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172-PT

PROFICIENCY TESTING 2012

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: 17 SEPTEMBER 2012

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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 'PT2012BRUSERNS1', 'PT2012BRUSERNS2' and 'PT2012BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2012BRUSERPS1', 'PT2012BRUSERPS2' and 'PT2012BRUSERPS3'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012BRUSERNS1, PT2012BRUSERNS2, PT2012BRUSERNS3, PT2012BRUSERPS2 and PT2012BRUSERPS3, and 5 aliquots of the reference serum sample PT2012BRUSERPS1. 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 earlier based on (i) the historical background of the animals and (ii) the results obtained by SAW with and without EDTA, RBT, an in-house BRU antibody ELISA test and the complement fixation test. The reference serum samples PT2012BRUSERNS1, PT2012BRUSERNS2 and PT2012BRUSERNS3 were derived from BRU-free farms, whereas the reference serum sample PT2012BRUSERPS3 was a 1/2 dilution of a serum obtained from a BRU-positive farm during a BRU outbreak in December 2010 in Belgium. The reference serum samples PT2012BRUSERPS1 and PT2012BRUSERPS2 were a 1/4 and a 1/2 dilution, respectively, of a serum derived from an animal that was experimentally infected with the *Brucella abortus* strain W99 (serum 3667). For each reference serum sample, the same qualitative result was obtained with all test methods used.
According to the procedure PRO/2.5/01 (cfr. Manual for the participant, section III.1), these reference serum samples were retested once shortly before the current PT with SAW with and without EDTA, RBT and an in-house BRU antibody ELISA test, hereby confirming the previously assigned status (pre-verification). Taken together, the reference serum samples PT2012BRUSERNS1, PT2012BRUSERNS2 and PT2012BRUSERNS3 were considered as negative sera, and the reference serum samples PT2012BRUSERPS1, PT2012BRUSERPS2 and PT2012BRUSERPS3 as (strong) positive sera.

A homogeneity check on the aliquoted and lyophilized samples was performed earlier on 10 aliquots of each reference serum sample using SAW-EDTA, RBT and an in-house BRU antibody ELISA test, hereby obtaining for each test the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of the participating laboratory to correctly identify the absence or presence of BRU-specific antibodies in bovine serum. In addition, all reference serum samples were tested once 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 'PT2012BRUSERNM1' and 'PT2012BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2012BRUSERPM1', 'PT2012BRUSERPM2', 'PT2012BRUSERPM3' and 'PT2012BRUSERPM4'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2012BRUSERNM1, PT2012BRUSERPM1, PT2012BRUSERPM3 and PT2012BRUSERPM4, and 4 aliquots of the reference milk samples PT2012BRUSERNM2 and PT2012BRUSERPM2. 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 using the BRU antibody ELISA test kit from IDEXX Montpellier SAS (pre-verification). The reference milk samples PT2012BRUSERNM1 and PT2012BRUSERNM2 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, PT2012BRUSERPM1 and PT2012BRUSERPM2 were spiked with serum 3667 in a 1/800 and a 1/1000 dilution, respectively, whereas PT2012BRUSERPM3 and PT2012BRUSERPM4 were spiked with serum 3467 in a 1/6400 and a 1/12800 dilution, respectively. Serum 3667 and serum 3467 were both obtained from animals that were experimentally infected with the *Brucella abortus* strain W99.
Taken together, the reference samples PT2012BRUSERNM1 and PT2012BRUSERNM2 were considered as negative milk samples, and the reference samples PT2012BRUSERPM1, PT2012BRUSERPM2, PT2012BRUSERPM3 and PT2012BRUSERPM4 as (strong) 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 tank milk. In addition, all reference milk samples were tested once 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 result equals the titre of the reference serum sample ± 1 titre; *failure* = the result does not equal the titre of the reference serum sample ± 1 titre.
- For RBT and ELISA: *success* = positive result when the reference serum sample is truly positive, negative result when the reference serum sample is truly negative; *failure* = positive result when the reference serum sample is truly negative, negative result when the reference serum sample is truly positive.

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* (i.e., the reported result matches with the assigned status) 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* (positive result when the reference milk sample is truly positive, negative result when the reference milk sample is truly negative) or *failure* (positive result when

the reference milk sample is truly negative, negative result when the reference milk sample is truly positive, non-interpretable result when the reference milk sample is truly negative or positive).

