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172-PT

PROFICIENCY TESTING 2014

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

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 14 JULY 2014

DATE REPORT: 10 OCTOBER 2014

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 'PT2014BRUSERNS1', 'PT2014BRUSERNS2' and 'PT2014BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2014BRUSERPS1', 'PT2014BRUSERPS2' and 'PT2014BRUSERPS3'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2014BRUSERNS1, PT2014BRUSERNS2, PT2014BRUSERPS2 and PT2014BRUSERPS3, and 4 aliquots of the reference serum samples PT2014BRUSERNS3 and PT2014BRUSERPS1. 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 PT2014BRUSERNS1 and PT2014BRUSERNS2 were derived from BRU-free farms. The reference serum sample PT2014BRUSERNS3 was a sample taken at abattoir and obtained from a BRU-free farm, whereas the reference serum sample PT2014BRUSERPS3 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 PT2014BRUSERPS1 and PT2014BRUSERPS2 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 PT2014BRUSERNS1, PT2014BRUSERNS2 and PT2013BRUSERNS3 were considered as negative sera, and the reference serum samples PT2013BRUSERPS1, PT2013BRUSERPS2 and PT2013BRUSERPS3 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 'PT2014BRUSERNM1' and 'PT2014BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2014BRUSERPM1', 'PT2014BRUSERPM2', 'PT2014BRUSERPM3' and 'PT2014BRUSERPM4'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2014BRUSERNM1, PT2014BRUSERPM2, PT2014BRUSERPM3 and PT2014BRUSERPM4, and 4 aliquots of the reference milk samples PT2014BRUSERNM2 and PT2014BRUSERPM1. 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 PT2014BRUSERNM1 and PT2014BRUSERNM2 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, PT2014BRUSERPM1 was spiked with serum 1275 in a 1/200 dilution and PT2014BRUSERPM2 was spiked with serum 3667 in a 1/1000 dilution, respectively, whereas PT2014BRUSERPM3 and PT2014BRUSERPM4 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 PT2014BRUSERNM1 and PT2014BRUSERNM2 were considered as negative milk samples, and the reference samples PT2014BRUSERPM1, PT2014BRUSERPM2, PT2014BRUSERPM3 and PT2014BRUSERPM4 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, 50 and ≥ 100. Any laboratory that reported other titers were translated accordingly.
- 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 the operational unit CVD-ERA of CODA-CERVA.

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

LAB1, LAB2, LAB3 and LAB4 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, LAB5, LAB6 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 (ambient temperature), whereas the reference milk samples were sent frozen (dry ice) to each of the participating laboratories by national courier on 14th of july 2014 (240 aliquots in total). All participants acknowledged receipt of the samples on the same day. Analyses were performed between 14th and 29th of July 2014 for serum and between 15th and 25th of July 2014 for milk (Table 1).

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results were submitted to the operational unit CVD-ERA between 15th and 31th of July 2014 (Table 1). Hereby, all laboratories respected the deadline of the 1st of August 2014 for submission of the results.

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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis serum			Start of analysis milk	Submission of the results (Excel file)
		SAW-EDTA	ELISA	RBT		
LAB1	14/07/2014	14 + 15/07/2014	15/07/2014	15/07/2014	15/07/2014	15/07/2014
LAB2	14/07/2014	NA	NA	15/07/2014	17/07/2014	18/07/2014
LAB 3	14/07/2014	NA	NA	17/07/2014	17/07/2014	17/07/2014
LAB4	14/07/2014	24/07/2014	24/07/2014	24/07/2014	25/07/2014	30/07/2014
LAB5	14/07/2014	29/07/2014	29/07/2014	28/07/2014	NA	31/07/2014
LAB6	14/07/2014	NA	22/07/2014	NA	NA	24/07/2014
LAB7	14/07/2014	14/07/2014	16/07/2014	NA	NA	16/07/2014
LAB8	14/07/2014	NA	NA	NA	24/07/2014	25/07/2014

Legend: NA = not applicable

IV.3. Compliance with the procedure

Except LAB1, all participating 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 all serological tests performed: SAW-EDTA (Table 2) and/or RBT (Table 3) and/or ELISA (Table 4).

A quantitative data analysis (including box plots) of the ELISA results is shown for educational purposes in Annex 1 and Annex 2.

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

	LABNR			
	1	**4***	**5**	**7**
failure	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)

* LAB4 performed a SAW without EDTA. The obtained titers were translated into the possibilities foreseen by the PT provider. A titer of 30, 80 and 140 was translated into 25, 50 and ≥100 respectively (see III 3.1.1).

Table 3. RBT: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by CODA-CERVA. 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 (0.0)	0 (0.0)
success	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 CODA-CERVA. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**4**	**5**	**6**	**7**
failure	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)

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).

A quantitative data analysis (including box plots) of the results is shown for educational purposes in Annex 1 and Annex 2.

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

	LABNR				
	1	**2**	**3**	**4**	**8**
failure	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)

IV.4.2. Variability among participating laboratories

IV.4.2.1. Reference serum samples

Since all participating laboratories reached 100% of agreement for the detection of BRU-specific antibodies in reference serum samples with each serological test performed, no variability between qualitative laboratory results could be observed.

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

Since all participating laboratories reached 100% of agreement for the detection of BRU-specific antibodies in reference milk samples, no variability between qualitative laboratory results could be observed.

