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172-PT

PROFICIENCY TESTING 2016

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: 12 SEPTEMBER 2016

DATE REPORT: 6 DECEMBER 2016

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 'PT2016BRUSERNS1', 'PT2016BRUSERNS2' and 'PT2016BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2016BRUSERPS1', 'PT2016BRUSERPS2' and 'PT2016BRUSERPS3'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference serum samples PT2016BRUSERPS1, 3 aliquots of the reference serum samples PT2016BRUSERNS1 and PT2016BRUSERNS2 and 4 aliquots of the reference serum samples PT2016BRUSERNS3, PT2016BRUSERPS2 and PT2016BRUSERPS3. 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 PT2016BRUSERNS1 and PT2016BRUSERNS2 were derived from BRU-free farms. The reference serum sample PT2016BRUSERNS3 was a sample taken at abattoir and obtained from a BRU-free farm, whereas the reference serum sample PT2016BRUSERPS3 was a 1/2 dilution of a serum obtained from a BRU-positive farm during a BRU breakdown in December 2010 in Belgium (serum 6459). The reference serum samples PT2016BRUSERPS1 and PT2016BRUSERPS2 were a 1/22,5 and a 1/2 dilution, respectively, of 2 different sera obtained from animals that were experimentally infected with the *Brucella abortus* strain W99 (serum 3467 and serum 3667, respectively). For each reference serum sample, the same qualitative result was obtained with all test methods used. Taken together, the reference serum samples PT2016BRUSERNS1, PT2016BRUSERNS2 and PT2016BRUSERNS3 were considered as negative sera, and the reference serum samples PT2016BRUSERPS1, PT2016BRUSERPS2 and PT2016BRUSERPS3 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. 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, 3 aliquots of each reference serum sample were 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 'PT2016BRUSERNM1' and 'PT2016BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2016BRUSERPM1', 'PT2016BRUSERPM2', 'PT2016BRUSERPM3' and 'PT2016BRUSERPM4'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference milk samples PT2016BRUSERNM2 and PT2016BRUSERPM4, 3 aliquots of the reference milk sample PT2016BRUSERPM1, 4 aliquots of the reference milk samples PT2016BRUSERNM1 and PT2016BRUSERPM3 and 5 aliquots of the reference milk sample PT2016BRUSERPM2. 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 PT2016BRUSERNM1 and PT2016BRUSERNM2 were derived from 2 different brands of commercial whole milk, whereas the other reference milk samples were commercial whole milk samples spiked with serum containing BRU-specific antibodies. More specifically, PT2016BRUSERPM1 was spiked with serum 1275 in a 1/200 dilution and PT2016BRUSERPM2 was spiked with serum 3667 in a 1/1000 dilution, respectively, whereas PT2016BRUSERPM3 and PT2016BRUSERPM4 were spiked with serum 3467 in a 1/6400 and a 1/12800 dilution. Serum 3467 and serum 3667 were both obtained from animals that were experimentally infected with the *Brucella abortus* strain W99 (see also III.2.1), whereas serum 1275 was derived from an animal that was experimentally infected with a *Brucella abortus* strain isolated in the field during a BRU outbreak in December 2010 in Belgium. Taken together, the reference samples PT2016BRUSERNM1 and PT2016BRUSERNM2 were considered as negative milk samples, and the reference samples PT2016BRUSERPM1, PT2016BRUSERPM2, PT2016BRUSERPM3 and PT2016BRUSERPM4 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. 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, 3 aliquots of each reference milk sample were 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, LAB5 and LAB6 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 LAB7 only participated in the PT serum, whereas LAB8 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 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 12th of September 2016 (240 aliquots in total). All participants acknowledged receipt of the samples on the same day except LAB3 which acknowledged receipt of the serum samples on 15th September 2016, LAB 5 which acknowledged receipt of the serum and milk samples still frozen on 13th September 2016 and LAB7 which acknowledged receipt of the serum samples on 21th September 2016.
LAB2 asked to resent two milk samples. These milk samples were resent by national courier on 29th of September 2016. LAB2 acknowledged receipt of the two samples on 30th of September 2016.
Analyses were performed between 12th and 30th of September 2016 for serum and between 14th of September and 3th of October 2016 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 21th of September and 10th of October 2016 (Table 1). Hereby, all laboratories except LAB1 and LAB2 respected the deadline of the 30th of September 2016 for submission of the results. It is noted that LAB2 received an extension of the deadline for submission of the results till 14th of October 2016.

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	12/09/2016	14-15/09/2016	15/09/2016	15/09/2016	14/09/2016	10/10/2016
LAB2	12/09/2016 + 30/09/2016	15/09/2016	16/09/2016	14/09/2016	14/09/2016 + 03/10/2016	03/10/2016
LAB 3	15/09/2016	22/09/2016	20/09/2016	22/09/2016	NA	30/09/2016
LAB4	12/09/2016	12/09/2016	13/09/2016	12/09/2016	NA	23/09/2016
LAB5	13/09/2016	NA	20/09/2016	20/09/2016	20/09/2016	21/09/2016
LAB6	12/09/2016	NA	NA	21/09/2016	20/09/2016	27/09/2016
LAB7	21/09/2016	NA	30/09/2016	NA	NA	30/09/2016
LAB8	12/09/2016	NA	NA	NA	20/09/2016	27/09/2016

Legend: NA = not applicable

IV.3. Compliance with the procedure

All laboratories except LAB1 and LAB6 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 except LAB1 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). LAB1 misclassified 1 aliquot (95% of agreement) of reference serum samples in the SAW-EDTA test.