III.3.2.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* (i.e., the reported result matches with the assigned status) 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 and LAB3 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, LAB4 and LAB5 only participated in the PT serum, whereas LAB6 only participated in the PT milk. These 3 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 17th of September 2012 (180 aliquots in total). All participants acknowledged receipt of the samples on the same day. Analyses were performed between 17th and 20th of September 2012 for serum (LAB3 did not provide the date of analysis) and between 18th and 24th of September 2012 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 19th of September and 1st of October 2012 (Table 1). LAB1 and LAB2 hereby (partially) exceeded the deadline of 28th of September 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	RB		
LAB1	17/09/2012	NA	18/09/2012	18/09/2012	24/09/2012	26/09/2012 & **01/10/2012** (*)
LAB2	17/09/2012	17/09/2012	18/09/2012	17/09/2012	21/09/2012	**01/10/2012**
LAB3	17/09/2012	NA	NA	**NOT PROVIDED**	18/09/2012	28/09/2012
LAB4	17/09/2012	17/09/2012	18/09/2012	NA	NA	19/09/2012
LAB5	17/09/2012	20/09/2012	20/09/2012	20/09/2012	NA	28/09/2012
LAB6	17/09/2012	NA	NA	NA	20/09/2012	21/09/2012

Legend: NA = not applicable; (*) = this laboratory used ELISA kits from 2 different kit producers

IV.3. Compliance with the procedure

Except LAB6, all participating laboratories have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

Since LAB1 analyzed the 20 aliquots of reference serum samples with ELISA kits from 2 different producers, this participating laboratory submitted 2 sets of results for the PT serum. Therefore, for the qualitative analysis of the ELISA results reported for the PT serum, LAB1 has been divided into 2 sublaboratories, namely LAB1.1 (kit1) and LAB1.2 (kit2).

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 true 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 generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of CODA-CERVA. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	2	**4**	**5**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. RBT: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of 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**	**5**
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)

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

	LABNR				
	1.1	**1.2**	**2**	**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)

IV.4.1.2. Reference milk samples

All participating laboratories provided qualitative results that were in full agreement with the true 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 generated by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the BRU reference laboratory of 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**	**6**
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)

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. Hereby, LAB1 obtained identical results using ELISA kits from 2 different producers.

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 the BRU reference laboratory of CODA-CERVA (STATUS). NEG: negative.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	2	1	PT2012BRUSERPS2	>=100	>=100	1
2	2	2	PT2012BRUSERNS3	NEG	NEG	1
3	2	3	PT2012BRUSERPS3	>=100	>=100	1
4	2	4	PT2012BRUSERNS2	NEG	NEG	1
5	2	5	PT2012BRUSERPS3	>=100	>=100	1
6	2	6	PT2012BRUSERPS1	50	50	1
7	2	7	PT2012BRUSERNS1	NEG	NEG	1
8	2	8	PT2012BRUSERPS2	>=100	>=100	1
9	2	9	PT2012BRUSERPS1	50	50	1
10	2	10	PT2012BRUSERPS1	50	50	1
11	2	11	PT2012BRUSERNS3	NEG	NEG	1
12	2	12	PT2012BRUSERPS2	>=100	>=100	1
13	2	13	PT2012BRUSERPS3	>=100	>=100	1
14	2	14	PT2012BRUSERNS1	NEG	NEG	1
15	2	15	PT2012BRUSERNS3	NEG	NEG	1
16	2	16	PT2012BRUSERPS1	50	50	1
17	2	17	PT2012BRUSERNS2	NEG	NEG	1
18	2	18	PT2012BRUSERNS2	NEG	NEG	1
19	2	19	PT2012BRUSERPS1	50	50	1
20	2	20	PT2012BRUSERNS1	NEG	NEG	1
21	4	1	PT2012BRUSERNS1	NEG	NEG	1
22	4	2	PT2012BRUSERPS2	>=100	>=100	1
23	4	3	PT2012BRUSERPS1	50	50	1
24	4	4	PT2012BRUSERPS1	50	50	1
25	4	5	PT2012BRUSERNS3	NEG	NEG	1
26	4	6	PT2012BRUSERPS2	>=100	>=100	1
27	4	7	PT2012BRUSERPS3	>=100	>=100	1
28	4	8	PT2012BRUSERNS1	NEG	NEG	1
29	4	9	PT2012BRUSERNS3	NEG	NEG	1
30	4	10	PT2012BRUSERPS1	50	50	1
31	4	11	PT2012BRUSERNS2	NEG	NEG	1
32	4	12	PT2012BRUSERNS2	NEG	NEG	1
33	4	13	PT2012BRUSERPS1	50	50	1
34	4	14	PT2012BRUSERNS1	NEG	NEG	1
35	4	15	PT2012BRUSERPS2	>=100	>=100	1
36	4	16	PT2012BRUSERNS3	NEG	NEG	1
37	4	17	PT2012BRUSERPS3	>=100	>=100	1
38	4	18	PT2012BRUSERNS2	NEG	NEG	1
39	4	19	PT2012BRUSERPS3	>=100	>=100	1
40	4	20	PT2012BRUSERPS1	50	50	1