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 CODA-CERVA (STATUS). NEG: negative.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BRUSERPS2	>=100	>=100	1
2	1	2	PT2014BRUSERNS1	NEG	NEG	1
3	1	3	PT2014BRUSERNS3	NEG	NEG	1
4	1	4	PT2014BRUSERPS3	>=100	>=100	1
5	1	5	PT2014BRUSERNS2	NEG	NEG	1
6	1	6	PT2014BRUSERPS1	50	50	1
7	1	7	PT2014BRUSERPS2	>=100	>=100	1
8	1	8	PT2014BRUSERPS3	>=100	>=100	1
9	1	9	PT2014BRUSERNS2	NEG	NEG	1
10	1	10	PT2014BRUSERNS1	NEG	NEG	1
11	1	11	PT2014BRUSERPS1	50	50	1
12	1	12	PT2014BRUSERNS3	NEG	NEG	1
13	1	13	PT2014BRUSERPS3	>=100	>=100	1
14	1	14	PT2014BRUSERPS2	>=100	>=100	1
15	1	15	PT2014BRUSERNS1	NEG	NEG	1
16	1	16	PT2014BRUSERPS1	50	50	1
17	1	17	PT2014BRUSERNS3	NEG	NEG	1
18	1	18	PT2014BRUSERNS2	NEG	NEG	1
19	1	19	PT2014BRUSERPS1	50	50	1
20	1	20	PT2014BRUSERNS3	NEG	NEG	1
21	4	1	PT2014BRUSERNS2	NEG	NEG	1
22	4	2	PT2014BRUSERPS1	50	25	1
23	4	3	PT2014BRUSERPS2	>=100	50	1
24	4	4	PT2014BRUSERNS1	NEG	NEG	1
25	4	5	PT2014BRUSERNS3	NEG	NEG	1
26	4	6	PT2014BRUSERPS3	>=100	>=100	1
27	4	7	PT2014BRUSERNS2	NEG	NEG	1
28	4	8	PT2014BRUSERNS1	NEG	NEG	1
29	4	9	PT2014BRUSERPS2	>=100	50	1
30	4	10	PT2014BRUSERNS3	NEG	NEG	1
31	4	11	PT2014BRUSERPS1	50	25	1
32	4	12	PT2014BRUSERNS3	NEG	NEG	1
33	4	13	PT2014BRUSERPS3	>=100	>=100	1
34	4	14	PT2014BRUSERPS2	>=100	50	1
35	4	15	PT2014BRUSERPS3	>=100	>=100	1
36	4	16	PT2014BRUSERPS1	50	25	1
37	4	17	PT2014BRUSERNS3	NEG	NEG	1
38	4	18	PT2014BRUSERNS2	NEG	NEG	1
39	4	19	PT2014BRUSERPS1	50	25	1
40	4	20	PT2014BRUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2014BRUSERPS2	>=100	>=100	1
42	5	2	PT2014BRUSERNS1	NEG	NEG	1
43	5	3	PT2014BRUSERNS3	NEG	NEG	1
44	5	4	PT2014BRUSERPS3	>=100	>=100	1
45	5	5	PT2014BRUSERNS2	NEG	NEG	1
46	5	6	PT2014BRUSERPS1	50	50	1
47	5	7	PT2014BRUSERPS2	>=100	>=100	1
48	5	8	PT2014BRUSERPS3	>=100	>=100	1
49	5	9	PT2014BRUSERNS2	NEG	NEG	1
50	5	10	PT2014BRUSERNS1	NEG	NEG	1
51	5	11	PT2014BRUSERPS1	50	50	1
52	5	12	PT2014BRUSERNS3	NEG	NEG	1
53	5	13	PT2014BRUSERPS3	>=100	>=100	1
54	5	14	PT2014BRUSERPS2	>=100	>=100	1
55	5	15	PT2014BRUSERNS1	NEG	NEG	1
56	5	16	PT2014BRUSERPS1	50	50	1
57	5	17	PT2014BRUSERNS3	NEG	NEG	1
58	5	18	PT2014BRUSERNS2	NEG	NEG	1
59	5	19	PT2014BRUSERPS1	50	50	1
60	5	20	PT2014BRUSERNS3	NEG	NEG	1
61	7	1	PT2014BRUSERPS2	>=100	>=100	1
62	7	2	PT2014BRUSERNS1	NEG	NEG	1
63	7	3	PT2014BRUSERNS3	NEG	NEG	1
64	7	4	PT2014BRUSERPS3	>=100	>=100	1
65	7	5	PT2014BRUSERNS2	NEG	NEG	1
66	7	6	PT2014BRUSERPS1	50	50	1
67	7	7	PT2014BRUSERPS2	>=100	>=100	1
68	7	8	PT2014BRUSERPS3	>=100	>=100	1
69	7	9	PT2014BRUSERNS2	NEG	NEG	1
70	7	10	PT2014BRUSERNS1	NEG	NEG	1
71	7	11	PT2014BRUSERPS1	50	50	1
72	7	12	PT2014BRUSERNS3	NEG	NEG	1
73	7	13	PT2014BRUSERPS3	>=100	>=100	1
74	7	14	PT2014BRUSERPS2	>=100	>=100	1
75	7	15	PT2014BRUSERNS1	NEG	NEG	1
76	7	16	PT2014BRUSERPS1	50	50	1
77	7	17	PT2014BRUSERNS3	NEG	NEG	1
78	7	18	PT2014BRUSERNS2	NEG	NEG	1
79	7	19	PT2014BRUSERPS1	50	50	1
80	7	20	PT2014BRUSERNS3	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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BRUSERPS2	POS	POS	1
2	1	2	PT2014BRUSERNS1	NEG	NEG	1
3	1	3	PT2014BRUSERNS3	NEG	NEG	1
4	1	4	PT2014BRUSERPS3	POS	POS	1
5	1	5	PT2014BRUSERNS2	NEG	NEG	1
6	1	6	PT2014BRUSERPS1	POS	POS	1
7	1	7	PT2014BRUSERPS2	POS	POS	1
8	1	8	PT2014BRUSERPS3	POS	POS	1
9	1	9	PT2014BRUSERNS2	NEG	NEG	1
10	1	10	PT2014BRUSERNS1	NEG	NEG	1
11	1	11	PT2014BRUSERPS1	POS	POS	1
12	1	12	PT2014BRUSERNS3	NEG	NEG	1
13	1	13	PT2014BRUSERPS3	POS	POS	1
14	1	14	PT2014BRUSERPS2	POS	POS	1
15	1	15	PT2014BRUSERNS1	NEG	NEG	1
16	1	16	PT2014BRUSERPS1	POS	POS	1
17	1	17	PT2014BRUSERNS3	NEG	NEG	1
18	1	18	PT2014BRUSERNS2	NEG	NEG	1
19	1	19	PT2014BRUSERPS1	POS	POS	1
20	1	20	PT2014BRUSERNS3	NEG	NEG	1
21	2	1	PT2014BRUSERNS2	NEG	NEG	1
22	2	2	PT2014BRUSERPS1	POS	POS	1
23	2	3	PT2014BRUSERPS2	POS	POS	1
24	2	4	PT2014BRUSERNS1	NEG	NEG	1
25	2	5	PT2014BRUSERNS3	NEG	NEG	1
26	2	6	PT2014BRUSERPS3	POS	POS	1
27	2	7	PT2014BRUSERNS2	NEG	NEG	1
28	2	8	PT2014BRUSERNS1	NEG	NEG	1
29	2	9	PT2014BRUSERPS2	POS	POS	1
30	2	10	PT2014BRUSERNS3	NEG	NEG	1
31	2	11	PT2014BRUSERPS1	POS	POS	1
32	2	12	PT2014BRUSERNS3	NEG	NEG	1
33	2	13	PT2014BRUSERPS3	POS	POS	1
34	2	14	PT2014BRUSERPS2	POS	POS	1
35	2	15	PT2014BRUSERPS3	POS	POS	1
36	2	16	PT2014BRUSERPS1	POS	POS	1
37	2	17	PT2014BRUSERNS3	NEG	NEG	1
38	2	18	PT2014BRUSERNS2	NEG	NEG	1
39	2	19	PT2014BRUSERPS1	POS	POS	1
40	2	20	PT2014BRUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2014BRUSERPS2	POS	POS	1
42	3	2	PT2014BRUSERNS1	NEG	NEG	1
43	3	3	PT2014BRUSERNS3	NEG	NEG	1
44	3	4	PT2014BRUSERPS3	POS	POS	1
45	3	5	PT2014BRUSERNS2	NEG	NEG	1
46	3	6	PT2014BRUSERPS1	POS	POS	1
47	3	7	PT2014BRUSERPS2	POS	POS	1
48	3	8	PT2014BRUSERPS3	POS	POS	1
49	3	9	PT2014BRUSERNS2	NEG	NEG	1
50	3	10	PT2014BRUSERNS1	NEG	NEG	1
51	3	11	PT2014BRUSERPS1	POS	POS	1
52	3	12	PT2014BRUSERNS3	NEG	NEG	1
53	3	13	PT2014BRUSERPS3	POS	POS	1