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**	**4**
failure	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	19 (95.0)	20 (100.0)	20 (100.0)	20 (100.0)

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**	**6**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

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**	**5**	**7**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.1.2. Reference milk samples

Four out of five participating laboratories (LAB1, LAB2, LAB5 and LAB8) provided qualitative results that were in full agreement with the assigned status of the reference milk samples and hence achieved 100% of agreement. In contrast, LAB6 misclassified 2 aliquots (90% of agreement) of reference milk samples (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**	**6**	**8**
failure	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	18 (90.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

IV.4.2.1. Reference serum samples

No variability between LAB2, LAB3, LAB4, LAB5, LAB6 and LAB7 could be observed since these participants correctly identified all reference serum samples with each serological test performed. In contrast, LAB1 misclassified 1 out of 4 aliquots of the reference serum sample PT2016BRUSERPS2 (NEG instead of >=100).

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 LAB8 could be observed since these participants correctly identified all reference milk samples. In contrast, LAB6 misclassified 2 out of 5 aliquots of the reference milk sample PT2016BRUSERPM2 (NI instead of POS).

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 POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016BRUSERNS3	NEG	NEG	1
2	1	2	PT2016BRUSERPS1	50	50	1
3	1	3	PT2016BRUSERNS1	NEG	NEG	1
4	1	4	PT2016BRUSERPS2	>=100	>=100	1
5	1	5	PT2016BRUSERPS3	>=100	>=100	1
6	1	6	PT2016BRUSERNS3	NEG	NEG	1
7	1	7	PT2016BRUSERNS2	NEG	NEG	1
8	1	8	PT2016BRUSERPS2	>=100	>=100	1
9	1	9	PT2016BRUSERNS3	NEG	NEG	1
10	1	10	PT2016BRUSERPS3	>=100	>=100	1
11	1	11	PT2016BRUSERNS2	NEG	NEG	1
12	1	12	PT2016BRUSERPS3	>=100	>=100	1
13	1	13	PT2016BRUSERPS2	**>=100**	**NEG**	**0**
14	1	14	PT2016BRUSERNS3	NEG	NEG	1
15	1	15	PT2016BRUSERNS1	NEG	NEG	1
16	1	16	PT2016BRUSERPS2	>=100	>=100	1
17	1	17	PT2016BRUSERNS2	NEG	NEG	1
18	1	18	PT2016BRUSERPS1	50	50	1
19	1	19	PT2016BRUSERNS1	NEG	NEG	1
20	1	20	PT2016BRUSERPS3	>=100	>=100	1

(Table 6 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016BRUSERNS3	NEG	NEG	1
22	2	2	PT2016BRUSERNS1	NEG	NEG	1
23	2	3	PT2016BRUSERPS2	>=100	>=100	1
24	2	4	PT2016BRUSERPS1	50	50	1
25	2	5	PT2016BRUSERNS3	NEG	NEG	1
26	2	6	PT2016BRUSERNS3	NEG	NEG	1
27	2	7	PT2016BRUSERPS3	>=100	>=100	1
28	2	8	PT2016BRUSERNS2	NEG	NEG	1
29	2	9	PT2016BRUSERNS1	NEG	NEG	1
30	2	10	PT2016BRUSERPS3	>=100	>=100	1
31	2	11	PT2016BRUSERPS2	>=100	>=100	1
32	2	12	PT2016BRUSERPS3	>=100	>=100	1
33	2	13	PT2016BRUSERNS2	NEG	NEG	1
34	2	14	PT2016BRUSERPS2	>=100	>=100	1
35	2	15	PT2016BRUSERNS3	NEG	NEG	1
36	2	16	PT2016BRUSERPS3	>=100	>=100	1
37	2	17	PT2016BRUSERNS1	NEG	NEG	1
38	2	18	PT2016BRUSERPS2	>=100	>=100	1
39	2	19	PT2016BRUSERPS1	50	50	1
40	2	20	PT2016BRUSERNS2	NEG	NEG	1
41	3	1	PT2016BRUSERNS3	NEG	NEG	1
42	3	2	PT2016BRUSERPS1	50	50	1
43	3	3	PT2016BRUSERNS1	NEG	NEG	1
44	3	4	PT2016BRUSERPS2	>=100	>=100	1
45	3	5	PT2016BRUSERPS3	>=100	>=100	1
46	3	6	PT2016BRUSERNS3	NEG	NEG	1
47	3	7	PT2016BRUSERNS2	NEG	NEG	1
48	3	8	PT2016BRUSERPS2	>=100	>=100	1
49	3	9	PT2016BRUSERNS3	NEG	NEG	1
50	3	10	PT2016BRUSERPS3	>=100	>=100	1
51	3	11	PT2016BRUSERNS2	NEG	NEG	1
52	3	12	PT2016BRUSERPS3	>=100	>=100	1
53	3	13	PT2016BRUSERPS2	>=100	>=100	1
54	3	14	PT2016BRUSERNS3	NEG	NEG	1
55	3	15	PT2016BRUSERNS1	NEG	NEG	1
56	3	16	PT2016BRUSERPS2	>=100	>=100	1
57	3	17	PT2016BRUSERNS2	NEG	NEG	1
58	3	18	PT2016BRUSERPS1	50	50	1
59	3	19	PT2016BRUSERNS1	NEG	NEG	1
60	3	20	PT2016BRUSERPS3	>=100	>=100	1