(Table 6 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2012BRUSERPS1	50	50	1
42	5	2	PT2012BRUSERNS3	NEG	NEG	1
43	5	3	PT2012BRUSERPS2	>=100	>=100	1
44	5	4	PT2012BRUSERPS3	>=100	>=100	1
45	5	5	PT2012BRUSERNS1	NEG	NEG	1
46	5	6	PT2012BRUSERNS3	NEG	NEG	1
47	5	7	PT2012BRUSERPS1	50	50	1
48	5	8	PT2012BRUSERNS2	NEG	NEG	1
49	5	9	PT2012BRUSERNS2	NEG	NEG	1
50	5	10	PT2012BRUSERPS1	50	50	1
51	5	11	PT2012BRUSERNS1	NEG	NEG	1
52	5	12	PT2012BRUSERPS2	>=100	>=100	1
53	5	13	PT2012BRUSERNS3	NEG	NEG	1
54	5	14	PT2012BRUSERPS3	>=100	>=100	1
55	5	15	PT2012BRUSERNS2	NEG	NEG	1
56	5	16	PT2012BRUSERPS3	>=100	>=100	1
57	5	17	PT2012BRUSERPS1	50	50	1
58	5	18	PT2012BRUSERNS1	NEG	NEG	1
59	5	19	PT2012BRUSERPS2	>=100	>=100	1
60	5	20	PT2012BRUSERPS1	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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012BRUSERNS2	NEG	NEG	1
2	1	2	PT2012BRUSERPS1	POS	POS	1
3	1	3	PT2012BRUSERNS1	NEG	NEG	1
4	1	4	PT2012BRUSERPS2	POS	POS	1
5	1	5	PT2012BRUSERNS3	NEG	NEG	1
6	1	6	PT2012BRUSERPS3	POS	POS	1
7	1	7	PT2012BRUSERNS2	NEG	NEG	1
8	1	8	PT2012BRUSERPS3	POS	POS	1
9	1	9	PT2012BRUSERPS1	POS	POS	1
10	1	10	PT2012BRUSERNS1	NEG	NEG	1
11	1	11	PT2012BRUSERPS2	POS	POS	1
12	1	12	PT2012BRUSERPS1	POS	POS	1
13	1	13	PT2012BRUSERPS1	POS	POS	1
14	1	14	PT2012BRUSERNS3	NEG	NEG	1
15	1	15	PT2012BRUSERPS2	POS	POS	1
16	1	16	PT2012BRUSERPS3	POS	POS	1
17	1	17	PT2012BRUSERNS1	NEG	NEG	1
18	1	18	PT2012BRUSERNS3	NEG	NEG	1
19	1	19	PT2012BRUSERPS1	POS	POS	1
20	1	20	PT2012BRUSERNS2	NEG	NEG	1
21	2	1	PT2012BRUSERPS2	POS	POS	1
22	2	2	PT2012BRUSERNS3	NEG	NEG	1
23	2	3	PT2012BRUSERPS3	POS	POS	1
24	2	4	PT2012BRUSERNS2	NEG	NEG	1
25	2	5	PT2012BRUSERPS3	POS	POS	1
26	2	6	PT2012BRUSERPS1	POS	POS	1
27	2	7	PT2012BRUSERNS1	NEG	NEG	1
28	2	8	PT2012BRUSERPS2	POS	POS	1
29	2	9	PT2012BRUSERPS1	POS	POS	1
30	2	10	PT2012BRUSERPS1	POS	POS	1
31	2	11	PT2012BRUSERNS3	NEG	NEG	1
32	2	12	PT2012BRUSERPS2	POS	POS	1
33	2	13	PT2012BRUSERPS3	POS	POS	1
34	2	14	PT2012BRUSERNS1	NEG	NEG	1
35	2	15	PT2012BRUSERNS3	NEG	NEG	1
36	2	16	PT2012BRUSERPS1	POS	POS	1
37	2	17	PT2012BRUSERNS2	NEG	NEG	1
38	2	18	PT2012BRUSERNS2	NEG	NEG	1
39	2	19	PT2012BRUSERPS1	POS	POS	1
40	2	20	PT2012BRUSERNS1	NEG	NEG	1