54	3	14	PT2014BRUSERPS2	POS	POS	1
55	3	15	PT2014BRUSERNS1	NEG	NEG	1
56	3	16	PT2014BRUSERPS1	POS	POS	1
57	3	17	PT2014BRUSERNS3	NEG	NEG	1
58	3	18	PT2014BRUSERNS2	NEG	NEG	1
59	3	19	PT2014BRUSERPS1	POS	POS	1
60	3	20	PT2014BRUSERNS3	NEG	NEG	1
61	4	1	PT2014BRUSERNS2	NEG	NEG	1
62	4	2	PT2014BRUSERPS1	POS	POS	1
63	4	3	PT2014BRUSERPS2	POS	POS	1
64	4	4	PT2014BRUSERNS1	NEG	NEG	1
65	4	5	PT2014BRUSERNS3	NEG	NEG	1
66	4	6	PT2014BRUSERPS3	POS	POS	1
67	4	7	PT2014BRUSERNS2	NEG	NEG	1
68	4	8	PT2014BRUSERNS1	NEG	NEG	1
69	4	9	PT2014BRUSERPS2	POS	POS	1
70	4	10	PT2014BRUSERNS3	NEG	NEG	1
71	4	11	PT2014BRUSERPS1	POS	POS	1
72	4	12	PT2014BRUSERNS3	NEG	NEG	1
73	4	13	PT2014BRUSERPS3	POS	POS	1
74	4	14	PT2014BRUSERPS2	POS	POS	1
75	4	15	PT2014BRUSERPS3	POS	POS	1
76	4	16	PT2014BRUSERPS1	POS	POS	1
77	4	17	PT2014BRUSERNS3	NEG	NEG	1
78	4	18	PT2014BRUSERNS2	NEG	NEG	1
79	4	19	PT2014BRUSERPS1	POS	POS	1
80	4	20	PT2014BRUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2014BRUSERPS2	POS	POS	1
82	5	2	PT2014BRUSERNS1	NEG	NEG	1
83	5	3	PT2014BRUSERNS3	NEG	NEG	1
84	5	4	PT2014BRUSERPS3	POS	POS	1
85	5	5	PT2014BRUSERNS2	NEG	NEG	1
86	5	6	PT2014BRUSERPS1	POS	POS	1
87	5	7	PT2014BRUSERPS2	POS	POS	1
88	5	8	PT2014BRUSERPS3	POS	POS	1
89	5	9	PT2014BRUSERNS2	NEG	NEG	1
90	5	10	PT2014BRUSERNS1	NEG	NEG	1
91	5	11	PT2014BRUSERPS1	POS	POS	1
92	5	12	PT2014BRUSERNS3	NEG	NEG	1
93	5	13	PT2014BRUSERPS3	POS	POS	1
94	5	14	PT2014BRUSERPS2	POS	POS	1
95	5	15	PT2014BRUSERNS1	NEG	NEG	1
96	5	16	PT2014BRUSERPS1	POS	POS	1
97	5	17	PT2014BRUSERNS3	NEG	NEG	1
98	5	18	PT2014BRUSERNS2	NEG	NEG	1
99	5	19	PT2014BRUSERPS1	POS	POS	1
100	5	20	PT2014BRUSERNS3	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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BRUSERPS2	POS	POS	1
2	1	2	PT2014BRUSERNS1	NEG	NEG	1
3	1	3	PT2014BRUSERNS3	NEG	NEG	1
4	1	4	PT2014BRUSERPS3	POS	POS	1
5	1	5	PT2014BRUSERNS2	NEG	NEG	1
6	1	6	PT2014BRUSERPS1	POS	POS	1
7	1	7	PT2014BRUSERPS2	POS	POS	1
8	1	8	PT2014BRUSERPS3	POS	POS	1
9	1	9	PT2014BRUSERNS2	NEG	NEG	1
10	1	10	PT2014BRUSERNS1	NEG	NEG	1
11	1	11	PT2014BRUSERPS1	POS	POS	1
12	1	12	PT2014BRUSERNS3	NEG	NEG	1
13	1	13	PT2014BRUSERPS3	POS	POS	1
14	1	14	PT2014BRUSERPS2	POS	POS	1
15	1	15	PT2014BRUSERNS1	NEG	NEG	1
16	1	16	PT2014BRUSERPS1	POS	POS	1
17	1	17	PT2014BRUSERNS3	NEG	NEG	1
18	1	18	PT2014BRUSERNS2	NEG	NEG	1
19	1	19	PT2014BRUSERPS1	POS	POS	1
20	1	20	PT2014BRUSERNS3	NEG	NEG	1
21	4	1	PT2014BRUSERNS2	NEG	NEG	1
22	4	2	PT2014BRUSERPS1	POS	POS	1
23	4	3	PT2014BRUSERPS2	POS	POS	1
24	4	4	PT2014BRUSERNS1	NEG	NEG	1
25	4	5	PT2014BRUSERNS3	NEG	NEG	1
26	4	6	PT2014BRUSERPS3	POS	POS	1
27	4	7	PT2014BRUSERNS2	NEG	NEG	1
28	4	8	PT2014BRUSERNS1	NEG	NEG	1
29	4	9	PT2014BRUSERPS2	POS	POS	1
30	4	10	PT2014BRUSERNS3	NEG	NEG	1
31	4	11	PT2014BRUSERPS1	POS	POS	1
32	4	12	PT2014BRUSERNS3	NEG	NEG	1
33	4	13	PT2014BRUSERPS3	POS	POS	1
34	4	14	PT2014BRUSERPS2	POS	POS	1
35	4	15	PT2014BRUSERPS3	POS	POS	1
36	4	16	PT2014BRUSERPS1	POS	POS	1
37	4	17	PT2014BRUSERNS3	NEG	NEG	1
38	4	18	PT2014BRUSERNS2	NEG	NEG	1
39	4	19	PT2014BRUSERPS1	POS	POS	1
40	4	20	PT2014BRUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2014BRUSERPS2	POS	POS	1
42	5	2	PT2014BRUSERNS1	NEG	NEG	1
43	5	3	PT2014BRUSERNS3	NEG	NEG	1
44	5	4	PT2014BRUSERPS3	POS	POS	1
45	5	5	PT2014BRUSERNS2	NEG	NEG	1
46	5	6	PT2014BRUSERPS1	POS	POS	1
47	5	7	PT2014BRUSERPS2	POS	POS	1
48	5	8	PT2014BRUSERPS3	POS	POS	1
49	5	9	PT2014BRUSERNS2	NEG	NEG	1
50	5	10	PT2014BRUSERNS1	NEG	NEG	1
51	5	11	PT2014BRUSERPS1	POS	POS	1
52	5	12	PT2014BRUSERNS3	NEG	NEG	1
53	5	13	PT2014BRUSERPS3	POS	POS	1
54	5	14	PT2014BRUSERPS2	POS	POS	1
55	5	15	PT2014BRUSERNS1	NEG	NEG	1
56	5	16	PT2014BRUSERPS1	POS	POS	1
57	5	17	PT2014BRUSERNS3	NEG	NEG	1
58	5	18	PT2014BRUSERNS2	NEG	NEG	1
59	5	19	PT2014BRUSERPS1	POS	POS	1
60	5	20	PT2014BRUSERNS3	NEG	NEG	1
61	6	1	PT2014BRUSERNS2	NEG	NEG	1
62	6	2	PT2014BRUSERPS1	POS	POS	1
63	6	3	PT2014BRUSERPS2	POS	POS	1
64	6	4	PT2014BRUSERNS1	NEG	NEG	1
65	6	5	PT2014BRUSERNS3	NEG	NEG	1
66	6	6	PT2014BRUSERPS3	POS	POS	1
67	6	7	PT2014BRUSERNS2	NEG	NEG	1
68	6	8	PT2014BRUSERNS1	NEG	NEG	1
69	6	9	PT2014BRUSERPS2	POS	POS	1
70	6	10	PT2014BRUSERNS3	NEG	NEG	1
71	6	11	PT2014BRUSERPS1	POS	POS	1
72	6	12	PT2014BRUSERNS3	NEG	NEG	1
73	6	13	PT2014BRUSERPS3	POS	POS	1
74	6	14	PT2014BRUSERPS2	POS	POS	1
75	6	15	PT2014BRUSERPS3	POS	POS	1
76	6	16	PT2014BRUSERPS1	POS	POS	1
77	6	17	PT2014BRUSERNS3	NEG	NEG	1
78	6	18	PT2014BRUSERNS2	NEG	NEG	1
79	6	19	PT2014BRUSERPS1	POS	POS	1
80	6	20	PT2014BRUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	7	1	PT2014BRUSERPS2	POS	POS	1
82	7	2	PT2014BRUSERNS1	NEG	NEG	1
83	7	3	PT2014BRUSERNS3	NEG	NEG	1
84	7	4	PT2014BRUSERPS3	POS	POS	1
85	7	5	PT2014BRUSERNS2	NEG	NEG	1
86	7	6	PT2014BRUSERPS1	POS	POS	1
87	7	7	PT2014BRUSERPS2	POS	POS	1
88	7	8	PT2014BRUSERPS3	POS	POS	1
89	7	9	PT2014BRUSERNS2	NEG	NEG	1
90	7	10	PT2014BRUSERNS1	NEG	NEG	1
91	7	11	PT2014BRUSERPS1	POS	POS	1