(Table 6 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2016BRUSERNS3	NEG	NEG	1
62	4	2	PT2016BRUSERNS1	NEG	NEG	1
63	4	3	PT2016BRUSERPS2	>=100	>=100	1
64	4	4	PT2016BRUSERPS1	50	30	1
65	4	5	PT2016BRUSERNS3	NEG	NEG	1
66	4	6	PT2016BRUSERNS3	NEG	NEG	1
67	4	7	PT2016BRUSERPS3	>=100	>=100	1
68	4	8	PT2016BRUSERNS2	NEG	NEG	1
69	4	9	PT2016BRUSERNS1	NEG	NEG	1
70	4	10	PT2016BRUSERPS3	>=100	>=100	1
71	4	11	PT2016BRUSERPS2	>=100	>=100	1
72	4	12	PT2016BRUSERPS3	>=100	>=100	1
73	4	13	PT2016BRUSERNS2	NEG	NEG	1
74	4	14	PT2016BRUSERPS2	>=100	>=100	1
75	4	15	PT2016BRUSERNS3	NEG	NEG	1
76	4	16	PT2016BRUSERPS3	>=100	>=100	1
77	4	17	PT2016BRUSERNS1	NEG	NEG	1
78	4	18	PT2016BRUSERPS2	>=100	>=100	1
79	4	19	PT2016BRUSERPS1	50	30	1
80	4	20	PT2016BRUSERNS2	NEG	NEG	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	PT2016BRUSERNS3	NEG	NEG	1
2	1	2	PT2016BRUSERPS1	POS	POS	1
3	1	3	PT2016BRUSERNS1	NEG	NEG	1
4	1	4	PT2016BRUSERPS2	POS	POS	1
5	1	5	PT2016BRUSERPS3	POS	POS	1
6	1	6	PT2016BRUSERNS3	NEG	NEG	1
7	1	7	PT2016BRUSERNS2	NEG	NEG	1
8	1	8	PT2016BRUSERPS2	POS	POS	1
9	1	9	PT2016BRUSERNS3	NEG	NEG	1
10	1	10	PT2016BRUSERPS3	POS	POS	1
11	1	11	PT2016BRUSERNS2	NEG	NEG	1
12	1	12	PT2016BRUSERPS3	POS	POS	1
13	1	13	PT2016BRUSERPS2	POS	POS	1
14	1	14	PT2016BRUSERNS3	NEG	NEG	1
15	1	15	PT2016BRUSERNS1	NEG	NEG	1
16	1	16	PT2016BRUSERPS2	POS	POS	1
17	1	17	PT2016BRUSERNS2	NEG	NEG	1
18	1	18	PT2016BRUSERPS1	POS	POS	1
19	1	19	PT2016BRUSERNS1	NEG	NEG	1
20	1	20	PT2016BRUSERPS3	POS	POS	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016BRUSERNS3	NEG	NEG	1
22	2	2	PT2016BRUSERNS1	NEG	NEG	1
23	2	3	PT2016BRUSERPS2	POS	POS	1
24	2	4	PT2016BRUSERPS1	POS	POS	1
25	2	5	PT2016BRUSERNS3	NEG	NEG	1
26	2	6	PT2016BRUSERNS3	NEG	NEG	1
27	2	7	PT2016BRUSERPS3	POS	POS	1
28	2	8	PT2016BRUSERNS2	NEG	NEG	1
29	2	9	PT2016BRUSERNS1	NEG	NEG	1
30	2	10	PT2016BRUSERPS3	POS	POS	1
31	2	11	PT2016BRUSERPS2	POS	POS	1
32	2	12	PT2016BRUSERPS3	POS	POS	1
33	2	13	PT2016BRUSERNS2	NEG	NEG	1
34	2	14	PT2016BRUSERPS2	POS	POS	1
35	2	15	PT2016BRUSERNS3	NEG	NEG	1
36	2	16	PT2016BRUSERPS3	POS	POS	1
37	2	17	PT2016BRUSERNS1	NEG	NEG	1
38	2	18	PT2016BRUSERPS2	POS	POS	1
39	2	19	PT2016BRUSERPS1	POS	POS	1
40	2	20	PT2016BRUSERNS2	NEG	NEG	1
41	3	1	PT2016BRUSERNS3	NEG	NEG	1
42	3	2	PT2016BRUSERPS1	POS	POS	1
43	3	3	PT2016BRUSERNS1	NEG	NEG	1
44	3	4	PT2016BRUSERPS2	POS	POS	1
45	3	5	PT2016BRUSERPS3	POS	POS	1
46	3	6	PT2016BRUSERNS3	NEG	NEG	1
47	3	7	PT2016BRUSERNS2	NEG	NEG	1
48	3	8	PT2016BRUSERPS2	POS	POS	1
49	3	9	PT2016BRUSERNS3	NEG	NEG	1
50	3	10	PT2016BRUSERPS3	POS	POS	1
51	3	11	PT2016BRUSERNS2	NEG	NEG	1
52	3	12	PT2016BRUSERPS3	POS	POS	1
53	3	13	PT2016BRUSERPS2	POS	POS	1
54	3	14	PT2016BRUSERNS3	NEG	NEG	1
55	3	15	PT2016BRUSERNS1	NEG	NEG	1
56	3	16	PT2016BRUSERPS2	POS	POS	1
57	3	17	PT2016BRUSERNS2	NEG	NEG	1
58	3	18	PT2016BRUSERPS1	POS	POS	1
59	3	19	PT2016BRUSERNS1	NEG	NEG	1
60	3	20	PT2016BRUSERPS3	POS	POS	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2016BRUSERNS3	NEG	NEG	1
62	4	2	PT2016BRUSERNS1	NEG	NEG	1
63	4	3	PT2016BRUSERPS2	POS	POS	1
64	4	4	PT2016BRUSERPS1	POS	POS	1
65	4	5	PT2016BRUSERNS3	NEG	NEG	1
66	4	6	PT2016BRUSERNS3	NEG	NEG	1
67	4	7	PT2016BRUSERPS3	POS	POS	1
68	4	8	PT2016BRUSERNS2	NEG	NEG	1
69	4	9	PT2016BRUSERNS1	NEG	NEG	1
70	4	10	PT2016BRUSERPS3	POS	POS	1
71	4	11	PT2016BRUSERPS2	POS	POS	1
72	4	12	PT2016BRUSERPS3	POS	POS	1
73	4	13	PT2016BRUSERNS2	NEG	NEG	1
74	4	14	PT2016BRUSERPS2	POS	POS	1
75	4	15	PT2016BRUSERNS3	NEG	NEG	1
76	4	16	PT2016BRUSERPS3	POS	POS	1