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012BRUSERNS2	NEG	NEG	1
42	3	2	PT2012BRUSERPS3	POS	POS	1
43	3	3	PT2012BRUSERPS1	POS	POS	1
44	3	4	PT2012BRUSERNS1	NEG	NEG	1
45	3	5	PT2012BRUSERPS2	POS	POS	1
46	3	6	PT2012BRUSERPS1	POS	POS	1
47	3	7	PT2012BRUSERPS1	POS	POS	1
48	3	8	PT2012BRUSERNS3	NEG	NEG	1
49	3	9	PT2012BRUSERPS2	POS	POS	1
50	3	10	PT2012BRUSERPS3	POS	POS	1
51	3	11	PT2012BRUSERNS1	NEG	NEG	1
52	3	12	PT2012BRUSERNS3	NEG	NEG	1
53	3	13	PT2012BRUSERPS1	POS	POS	1
54	3	14	PT2012BRUSERNS2	NEG	NEG	1
55	3	15	PT2012BRUSERNS2	NEG	NEG	1
56	3	16	PT2012BRUSERPS1	POS	POS	1
57	3	17	PT2012BRUSERNS1	NEG	NEG	1
58	3	18	PT2012BRUSERPS2	POS	POS	1
59	3	19	PT2012BRUSERNS3	NEG	NEG	1
60	3	20	PT2012BRUSERPS3	POS	POS	1
61	5	1	PT2012BRUSERPS1	POS	POS	1
62	5	2	PT2012BRUSERNS3	NEG	NEG	1
63	5	3	PT2012BRUSERPS2	POS	POS	1
64	5	4	PT2012BRUSERPS3	POS	POS	1
65	5	5	PT2012BRUSERNS1	NEG	NEG	1
66	5	6	PT2012BRUSERNS3	NEG	NEG	1
67	5	7	PT2012BRUSERPS1	POS	POS	1
68	5	8	PT2012BRUSERNS2	NEG	NEG	1
69	5	9	PT2012BRUSERNS2	NEG	NEG	1
70	5	10	PT2012BRUSERPS1	POS	POS	1
71	5	11	PT2012BRUSERNS1	NEG	NEG	1
72	5	12	PT2012BRUSERPS2	POS	POS	1
73	5	13	PT2012BRUSERNS3	NEG	NEG	1
74	5	14	PT2012BRUSERPS3	POS	POS	1
75	5	15	PT2012BRUSERNS2	NEG	NEG	1
76	5	16	PT2012BRUSERPS3	POS	POS	1
77	5	17	PT2012BRUSERPS1	POS	POS	1
78	5	18	PT2012BRUSERNS1	NEG	NEG	1
79	5	19	PT2012BRUSERPS2	POS	POS	1
80	5	20	PT2012BRUSERPS1	POS	POS	1