92	7	12	PT2014BRUSERNS3	NEG	NEG	1
93	7	13	PT2014BRUSERPS3	POS	POS	1
94	7	14	PT2014BRUSERPS2	POS	POS	1
95	7	15	PT2014BRUSERNS1	NEG	NEG	1
96	7	16	PT2014BRUSERPS1	POS	POS	1
97	7	17	PT2014BRUSERNS3	NEG	NEG	1
98	7	18	PT2014BRUSERNS2	NEG	NEG	1
99	7	19	PT2014BRUSERPS1	POS	POS	1
100	7	20	PT2014BRUSERNS3	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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BRUSERPM1	POS	POS	1
2	1	2	PT2014BRUSERPM3	POS	POS	1
3	1	3	PT2014BRUSERNM1	NEG	NEG	1
4	1	4	PT2014BRUSERPM4	POS	POS	1
5	1	5	PT2014BRUSERPM1	POS	POS	1
6	1	6	PT2014BRUSERNM2	NEG	NEG	1
7	1	7	PT2014BRUSERPM3	POS	POS	1
8	1	8	PT2014BRUSERPM2	POS	POS	1
9	1	9	PT2014BRUSERNM1	NEG	NEG	1
10	1	10	PT2014BRUSERPM4	POS	POS	1
11	1	11	PT2014BRUSERPM2	POS	POS	1
12	1	12	PT2014BRUSERNM1	NEG	NEG	1
13	1	13	PT2014BRUSERPM3	POS	POS	1
14	1	14	PT2014BRUSERNM2	NEG	NEG	1
15	1	15	PT2014BRUSERPM2	POS	POS	1
16	1	16	PT2014BRUSERPM1	POS	POS	1
17	1	17	PT2014BRUSERNM2	NEG	NEG	1
18	1	18	PT2014BRUSERPM4	POS	POS	1
19	1	19	PT2014BRUSERPM1	POS	POS	1
20	1	20	PT2014BRUSERNM2	NEG	NEG	1
21	2	1	PT2014BRUSERNM2	NEG	NEG	1
22	2	2	PT2014BRUSERPM1	POS	POS	1
23	2	3	PT2014BRUSERPM3	POS	POS	1
24	2	4	PT2014BRUSERNM2	NEG	NEG	1
25	2	5	PT2014BRUSERPM4	POS	POS	1
26	2	6	PT2014BRUSERNM1	NEG	NEG	1
27	2	7	PT2014BRUSERPM2	POS	POS	1
28	2	8	PT2014BRUSERPM1	POS	POS	1
29	2	9	PT2014BRUSERNM2	NEG	NEG	1
30	2	10	PT2014BRUSERNM1	NEG	NEG	1
31	2	11	PT2014BRUSERPM4	POS	POS	1
32	2	12	PT2014BRUSERPM2	POS	POS	1
33	2	13	PT2014BRUSERPM1	POS	POS	1
34	2	14	PT2014BRUSERPM4	POS	POS	1
35	2	15	PT2014BRUSERPM3	POS	POS	1
36	2	16	PT2014BRUSERNM2	NEG	NEG	1
37	2	17	PT2014BRUSERPM1	POS	POS	1
38	2	18	PT2014BRUSERPM2	POS	POS	1
39	2	19	PT2014BRUSERNM1	NEG	NEG	1
40	2	20	PT2014BRUSERPM3	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2014BRUSERPM1	POS	POS	1
42	3	2	PT2014BRUSERPM3	POS	POS	1
43	3	3	PT2014BRUSERNM1	NEG	NEG	1
44	3	4	PT2014BRUSERPM4	POS	POS	1
45	3	5	PT2014BRUSERPM1	POS	POS	1
46	3	6	PT2014BRUSERNM2	NEG	NEG	1
47	3	7	PT2014BRUSERPM3	POS	POS	1
48	3	8	PT2014BRUSERPM2	POS	POS	1
49	3	9	PT2014BRUSERNM1	NEG	NEG	1
50	3	10	PT2014BRUSERPM4	POS	POS	1
51	3	11	PT2014BRUSERPM2	POS	POS	1
52	3	12	PT2014BRUSERNM1	NEG	NEG	1
53	3	13	PT2014BRUSERPM3	POS	POS	1
54	3	14	PT2014BRUSERNM2	NEG	NEG	1
55	3	15	PT2014BRUSERPM2	POS	POS	1
56	3	16	PT2014BRUSERPM1	POS	POS	1
57	3	17	PT2014BRUSERNM2	NEG	NEG	1
58	3	18	PT2014BRUSERPM4	POS	POS	1
59	3	19	PT2014BRUSERPM1	POS	POS	1
60	3	20	PT2014BRUSERNM2	NEG	NEG	1
61	4	1	PT2014BRUSERNM2	NEG	NEG	1
62	4	2	PT2014BRUSERPM1	POS	POS	1
63	4	3	PT2014BRUSERPM3	POS	POS	1
64	4	4	PT2014BRUSERNM2	NEG	NEG	1
65	4	5	PT2014BRUSERPM4	POS	POS	1
66	4	6	PT2014BRUSERNM1	NEG	NEG	1
67	4	7	PT2014BRUSERPM2	POS	POS	1
68	4	8	PT2014BRUSERPM1	POS	POS	1
69	4	9	PT2014BRUSERNM2	NEG	NEG	1
70	4	10	PT2014BRUSERNM1	NEG	NEG	1
71	4	11	PT2014BRUSERPM4	POS	POS	1
72	4	12	PT2014BRUSERPM2	POS	POS	1
73	4	13	PT2014BRUSERPM1	POS	POS	1
74	4	14	PT2014BRUSERPM4	POS	POS	1
75	4	15	PT2014BRUSERPM3	POS	POS	1
76	4	16	PT2014BRUSERNM2	NEG	NEG	1
77	4	17	PT2014BRUSERPM1	POS	POS	1
78	4	18	PT2014BRUSERPM2	POS	POS	1
79	4	19	PT2014BRUSERNM1	NEG	NEG	1
80	4	20	PT2014BRUSERPM3	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	8	1	PT2014BRUSERPM1	POS	POS	1
82	8	2	PT2014BRUSERPM3	POS	POS	1
83	8	3	PT2014BRUSERNM1	NEG	NEG	1
84	8	4	PT2014BRUSERPM4	POS	POS	1
85	8	5	PT2014BRUSERPM1	POS	POS	1
86	8	6	PT2014BRUSERNM2	NEG	NEG	1
87	8	7	PT2014BRUSERPM3	POS	POS	1
88	8	8	PT2014BRUSERPM2	POS	POS	1
89	8	9	PT2014BRUSERNM1	NEG	NEG	1
90	8	10	PT2014BRUSERPM4	POS	POS	1
91	8	11	PT2014BRUSERPM2	POS	POS	1
92	8	12	PT2014BRUSERNM1	NEG	NEG	1
93	8	13	PT2014BRUSERPM3	POS	POS	1
94	8	14	PT2014BRUSERNM2	NEG	NEG	1
95	8	15	PT2014BRUSERPM2	POS	POS	1
96	8	16	PT2014BRUSERPM1	POS	POS	1
97	8	17	PT2014BRUSERNM2	NEG	NEG	1
98	8	18	PT2014BRUSERPM4	POS	POS	1
99	8	19	PT2014BRUSERPM1	POS	POS	1
100	8	20	PT2014BRUSERNM2	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, the 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) (Table 2, Table 3, Table 4, Table 6, Table 7 and Table 8). Three of the 4 participating laboratories that performed SAW-EDTA used the same batch of the SAW antigen from Synbiotics (batch 12SAW12), LAB4 used the SAW antigen from a different producer, IDEXX (batch 212). The 4 participating laboratories that performed RBT used a RBT antigen from 2 different producers, namely Synbiotics Europe (1 batch: 11BGT59) and IDEXX Montpellier SAS (3 batches: 381-100, 383-100 and 377-10). Hereby, LAB1, LAB4 and LAB5 used the same antigen. From the 5 participating laboratories that performed ELISA, LAB1 used an in-house developed BRU antibody ELISA kit, whereas LAB 4 used a commercially available BRU antibody ELISA kit from IDEXX (batch: 4011). Furthermore LAB5, LAB6 and LAB7 used a commercially available BRU antibody ELISA kit from Synbiotics (1 batch: 14SBRU30CB56).