77	4	17	PT2016BRUSERNS1	NEG	NEG	1
78	4	18	PT2016BRUSERPS2	POS	POS	1
79	4	19	PT2016BRUSERPS1	POS	POS	1
80	4	20	PT2016BRUSERNS2	NEG	NEG	1
81	5	1	PT2016BRUSERNS3	NEG	NEG	1
82	5	2	PT2016BRUSERPS1	POS	POS	1
83	5	3	PT2016BRUSERNS1	NEG	NEG	1
84	5	4	PT2016BRUSERPS2	POS	POS	1
85	5	5	PT2016BRUSERPS3	POS	POS	1
86	5	6	PT2016BRUSERNS3	NEG	NEG	1
87	5	7	PT2016BRUSERNS2	NEG	NEG	1
88	5	8	PT2016BRUSERPS2	POS	POS	1
89	5	9	PT2016BRUSERNS3	NEG	NEG	1
90	5	10	PT2016BRUSERPS3	POS	POS	1
91	5	11	PT2016BRUSERNS2	NEG	NEG	1
92	5	12	PT2016BRUSERPS3	POS	POS	1
93	5	13	PT2016BRUSERPS2	POS	POS	1
94	5	14	PT2016BRUSERNS3	NEG	NEG	1
95	5	15	PT2016BRUSERNS1	NEG	NEG	1
96	5	16	PT2016BRUSERPS2	POS	POS	1
97	5	17	PT2016BRUSERNS2	NEG	NEG	1
98	5	18	PT2016BRUSERPS1	POS	POS	1
99	5	19	PT2016BRUSERNS1	NEG	NEG	1
100	5	20	PT2016BRUSERPS3	POS	POS	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
101	6	1	PT2016BRUSERNS3	NEG	NEG	1
102	6	2	PT2016BRUSERNS1	NEG	NEG	1
103	6	3	PT2016BRUSERPS2	POS	POS	1
104	6	4	PT2016BRUSERPS1	POS	POS	1
105	6	5	PT2016BRUSERNS3	NEG	NEG	1
106	6	6	PT2016BRUSERNS3	NEG	NEG	1
107	6	7	PT2016BRUSERPS3	POS	POS	1
108	6	8	PT2016BRUSERNS2	NEG	NEG	1
109	6	9	PT2016BRUSERNS1	NEG	NEG	1
110	6	10	PT2016BRUSERPS3	POS	POS	1
111	6	11	PT2016BRUSERPS2	POS	POS	1
112	6	12	PT2016BRUSERPS3	POS	POS	1
113	6	13	PT2016BRUSERNS2	NEG	NEG	1
114	6	14	PT2016BRUSERPS2	POS	POS	1
115	6	15	PT2016BRUSERNS3	NEG	NEG	1
116	6	16	PT2016BRUSERPS3	POS	POS	1
117	6	17	PT2016BRUSERNS1	NEG	NEG	1
118	6	18	PT2016BRUSERPS2	POS	POS	1
119	6	19	PT2016BRUSERPS1	POS	POS	1
120	6	20	PT2016BRUSERNS2	NEG	NEG	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	PT2016BRUSERNS3	NEG	NEG	1
2	1	2	PT2016BRUSERPS1	POS	POS	1
3	1	3	PT2016BRUSERNS1	NEG	NEG	1
4	1	4	PT2016BRUSERPS2	POS	POS	1
5	1	5	PT2016BRUSERPS3	POS	POS	1
6	1	6	PT2016BRUSERNS3	NEG	NEG	1
7	1	7	PT2016BRUSERNS2	NEG	NEG	1
8	1	8	PT2016BRUSERPS2	POS	POS	1
9	1	9	PT2016BRUSERNS3	NEG	NEG	1
10	1	10	PT2016BRUSERPS3	POS	POS	1
11	1	11	PT2016BRUSERNS2	NEG	NEG	1
12	1	12	PT2016BRUSERPS3	POS	POS	1
13	1	13	PT2016BRUSERPS2	POS	POS	1
14	1	14	PT2016BRUSERNS3	NEG	NEG	1
15	1	15	PT2016BRUSERNS1	NEG	NEG	1
16	1	16	PT2016BRUSERPS2	POS	POS	1
17	1	17	PT2016BRUSERNS2	NEG	NEG	1
18	1	18	PT2016BRUSERPS1	POS	POS	1
19	1	19	PT2016BRUSERNS1	NEG	NEG	1
20	1	20	PT2016BRUSERPS3	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016BRUSERNS3	NEG	NEG	1
22	2	2	PT2016BRUSERNS1	NEG	NEG	1
23	2	3	PT2016BRUSERPS2	POS	POS	1
24	2	4	PT2016BRUSERPS1	POS	POS	1
25	2	5	PT2016BRUSERNS3	NEG	NEG	1
26	2	6	PT2016BRUSERNS3	NEG	NEG	1
27	2	7	PT2016BRUSERPS3	POS	POS	1
28	2	8	PT2016BRUSERNS2	NEG	NEG	1
29	2	9	PT2016BRUSERNS1	NEG	NEG	1
30	2	10	PT2016BRUSERPS3	POS	POS	1
31	2	11	PT2016BRUSERPS2	POS	POS	1
32	2	12	PT2016BRUSERPS3	POS	POS	1
33	2	13	PT2016BRUSERNS2	NEG	NEG	1
34	2	14	PT2016BRUSERPS2	POS	POS	1
35	2	15	PT2016BRUSERNS3	NEG	NEG	1
36	2	16	PT2016BRUSERPS3	POS	POS	1
37	2	17	PT2016BRUSERNS1	NEG	NEG	1
38	2	18	PT2016BRUSERPS2	POS	POS	1
39	2	19	PT2016BRUSERPS1	POS	POS	1
40	2	20	PT2016BRUSERNS2	NEG	NEG	1
41	3	1	PT2016BRUSERNS3	NEG	NEG	1
42	3	2	PT2016BRUSERPS1	POS	POS	1
43	3	3	PT2016BRUSERNS1	NEG	NEG	1
44	3	4	PT2016BRUSERPS2	POS	POS	1
45	3	5	PT2016BRUSERPS3	POS	POS	1
46	3	6	PT2016BRUSERNS3	NEG	NEG	1
47	3	7	PT2016BRUSERNS2	NEG	NEG	1
48	3	8	PT2016BRUSERPS2	POS	POS	1
49	3	9	PT2016BRUSERNS3	NEG	NEG	1
50	3	10	PT2016BRUSERPS3	POS	POS	1
51	3	11	PT2016BRUSERNS2	NEG	NEG	1
52	3	12	PT2016BRUSERPS3	POS	POS	1
53	3	13	PT2016BRUSERPS2	POS	POS	1
