6 used a commercially available BRU antibody ELISA kit from two different producers, namely Zoetis (Synbiotics) (batches : 1ZEAJ002, 16ZEAJ002 and 17ZEAJ003) and IDEXX (batch 6094).

For the detection of BRU-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).
The 4 participating laboratories that performed ELISA used the BRU antibody ELISA kit from IDEXX (batches 5078 and 6039).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if the level of agreement is 100% for SAW-EDTA (PT serum) and at least 90% for RBT (PT serum) and ELISA (PT serum and PT milk) (see III.3.1.3. and III.3.2.3.). Consequently, all partipants achieved a satisfactory performance for the detection of BRU-specific antibodies in reference serum samples by SAW-EDTA and/or RBT and/or ELISA. For milk, all partipants achivied satisfactory performance for the detection of BRU-specific antibodies in milk.

Coordinator proficiency tests
Katia Knapen

Appendix

Name of the participating laboratories

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE) (Maisons-Alfort, France)

Melkcontrolecentrum Vlaanderen (MCC-Vlaanderen) (Lier, Belgium)

Zoetis France (Lyon, France)

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

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software R. All quantitative data analyses were performed on normalized data, namely the percentages blocking calculated according to the instructions of the PT provider: [1- (OD_{Sample} / mean $OD_{Negative\ Kit\ Controls}$)] x 100.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