For the detection of BRU-specific antibodies in reference milk samples, the 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 5 and Table 9). All participating laboratories used the BRU antibody ELISA kit from IDEXX Montpellier SAS, but 2 different batches were used: batch 3248 (LAB1) and batch 3131 (LAB2, LAB3, LAB4 and LAB8).

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 participants achieved a satisfactory performance for the detection of BRU-specific antibodies in (i) reference serum samples by SAW-EDTA and/or RBT and/or ELISA, and (ii) reference milk samples by ELISA.

Head CVD-ERA
Yves Van der Stede

Appendix

Name of the participating laboratories

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

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

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

ANSES Maisons-Alfort (Unité Zoonose Bactériennes, LNR/LREU Brucellose) (ANSES) (Maisons-Alfort, France)

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 on the results obtained by ELISA in both the PT serum and the PT milk. Therefore, the statistical software programs R (box plots) and SAS 9.2. (summary statistics) were used. All quantitative data analyses were performed on the normalized data, namely the percentages S/P ratio calculated according to the instructions for this PT: [(OD_{Sample} - mean $OD_{Negative\ Kit\ Controls}$) / (mean $OD_{Positive\ Kit\ Controls}$ - 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.

Remark: For the PT serum, the normalized data obtained by LAB1 (in-house developed BRU antibody ELISA kit) were not comparable with the normalized data obtained by the other participating laboratories (commercially available BRU antibody ELISA kit) and could hence not be included into the comparative quantitative data analysis for the PT serum.