Table 8. ELISA: 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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1.1	1	PT2012BRUSERNS2	NEG	NEG	1
2	1.1	2	PT2012BRUSERPS1	POS	POS	1
3	1.1	3	PT2012BRUSERNS1	NEG	NEG	1
4	1.1	4	PT2012BRUSERPS2	POS	POS	1
5	1.1	5	PT2012BRUSERNS3	NEG	NEG	1
6	1.1	6	PT2012BRUSERPS3	POS	POS	1
7	1.1	7	PT2012BRUSERNS2	NEG	NEG	1
8	1.1	8	PT2012BRUSERPS3	POS	POS	1
9	1.1	9	PT2012BRUSERPS1	POS	POS	1
10	1.1	10	PT2012BRUSERNS1	NEG	NEG	1
11	1.1	11	PT2012BRUSERPS2	POS	POS	1
12	1.1	12	PT2012BRUSERPS1	POS	POS	1
13	1.1	13	PT2012BRUSERPS1	POS	POS	1
14	1.1	14	PT2012BRUSERNS3	NEG	NEG	1
15	1.1	15	PT2012BRUSERPS2	POS	POS	1
16	1.1	16	PT2012BRUSERPS3	POS	POS	1
17	1.1	17	PT2012BRUSERNS1	NEG	NEG	1
18	1.1	18	PT2012BRUSERNS3	NEG	NEG	1
19	1.1	19	PT2012BRUSERPS1	POS	POS	1
20	1.1	20	PT2012BRUSERNS2	NEG	NEG	1
21	1.2	1	PT2012BRUSERNS2	NEG	NEG	1
22	1.2	2	PT2012BRUSERPS1	POS	POS	1
23	1.2	3	PT2012BRUSERNS1	NEG	NEG	1
24	1.2	4	PT2012BRUSERPS2	POS	POS	1
25	1.2	5	PT2012BRUSERNS3	NEG	NEG	1
26	1.2	6	PT2012BRUSERPS3	POS	POS	1
27	1.2	7	PT2012BRUSERNS2	NEG	NEG	1
28	1.2	8	PT2012BRUSERPS3	POS	POS	1
29	1.2	9	PT2012BRUSERPS1	POS	POS	1
30	1.2	10	PT2012BRUSERNS1	NEG	NEG	1
31	1.2	11	PT2012BRUSERPS2	POS	POS	1
32	1.2	12	PT2012BRUSERPS1	POS	POS	1
33	1.2	13	PT2012BRUSERPS1	POS	POS	1
34	1.2	14	PT2012BRUSERNS3	NEG	NEG	1
35	1.2	15	PT2012BRUSERPS2	POS	POS	1
36	1.2	16	PT2012BRUSERPS3	POS	POS	1
37	1.2	17	PT2012BRUSERNS1	NEG	NEG	1
38	1.2	18	PT2012BRUSERNS3	NEG	NEG	1
39	1.2	19	PT2012BRUSERPS1	POS	POS	1
40	1.2	20	PT2012BRUSERNS2	NEG	NEG	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	2	1	PT2012BRUSERPS2	POS	POS	1
42	2	2	PT2012BRUSERNS3	NEG	NEG	1
43	2	3	PT2012BRUSERPS3	POS	POS	1
44	2	4	PT2012BRUSERNS2	NEG	NEG	1
45	2	5	PT2012BRUSERPS3	POS	POS	1
46	2	6	PT2012BRUSERPS1	POS	POS	1
47	2	7	PT2012BRUSERNS1	NEG	NEG	1