54	3	14	PT2016BRUSERNS3	NEG	NEG	1
55	3	15	PT2016BRUSERNS1	NEG	NEG	1
56	3	16	PT2016BRUSERPS2	POS	POS	1
57	3	17	PT2016BRUSERNS2	NEG	NEG	1
58	3	18	PT2016BRUSERPS1	POS	POS	1
59	3	19	PT2016BRUSERNS1	NEG	NEG	1
60	3	20	PT2016BRUSERPS3	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2016BRUSERNS3	NEG	NEG	1
62	4	2	PT2016BRUSERNS1	NEG	NEG	1
63	4	3	PT2016BRUSERPS2	POS	POS	1
64	4	4	PT2016BRUSERPS1	POS	POS	1
65	4	5	PT2016BRUSERNS3	NEG	NEG	1
66	4	6	PT2016BRUSERNS3	NEG	NEG	1
67	4	7	PT2016BRUSERPS3	POS	POS	1
68	4	8	PT2016BRUSERNS2	NEG	NEG	1
69	4	9	PT2016BRUSERNS1	NEG	NEG	1
70	4	10	PT2016BRUSERPS3	POS	POS	1
71	4	11	PT2016BRUSERPS2	POS	POS	1
72	4	12	PT2016BRUSERPS3	POS	POS	1
73	4	13	PT2016BRUSERNS2	NEG	NEG	1
74	4	14	PT2016BRUSERPS2	POS	POS	1
75	4	15	PT2016BRUSERNS3	NEG	NEG	1
76	4	16	PT2016BRUSERPS3	POS	POS	1
77	4	17	PT2016BRUSERNS1	NEG	NEG	1
78	4	18	PT2016BRUSERPS2	POS	POS	1
79	4	19	PT2016BRUSERPS1	POS	POS	1
80	4	20	PT2016BRUSERNS2	NEG	NEG	1
81	5	1	PT2016BRUSERNS3	NEG	NEG	1
82	5	2	PT2016BRUSERPS1	POS	POS	1
83	5	3	PT2016BRUSERNS1	NEG	NEG	1
84	5	4	PT2016BRUSERPS2	POS	POS	1
85	5	5	PT2016BRUSERPS3	POS	POS	1
86	5	6	PT2016BRUSERNS3	NEG	NEG	1
87	5	7	PT2016BRUSERNS2	NEG	NEG	1
88	5	8	PT2016BRUSERPS2	POS	POS	1
89	5	9	PT2016BRUSERNS3	NEG	NEG	1
90	5	10	PT2016BRUSERPS3	POS	POS	1
91	5	11	PT2016BRUSERNS2	NEG	NEG	1
92	5	12	PT2016BRUSERPS3	POS	POS	1
93	5	13	PT2016BRUSERPS2	POS	POS	1
94	5	14	PT2016BRUSERNS3	NEG	NEG	1
95	5	15	PT2016BRUSERNS1	NEG	NEG	1
96	5	16	PT2016BRUSERPS2	POS	POS	1
97	5	17	PT2016BRUSERNS2	NEG	NEG	1
98	5	18	PT2016BRUSERPS1	POS	POS	1
99	5	19	PT2016BRUSERNS1	NEG	NEG	1
100	5	20	PT2016BRUSERPS3	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
101	7	1	PT2016BRUSERNS3	NEG	NEG	1
102	7	2	PT2016BRUSERPS1	POS	POS	1
103	7	3	PT2016BRUSERNS1	NEG	NEG	1
104	7	4	PT2016BRUSERPS2	POS	POS	1
105	7	5	PT2016BRUSERPS3	POS	POS	1
106	7	6	PT2016BRUSERNS3	NEG	NEG	1
107	7	7	PT2016BRUSERNS2	NEG	NEG	1
108	7	8	PT2016BRUSERPS2	POS	POS	1
109	7	9	PT2016BRUSERNS3	NEG	NEG	1
110	7	10	PT2016BRUSERPS3	POS	POS	1
111	7	11	PT2016BRUSERNS2	NEG	NEG	1
112	7	12	PT2016BRUSERPS3	POS	POS	1
113	7	13	PT2016BRUSERPS2	POS	POS	1
114	7	14	PT2016BRUSERNS3	NEG	NEG	1
115	7	15	PT2016BRUSERNS1	NEG	NEG	1
116	7	16	PT2016BRUSERPS2	POS	POS	1
117	7	17	PT2016BRUSERNS2	NEG	NEG	1
118	7	18	PT2016BRUSERPS1	POS	POS	1
119	7	19	PT2016BRUSERNS1	NEG	NEG	1
120	7	20	PT2016BRUSERPS3	POS	POS	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	PT2016BRUSERPM2	POS	POS	1
2	1	2	PT2016BRUSERNM2	NEG	NEG	1
3	1	3	PT2016BRUSERNM1	NEG	NEG	1
4	1	4	PT2016BRUSERPM3	POS	POS	1
5	1	5	PT2016BRUSERPM4	POS	POS	1
6	1	6	PT2016BRUSERPM1	POS	POS	1
7	1	7	PT2016BRUSERPM3	POS	POS	1
8	1	8	PT2016BRUSERNM2	NEG	NEG	1
9	1	9	PT2016BRUSERPM2	POS	POS	1
10	1	10	PT2016BRUSERPM2	POS	POS	1
11	1	11	PT2016BRUSERPM1	POS	POS	1
12	1	12	PT2016BRUSERPM4	POS	POS	1
13	1	13	PT2016BRUSERPM1	POS	POS	1
14	1	14	PT2016BRUSERNM1	NEG	NEG	1
15	1	15	PT2016BRUSERPM3	POS	POS	1
16	1	16	PT2016BRUSERPM2	POS	POS	1
17	1	17	PT2016BRUSERNM1	NEG	NEG	1
18	1	18	PT2016BRUSERPM3	POS	POS	1
19	1	19	PT2016BRUSERPM2	POS	POS	1
20	1	20	PT2016BRUSERNM1	NEG	NEG	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2016BRUSERNM1	POS	NEG	1
22	2	2	PT2016BRUSERPM1	POS	POS	1
23	2	3	PT2016BRUSERPM2	POS	POS	1
24	2	4	PT2016BRUSERPM3	POS	POS	1
25	2	5	PT2016BRUSERNM1	NEG	NEG	1
26	2	6	PT2016BRUSERPM4	POS	POS	1
27	2	7	PT2016BRUSERNM2	NEG	NEG	1
28	2	8	PT2016BRUSERPM3	POS	POS	1
29	2	9	PT2016BRUSERPM2	POS	POS	1
30	2	10	PT2016BRUSERPM4	POS	POS	1