I. Box plots

Box plots of the percentages S/P ratio per reference sample and per participating laboratory were made using the statistical software R and are shown in Figure 1 for the PT serum and in Figure 2 for the PT milk.

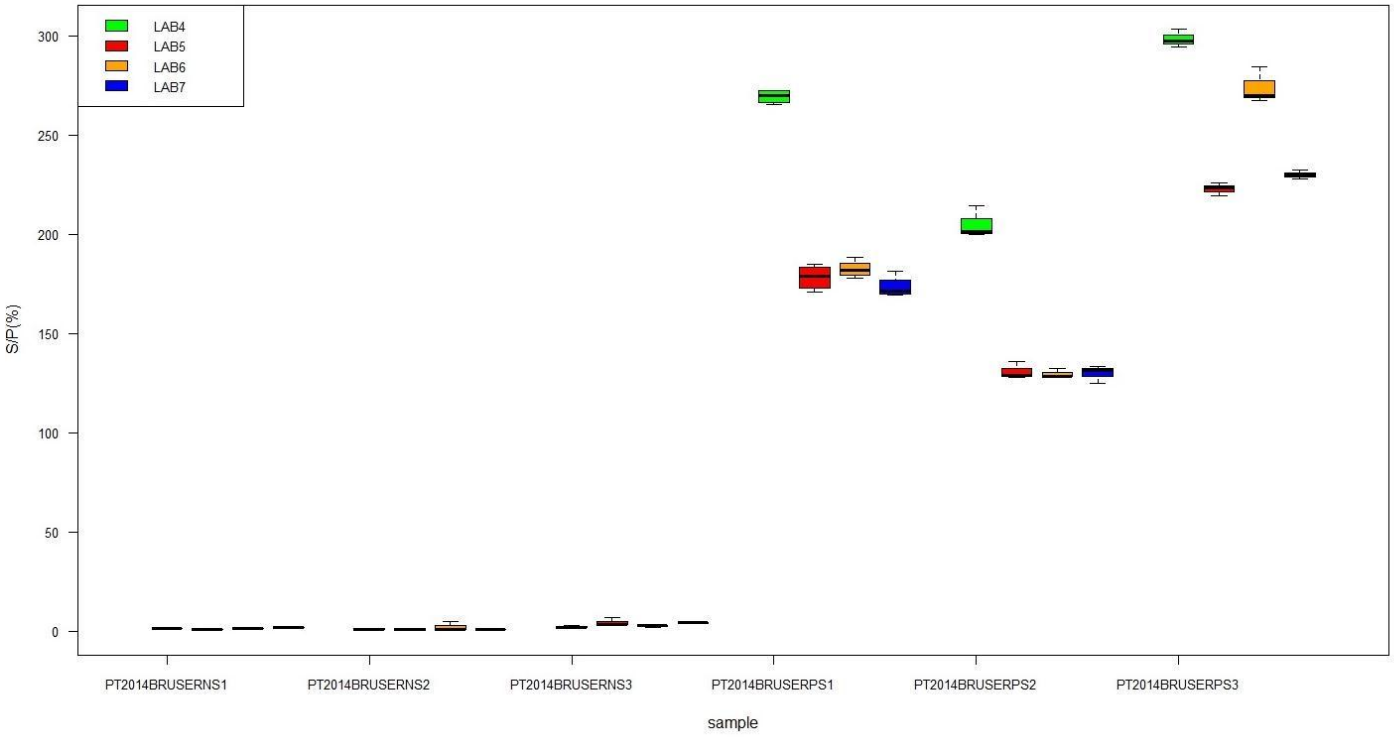

Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. The participating laboratories used 2 differents BRU antibody ELISA kits, LAB 4 used a commercially available BRU antibody ELISA kit from IDEXX (batch: 4011), whereas LAB5, LAB6 and LAB7 used the same batch of a commercially available BRU antibody ELISA kit from Synbiotics (batch: 14SBRU30CB56). Cut-off values are not shown since these were based on the mean OD values of the positive kit controls and are hence different for each participant.