48	2	8	PT2012BRUSERPS2	POS	POS	1
49	2	9	PT2012BRUSERPS1	POS	POS	1
50	2	10	PT2012BRUSERPS1	POS	POS	1
51	2	11	PT2012BRUSERNS3	NEG	NEG	1
52	2	12	PT2012BRUSERPS2	POS	POS	1
53	2	13	PT2012BRUSERPS3	POS	POS	1
54	2	14	PT2012BRUSERNS1	NEG	NEG	1
55	2	15	PT2012BRUSERNS3	NEG	NEG	1
56	2	16	PT2012BRUSERPS1	POS	POS	1
57	2	17	PT2012BRUSERNS2	NEG	NEG	1
58	2	18	PT2012BRUSERNS2	NEG	NEG	1
59	2	19	PT2012BRUSERPS1	POS	POS	1
60	2	20	PT2012BRUSERNS1	NEG	NEG	1
61	4	1	PT2012BRUSERNS1	NEG	NEG	1
62	4	2	PT2012BRUSERPS2	POS	POS	1
63	4	3	PT2012BRUSERPS1	POS	POS	1
64	4	4	PT2012BRUSERPS1	POS	POS	1
65	4	5	PT2012BRUSERNS3	NEG	NEG	1
66	4	6	PT2012BRUSERPS2	POS	POS	1
67	4	7	PT2012BRUSERPS3	POS	POS	1
68	4	8	PT2012BRUSERNS1	NEG	NEG	1
69	4	9	PT2012BRUSERNS3	NEG	NEG	1
70	4	10	PT2012BRUSERPS1	POS	POS	1
71	4	11	PT2012BRUSERNS2	NEG	NEG	1
72	4	12	PT2012BRUSERNS2	NEG	NEG	1
73	4	13	PT2012BRUSERPS1	POS	POS	1
74	4	14	PT2012BRUSERNS1	NEG	NEG	1
75	4	15	PT2012BRUSERPS2	POS	POS	1
76	4	16	PT2012BRUSERNS3	NEG	NEG	1
77	4	17	PT2012BRUSERPS3	POS	POS	1
78	4	18	PT2012BRUSERNS2	NEG	NEG	1
79	4	19	PT2012BRUSERPS3	POS	POS	1
80	4	20	PT2012BRUSERPS1	POS	POS	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2012BRUSERPS1	POS	POS	1
82	5	2	PT2012BRUSERNS3	NEG	NEG	1
83	5	3	PT2012BRUSERPS2	POS	POS	1
84	5	4	PT2012BRUSERPS3	POS	POS	1
85	5	5	PT2012BRUSERNS1	NEG	NEG	1
86	5	6	PT2012BRUSERNS3	NEG	NEG	1
87	5	7	PT2012BRUSERPS1	POS	POS	1
88	5	8	PT2012BRUSERNS2	NEG	NEG	1
89	5	9	PT2012BRUSERNS2	NEG	NEG	1
90	5	10	PT2012BRUSERPS1	POS	POS	1
91	5	11	PT2012BRUSERNS1	NEG	NEG	1
92	5	12	PT2012BRUSERPS2	POS	POS	1
93	5	13	PT2012BRUSERNS3	NEG	NEG	1
94	5	14	PT2012BRUSERPS3	POS	POS	1
95	5	15	PT2012BRUSERNS2	NEG	NEG	1
96	5	16	PT2012BRUSERPS3	POS	POS	1
97	5	17	PT2012BRUSERPS1	POS	POS	1
98	5	18	PT2012BRUSERNS1	NEG	NEG	1
99	5	19	PT2012BRUSERPS2	POS	POS	1
100	5	20	PT2012BRUSERPS1	POS	POS	1