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

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

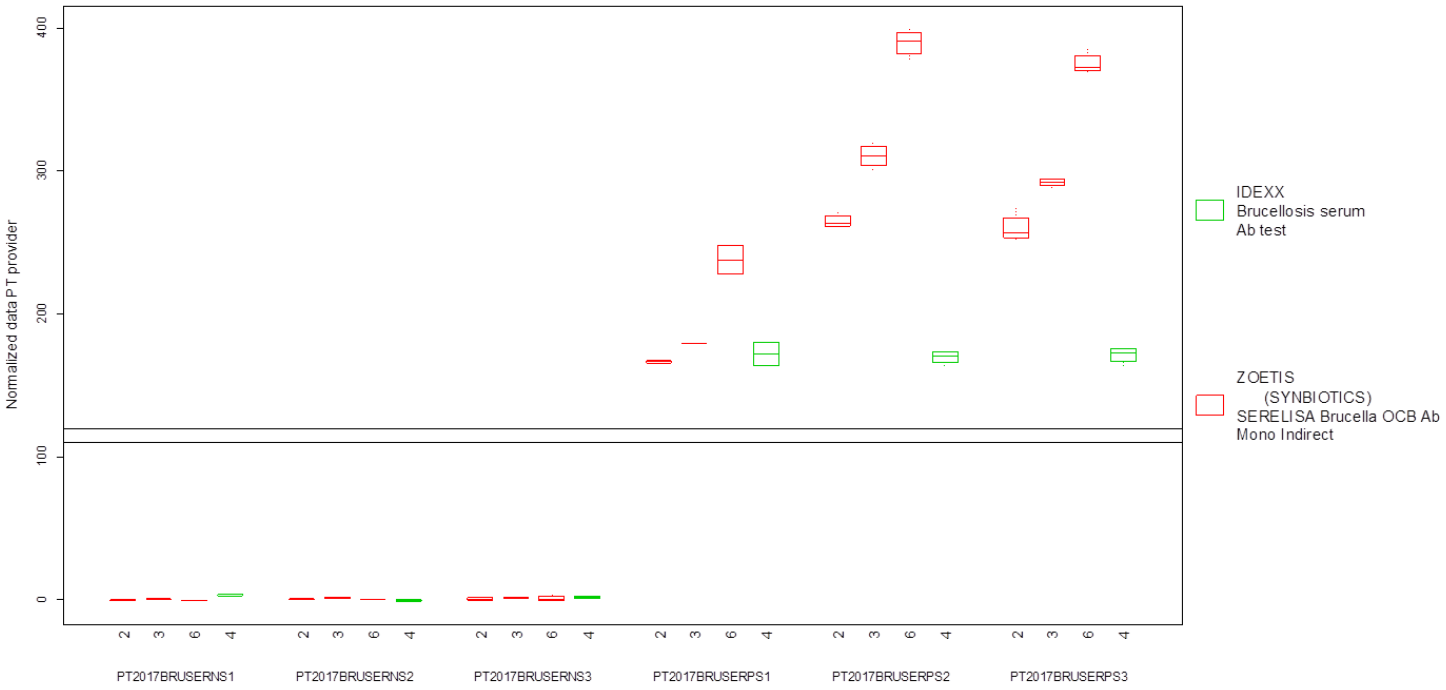

Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory except LAB1 which used an home made developed BRU antibody ELISA kit. LAB2, LAB3, LAB4 and LAB6 used a commercially available BRU antibody ELISA kit from two different producers, namely Zoetis (Synbiotics) (batches : 1ZEAJ002, 16ZEAJ002 and 17ZEAJ003) and IDEXX (batch 6094). The cut-off values (110-120) of IDEXX are shown by horizontal lines. The cut-off values of Zoetis aren't shown because they are depending on the OD value of the positive control (variable cut-off)

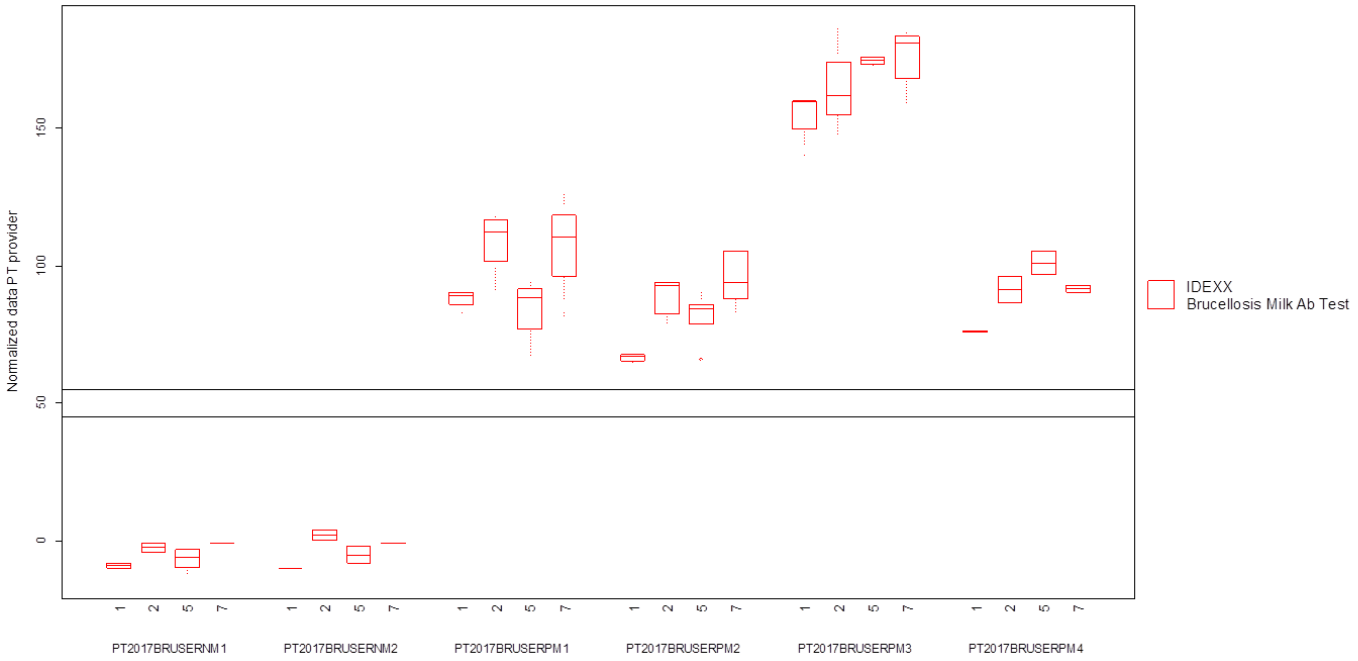

Figure 2. Box plots showing the percentage S/P ratio per reference milk sample and per participating laboratory. The 4 participating laboratories that performed ELISA used the BRU antibody ELISA kit from IDEXX (batches 5078 and 6039).
In addition, the cut-off value of 45-55% is shown by horizontal lines.