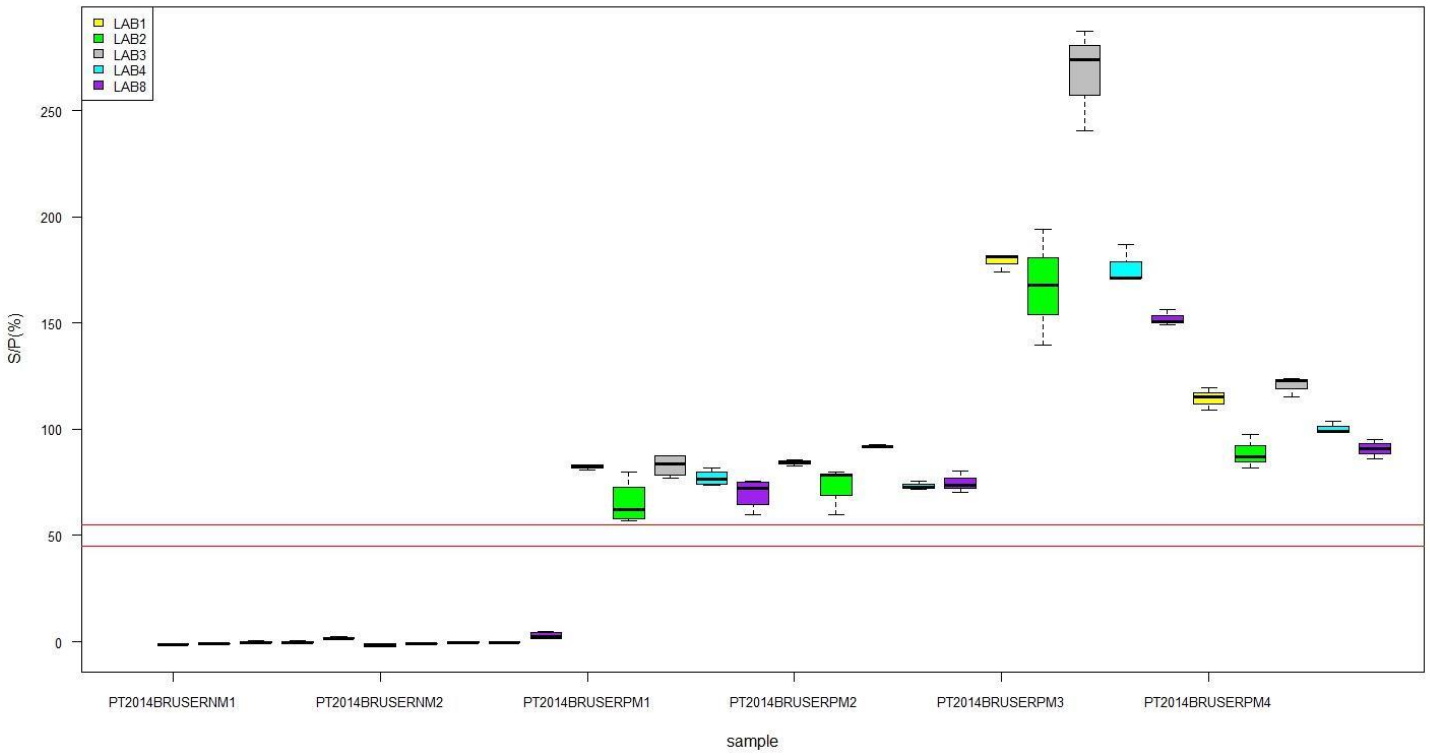

Figure 2. Box plots showing the percentage S/P ratio per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. All participating laboratories used the BRU antibody ELISA kit from IDEXX Montpellier SAS, but 2 different batches were used: batch 3248 (LAB1) and batch 3131 (LAB2, LAB3, LAB4 and LAB8). In addition, The cut-off value of 45-55% is shown in red.

II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-laboratory variability (reproducibility) and within-laboratory variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated per reference serum/milk sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,40	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic is 1,42 for the PT serum (p=4) and 1,57 the PT milk (p=5). For the PT serum, the maximum value for Mandel's k-statistic is 1,59 for the reference serum samples PT2014BRUSERNS1, PT2014BRUSERNS2, PT2014BRUSERPS2 and PT2014BRUSERPS3 (p=4 and n=3) and 1,50 for the reference serum samples PT2014BRUSERNS3 and PT2014BRUSERPS1 (p=4 and n=4). For the PT milk, the maximum value for Mandel's k-statistic is 1,62 for the reference milk samples PT2014BRUSERNM1, PT2014BRUSERPM2, PT2014BRUSERPM3 and PT2014BRUSERPM4 (p=5 and n=3) and 1,53 for the reference milk samples PT2014BRUSERNM2 and PT2014BRUSERPM1 (p=5 and n=4).

For the detection of BRU-specific antibodies in serum, only 2 of the 4 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. In contrast, the other participants showed an increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB 4 for the positive reference serum samples PT2014BRUSERPS1 (h=1,50) and PT2014BRUSERPS2 (h=1,50), and LAB6 for the negative reference serum sample PT2014BRUSERNS2 (h=1,47). The participating laboratories used 2 differents BRU antibody ELISA kits, LAB 4 used a commercially available BRU antibody ELISA kit from IDEXX (batch: 4011), whereas LAB5, LAB6 and LAB7 used the same batch of a commercially available BRU antibody ELISA kit from Synbiotics (batch: 14SBRU30CB56).
Only 2 out of 4 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples (LAB2). In contrast, LAB5 and LAB6 showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB5 for the negative reference serum sample PT2014BRUSERNS3 (k=1,80) and LAB6 for the negative reference serum sample PT2014BRUSERNS2 (k=1,90) and the positive reference serum sample PT2014BRUSERPS3 (k=1,68).

For the detection of BRU-specific antibodies in milk, 3 out of 5 participating laboratories obtained a satisfactory between-laboratory consistency for all reference milk samples. This was not the case for LAB3 and LAB8, which showed an increased value for Mandel's h-statistic: LAB3 for the positive reference milk sample PT2014BRUSERPM3 (h=1,74), and LAB8 for the negative reference milk samples PT2014BRUSERNM1 (h= 1,59) and PT2014BRUSERNM2 (h=1,71). All participating laboratories used the BRU antibody ELISA kit from IDEXX Montpellier SAS, but 2 different batches were used: batch 3248 (LAB1) and batch 3131 (LAB2, LAB3, LAB4 and LAB8).
Furthermore, 3 out of 5 participating laboratories obtained a satisfactory within-laboratory consistency for all reference milk samples (LAB1, LAB3 and LAB4). In contrast, LAB2 and LAB8 showed an increased value for Mandel's k-statistic for

at least 1 reference milk sample: LAB2 for the positive reference milk samples PT2014BRUSERPM1 (k=1,65) and PT2014BRUSERPM2 (k=2,00) and LAB8 for the negative reference milk sample PT2014BRUSERNM2 (k=1,87).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values as a dependent variable, it was investigated whether statistically significant differences exist (α=0,05) between participating laboratories. Comparisons were made at the global level (all reference samples were analysed together), status level (all reference samples with the same status were analysed together) and sample level (all reference samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

III.1. Serum

For the PT serum, no statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were observed for the positive but not for the negative reference serum samples. For the positive reference serum samples, LAB4 reported percentages S/P ratio that were significantly higher than those reported by the other labs.

III.2. Milk

For the PT milk, no statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were observed for the negative but not for the positive reference milk samples. For the negative reference milk samples, LAB8 reported percentages S/P ratio that were significantly higher than those reported by the other laboratories. For samples PT2014BRUSERPM3 and . PT2014BRUSERPM4, LAB3 reported clearly higher S/P ratio's compared to the other laboratories.

Annex 2: Calculations of Mandel's h- and k-statistics – ELISA (based on % S/P)