31	2	11	PT2016BRUSERPM3	POS	POS	1
32	2	12	PT2016BRUSERNM1	NEG	NEG	1
33	2	13	PT2016BRUSERPM2	POS	POS	1
34	2	14	PT2016BRUSERPM3	POS	POS	1
35	2	15	PT2016BRUSERPM1	POS	POS	1
36	2	16	PT2016BRUSERNM2	NEG	NEG	1
37	2	17	PT2016BRUSERPM2	POS	POS	1
38	2	18	PT2016BRUSERPM2	POS	POS	1
39	2	19	PT2016BRUSERNM1	NEG	NEG	1
40	2	20	PT2016BRUSERPM1	POS	POS	1
41	5	1	PT2016BRUSERPM2	POS	POS	1
42	5	2	PT2016BRUSERNM2	NEG	NEG	1
43	5	3	PT2016BRUSERNM1	NEG	NEG	1
44	5	4	PT2016BRUSERPM3	POS	POS	1
45	5	5	PT2016BRUSERPM4	POS	POS	1
46	5	6	PT2016BRUSERPM1	POS	POS	1
47	5	7	PT2016BRUSERPM3	POS	POS	1
48	5	8	PT2016BRUSERNM2	NEG	NEG	1
49	5	9	PT2016BRUSERPM2	POS	POS	1
50	5	10	PT2016BRUSERPM2	POS	POS	1
51	5	11	PT2016BRUSERPM1	POS	POS	1
52	5	12	PT2016BRUSERPM4	POS	POS	1
53	5	13	PT2016BRUSERPM1	POS	POS	1
54	5	14	PT2016BRUSERNM1	NEG	NEG	1
55	5	15	PT2016BRUSERPM3	POS	POS	1
56	5	16	PT2016BRUSERPM2	POS	POS	1
57	5	17	PT2016BRUSERNM1	NEG	NEG	1
58	5	18	PT2016BRUSERPM3	POS	POS	1
59	5	19	PT2016BRUSERPM2	POS	POS	1
60	5	20	PT2016BRUSERNM1	NEG	NEG	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	6	1	PT2016BRUSERNM1	NEG	NEG	1
62	6	2	PT2016BRUSERPM1	POS	POS	1
63	6	3	PT2016BRUSERPM2	**POS**	**NI**	**0**
64	6	4	PT2016BRUSERPM3	POS	POS	1
65	6	5	PT2016BRUSERNM1	NEG	NEG	1
66	6	6	PT2016BRUSERPM4	POS	POS	1
67	6	7	PT2016BRUSERNM2	NEG	NEG	1
68	6	8	PT2016BRUSERPM3	POS	POS	1
69	6	9	PT2016BRUSERPM2	POS	POS	1
70	6	10	PT2016BRUSERPM4	POS	POS	1
71	6	11	PT2016BRUSERPM3	POS	POS	1
72	6	12	PT2016BRUSERNM1	NEG	NEG	1
73	6	13	PT2016BRUSERPM2	POS	POS	1
74	6	14	PT2016BRUSERPM3	POS	POS	1
75	6	15	PT2016BRUSERPM1	POS	POS	1
76	6	16	PT2016BRUSERNM2	NEG	NEG	1
77	6	17	PT2016BRUSERPM2	POS	POS	1
78	6	18	PT2016BRUSERPM2	**POS**	**NI**	**0**
79	6	19	PT2016BRUSERNM1	NEG	NEG	1
80	6	20	PT2016BRUSERPM1	POS	POS	1
81	8	1	PT2016BRUSERPM2	POS	POS	1
82	8	2	PT2016BRUSERNM2	NEG	NEG	1
83	8	3	PT2016BRUSERNM1	NEG	NEG	1
84	8	4	PT2016BRUSERPM3	POS	POS	1
85	8	5	PT2016BRUSERPM4	POS	POS	1
86	8	6	PT2016BRUSERPM1	POS	POS	1
87	8	7	PT2016BRUSERPM3	POS	POS	1
88	8	8	PT2016BRUSERNM2	NEG	NEG	1
89	8	9	PT2016BRUSERPM2	POS	POS	1
90	8	10	PT2016BRUSERPM2	POS	POS	1
91	8	11	PT2016BRUSERPM1	POS	POS	1
92	8	12	PT2016BRUSERPM4	POS	POS	1
93	8	13	PT2016BRUSERPM1	POS	POS	1
94	8	14	PT2016BRUSERNM1	NEG	NEG	1
95	8	15	PT2016BRUSERPM3	POS	POS	1
96	8	16	PT2016BRUSERPM2	POS	POS	1
97	8	17	PT2016BRUSERNM1	NEG	NEG	1
98	8	18	PT2016BRUSERPM3	POS	POS	1
99	8	19	PT2016BRUSERPM2	POS	POS	1
100	8	20	PT2016BRUSERNM1	NEG	NEG	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, 6 out of 7 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). In contrast LAB1 misclassified 1 aliquot (95% of agreement) of reference serum samples in the SAW-EDTA test.
The 4 participating laboratories that performed SAW-EDTA used a SAW antigen from 2 different producers, namely Synbiotics (batches : 14SAW13 and 15SAW14) and Institut Pourquier (batch 214). The 6 participating laboratories that performed RBT used a RBT antigen from 3 different producers, namely Synbiotics Europe (2 batches: 14BGT75 and 15BGT82), Institut Pourquier (2 batches: 381-100 and 404) and IDEXX (2 batches: 395-10 and 400-100). From the 6 participating laboratories that performed ELISA, LAB1 used an home made developed BRU antibody ELISA kit, whereas LAB2, LAB3, LAB4, LAB5 and LAB7 used a commercially available BRU antibody ELISA kit from two different producers, namely Synbiotics Europe (batch 16SBRU3OCB61) and IDEXX (batch 4108).