A. PT serum

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
PT2014BRUSERNS1	4	3	0,05	1,19	1,19	0,35	0,26	0,32	0,19	0,00	0,81	17,87
PT2014BRUSERNS1	5	3	0,17	0,75	1,19	0,35	0,26	0,32	0,19	-1,20	1,56	54,28
PT2014BRUSERNS1	6	3	0,05	1,17	1,19	0,35	0,26	0,32	0,19	-0,04	0,85	18,88
PT2014BRUSERNS1	7	3	0,01	1,64	1,19	0,35	0,26	0,32	0,19	1,25	0,43	6,85
PT2014BRUSERNS2	4	3	0,02	1,05	1,19	0,00	1,20	1,20	0,00	-0,24	0,13	14,81
PT2014BRUSERNS2	5	3	0,47	0,79	1,19	0,00	1,20	1,20	0,00	-0,67	0,57	86,33
PT2014BRUSERNS2	**6**	3	5,21	2,07	1,19	0,00	1,20	1,20	0,00	**1,47**	**1,90**	110,31
PT2014BRUSERNS2	7	3	0,09	0,86	1,19	0,00	1,20	1,20	0,00	-0,56	0,25	34,63
PT2014BRUSERNS3	4	4	0,34	2,11	3,25	0,24	0,96	1,10	0,54	-1,08	0,61	27,65
PT2014BRUSERNS3	**5**	4	3,00	4,10	3,25	0,24	0,96	1,10	0,54	0,81	**1,80**	42,26
PT2014BRUSERNS3	6	4	0,29	2,60	3,25	0,24	0,96	1,10	0,54	-0,62	0,56	20,56
PT2014BRUSERNS3	7	4	0,08	4,19	3,25	0,24	0,96	1,10	0,54	0,89	0,30	6,86
PT2014BRUSERPS1	**4**	4	12,42	269,42	200,81	0,96	5,13	26,95	26,45	**1,50**	0,69	1,31
PT2014BRUSERPS1	5	4	42,23	178,24	200,81	0,96	5,13	26,95	26,45	-0,49	1,27	3,65
PT2014BRUSERPS1	6	4	19,71	182,28	200,81	0,96	5,13	26,95	26,45	-0,40	0,86	2,44
PT2014BRUSERPS1	7	4	31,03	173,31	200,81	0,96	5,13	26,95	26,45	-0,60	1,09	3,21
PT2014BRUSERPS2	**4**	3	62,73	205,06	148,83	0,95	5,17	22,19	21,58	**1,50**	1,53	3,86
PT2014BRUSERPS2	5	3	19,34	130,89	148,83	0,95	5,17	22,19	21,58	-0,48	0,85	3,36
PT2014BRUSERPS2	6	3	5,34	129,56	148,83	0,95	5,17	22,19	21,58	-0,51	0,45	1,78
PT2014BRUSERPS2	7	3	19,41	129,83	148,83	0,95	5,17	22,19	21,58	-0,51	0,85	3,39
PT2014BRUSERPS3	4	3	20,49	298,20	256,18	0,94	5,46	21,42	20,72	1,17	0,83	1,52
PT2014BRUSERPS3	5	3	10,16	222,67	256,18	0,94	5,46	21,42	20,72	-0,93	0,58	1,43
PT2014BRUSERPS3	**6**	3	84,17	273,91	256,18	0,94	5,46	21,42	20,72	0,49	**1,68**	3,35
PT2014BRUSERPS3	7	3	4,30	229,93	256,18	0,94	5,46	21,42	20,72	-0,73	0,38	0,90

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalized data (% S/P); **x_i_m** = mean of normalized data (% S/P); **x_g_m** = mean of normalized data (% S/P) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).

B. PT milk

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_lab_coeff	STDEV_repeat	STDEV_repro	STDEV_betweenlab	h	k	cv
PT2014BRUSERNM1	1	3	0,29	-1,43	-0,24	0,52	0,55	0,79	0,57	-1,01	0,99	-37,96
PT2014BRUSERNM1	2	3	0,09	-1,01	-0,24	0,52	0,55	0,79	0,57	-0,66	0,55	-29,54
PT2014BRUSERNM1	3	3	0,27	-0,18	-0,24	0,52	0,55	0,79	0,57	0,05	0,94	-290,34
PT2014BRUSERNM1	4	3	0,18	-0,21	-0,24	0,52	0,55	0,79	0,57	0,02	0,77	-197,83
PT2014BRUSERNM1	**8**	3	0,67	1,64	-0,24	0,52	0,55	0,79	0,57	**1,59**	1,50	49,99
PT2014BRUSERNM2	1	4	0,82	-1,60	-0,07	0,50	0,84	1,18	0,83	-0,88	1,08	-56,64
PT2014BRUSERNM2	2	4	0,03	-0,89	-0,07	0,50	0,84	1,18	0,83	-0,47	0,19	-17,98
PT2014BRUSERNM2	3	4	0,18	-0,41	-0,07	0,50	0,84	1,18	0,83	-0,20	0,50	-101,73
PT2014BRUSERNM2	4	4	0,05	-0,34	-0,07	0,50	0,84	1,18	0,83	-0,16	0,26	-62,97
PT2014BRUSERNM2	**8**	4	2,46	2,87	-0,07	0,50	0,84	1,18	0,83	**1,71**	**1,87**	54,72
PT2014BRUSERPM1	1	4	1,17	82,42	75,51	0,24	6,40	7,33	3,57	0,88	0,17	1,31
PT2014BRUSERPM1	**2**	4	110,96	65,17	75,51	0,24	6,40	7,33	3,57	-1,32	**1,65**	16,16
PT2014BRUSERPM1	3	4	27,91	83,01	75,51	0,24	6,40	7,33	3,57	0,96	0,83	6,36
PT2014BRUSERPM1	4	4	13,59	77,06	75,51	0,24	6,40	7,33	3,57	0,20	0,58	4,78
PT2014BRUSERPM1	8	4	51,23	69,88	75,51	0,24	6,40	7,33	3,57	-0,72	1,12	10,24
PT2014BRUSERPM2	1	3	2,56	84,22	79,37	0,32	5,61	6,81	3,87	0,58	0,29	1,90
PT2014BRUSERPM2	**2**	3	125,99	72,64	79,37	0,32	5,61	6,81	3,87	-0,80	**2,00**	15,45
PT2014BRUSERPM2	3	3	0,39	91,83	79,37	0,32	5,61	6,81	3,87	1,49	0,11	0,68
PT2014BRUSERPM2	4	3	3,17	73,31	79,37	0,32	5,61	6,81	3,87	-0,72	0,32	2,43
PT2014BRUSERPM2	8	3	25,08	74,86	79,37	0,32	5,61	6,81	3,87	-0,54	0,89	6,69

PT2014BRUSERPM3	1	3	18,69	179,06	188,38	0,63	16,95	27,86	22,10	-0,21	0,26	2,41
PT2014BRUSERPM3	2	3	739,64	167,11	188,38	0,63	16,95	27,86	22,10	-0,47	1,60	16,27
PT2014BRUSERPM3	**3**	3	582,71	267,16	188,38	0,63	16,95	27,86	22,10	**1,74**	1,42	9,04
PT2014BRUSERPM3	4	3	80,98	176,36	188,38	0,63	16,95	27,86	22,10	-0,27	0,53	5,10
PT2014BRUSERPM3	8	3	15,00	152,19	188,38	0,63	16,95	27,86	22,10	-0,80	0,23	2,55
PT2014BRUSERPM4	1	3	27,61	114,50	103,00	0,62	5,37	8,76	6,92	0,81	0,98	4,59
PT2014BRUSERPM4	2	3	64,29	88,84	103,00	0,62	5,37	8,76	6,92	-1,00	1,49	9,03
PT2014BRUSERPM4	3	3	22,36	120,68	103,00	0,62	5,37	8,76	6,92	1,25	0,88	3,92
PT2014BRUSERPM4	4	3	9,58	100,27	103,00	0,62	5,37	8,76	6,92	-0,19	0,58	3,09
PT2014BRUSERPM4	8	3	20,38	90,71	103,00	0,62	5,37	8,76	6,92	-0,87	0,84	4,98

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalized data (% S/P); **x_i_m** = mean of normalized data (% S/P); **x_g_m** = mean of normalized data (% S/P) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).