For the detection of BRU-specific antibodies in reference milk samples, 4 out of the 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). In contrast, LAB6 misclassified 2 aliquots (90% of agreement) of reference milk samples.
The 5 participating laboratories that performed ELISA used the BRU antibody ELISA kit from IDEXX (batches 4121 and 5078).

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, 6 of the 7 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

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (Maisons-Alfort, France)

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)

Synbiotics Europe (Synbiotics) (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.

<u>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.</u>

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.

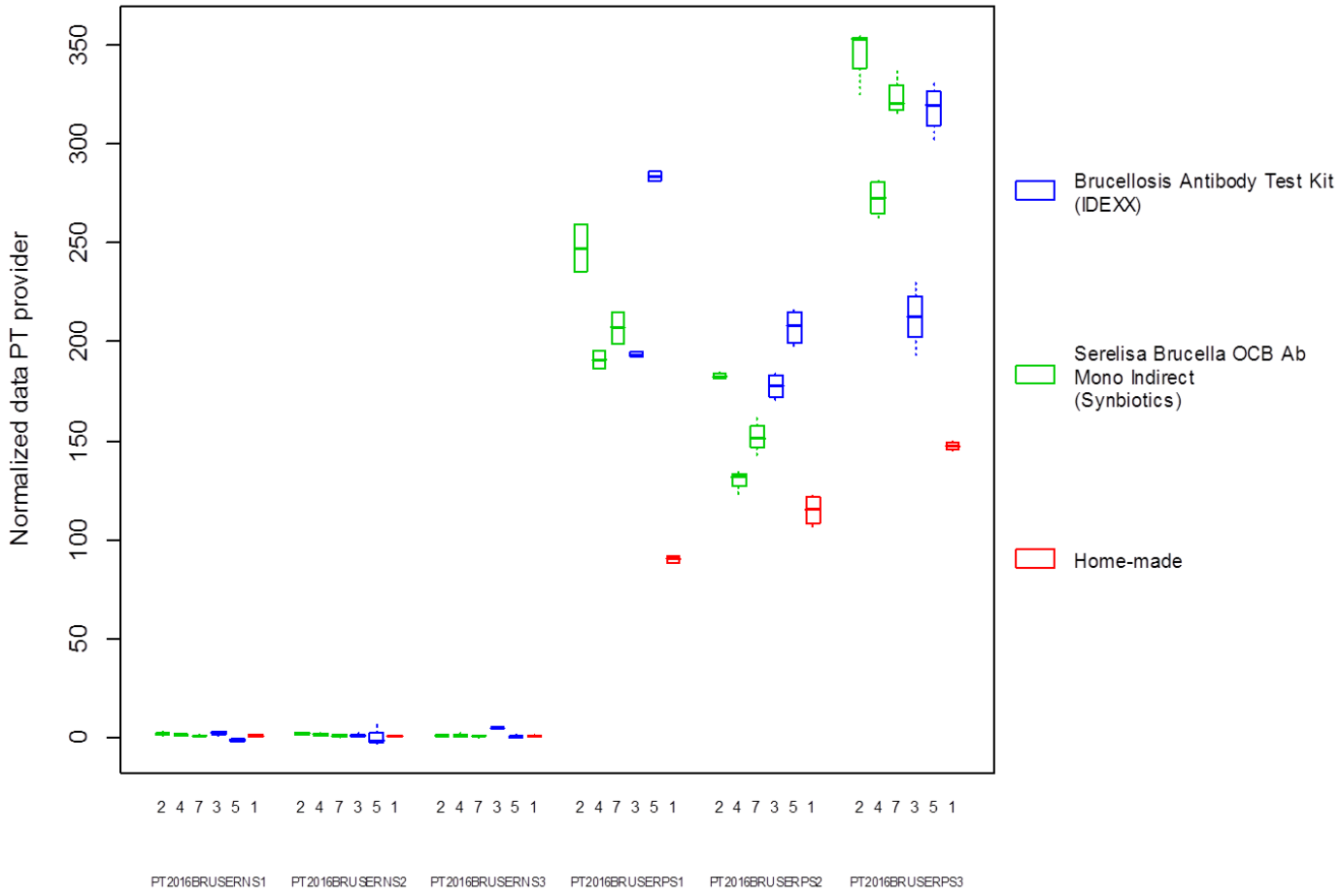

Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory. From the 6 participating laboratories that performed ELISA, LAB1 used an home made developed BRU antibody ELISA kit, whereas LAB2, LAB3, LAB4, LAB5 and LAB7 used a commercially available BRU antibody ELISA kit from two different producers, namely Synbiotics Europe (batch 16SBRU3OCB61) and IDEXX (batch 4108). Cut-off values are not shown since these were different for each participant.

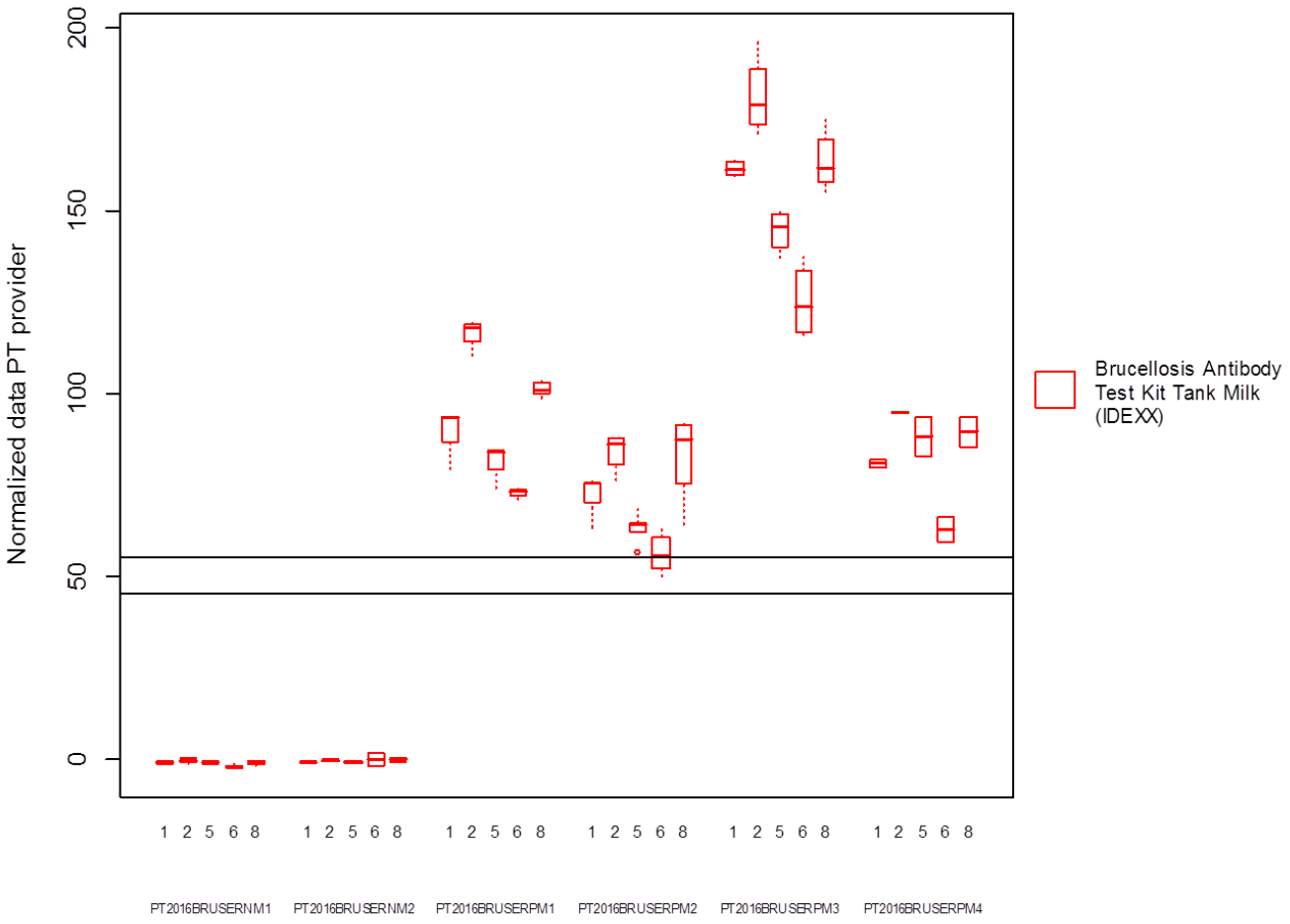

Figure 2. Box plots showing the percentage S/P ratio per reference milk sample and per participating laboratory. The 5 participating laboratories that performed ELISA used the BRU antibody ELISA kit from IDEXX (batches 4121 and 5078). In addition, the cut-off value of 45-55% is shown by horizontal lines.