Table 9. 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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012BRUSERPM2	POS	POS	1
2	1	2	PT2012BRUSERNM1	NEG	NEG	1
3	1	3	PT2012BRUSERPM3	POS	POS	1
4	1	4	PT2012BRUSERNM2	NEG	NEG	1
5	1	5	PT2012BRUSERPM4	POS	POS	1
6	1	6	PT2012BRUSERPM1	POS	POS	1
7	1	7	PT2012BRUSERPM2	POS	POS	1
8	1	8	PT2012BRUSERPM4	POS	POS	1
9	1	9	PT2012BRUSERNM2	NEG	NEG	1
10	1	10	PT2012BRUSERPM3	POS	POS	1
11	1	11	PT2012BRUSERPM2	POS	POS	1
12	1	12	PT2012BRUSERNM1	NEG	NEG	1
13	1	13	PT2012BRUSERPM1	POS	POS	1
14	1	14	PT2012BRUSERNM2	NEG	NEG	1
15	1	15	PT2012BRUSERPM3	POS	POS	1
16	1	16	PT2012BRUSERPM1	POS	POS	1
17	1	17	PT2012BRUSERPM2	POS	POS	1
18	1	18	PT2012BRUSERNM1	NEG	NEG	1
19	1	19	PT2012BRUSERNM2	NEG	NEG	1
20	1	20	PT2012BRUSERPM4	POS	POS	1
21	2	1	PT2012BRUSERNM2	NEG	NEG	1
22	2	2	PT2012BRUSERPM4	POS	POS	1
23	2	3	PT2012BRUSERPM1	POS	POS	1
24	2	4	PT2012BRUSERPM2	POS	POS	1
25	2	5	PT2012BRUSERPM4	POS	POS	1
26	2	6	PT2012BRUSERNM2	NEG	NEG	1
27	2	7	PT2012BRUSERPM3	POS	POS	1
28	2	8	PT2012BRUSERPM2	POS	POS	1
29	2	9	PT2012BRUSERNM1	NEG	NEG	1
30	2	10	PT2012BRUSERPM1	POS	POS	1
31	2	11	PT2012BRUSERNM2	NEG	NEG	1
32	2	12	PT2012BRUSERPM3	POS	POS	1
33	2	13	PT2012BRUSERPM1	POS	POS	1
34	2	14	PT2012BRUSERPM2	POS	POS	1
35	2	15	PT2012BRUSERNM1	NEG	NEG	1
36	2	16	PT2012BRUSERNM2	NEG	NEG	1
37	2	17	PT2012BRUSERPM4	POS	POS	1
38	2	18	PT2012BRUSERPM2	POS	POS	1
39	2	19	PT2012BRUSERNM1	NEG	NEG	1
40	2	20	PT2012BRUSERPM3	POS	POS	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012BRUSERPM2	POS	POS	1
42	3	2	PT2012BRUSERPM4	POS	POS	1
43	3	3	PT2012BRUSERNM2	NEG	NEG	1
44	3	4	PT2012BRUSERPM3	POS	POS	1
45	3	5	PT2012BRUSERPM2	POS	POS	1
46	3	6	PT2012BRUSERNM1	NEG	NEG	1
47	3	7	PT2012BRUSERPM1	POS	POS	1
48	3	8	PT2012BRUSERNM2	NEG	NEG	1
49	3	9	PT2012BRUSERPM3	POS	POS	1
50	3	10	PT2012BRUSERPM1	POS	POS	1
51	3	11	PT2012BRUSERPM2	POS	POS	1
52	3	12	PT2012BRUSERNM1	NEG	NEG	1
53	3	13	PT2012BRUSERNM2	NEG	NEG	1
54	3	14	PT2012BRUSERPM4	POS	POS	1
55	3	15	PT2012BRUSERPM2	POS	POS	1
56	3	16	PT2012BRUSERNM1	NEG	NEG	1
57	3	17	PT2012BRUSERPM3	POS	POS	1
58	3	18	PT2012BRUSERNM2	NEG	NEG	1
59	3	19	PT2012BRUSERPM4	POS	POS	1
60	3	20	PT2012BRUSERPM1	POS	POS	1
61	6	1	PT2012BRUSERPM3	POS	POS	1
62	6	2	PT2012BRUSERPM2	POS	POS	1
63	6	3	PT2012BRUSERNM1	NEG	NEG	1
64	6	4	PT2012BRUSERPM1	POS	POS	1
65	6	5	PT2012BRUSERNM2	NEG	NEG	1
66	6	6	PT2012BRUSERPM3	POS	POS	1
67	6	7	PT2012BRUSERPM1	POS	POS	1
68	6	8	PT2012BRUSERPM2	POS	POS	1
69	6	9	PT2012BRUSERNM1	NEG	NEG	1
70	6	10	PT2012BRUSERNM2	NEG	NEG	1
71	6	11	PT2012BRUSERPM4	POS	POS	1
72	6	12	PT2012BRUSERPM2	POS	POS	1
73	6	13	PT2012BRUSERNM1	NEG	NEG	1
74	6	14	PT2012BRUSERPM3	POS	POS	1
75	6	15	PT2012BRUSERNM2	NEG	NEG	1
76	6	16	PT2012BRUSERPM4	POS	POS	1
77	6	17	PT2012BRUSERPM1	POS	POS	1
78	6	18	PT2012BRUSERPM2	POS	POS	1
79	6	19	PT2012BRUSERPM4	POS	POS	1
80	6	20	PT2012BRUSERNM2	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 5 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement) for all serological tests performed. Hereby, LAB1 obtained identical qualitative results using ELISA kits from 2 different producers (Table 2, Table 3, Table 4, Table 6, Table 7 and Table 8).
All 3 laboratories that performed SAW-EDTA used the same batch of the SAW antigen from Synbiotics (batch 10SAW11). For the RBT, antigen from at least 2 different producers was used: Synbiotics (1 batch: 9BGT46) by LAB1 and IDEXX Montpellier SAS (2 batches: 369-10 and 372-100) by LAB2 and LAB5. LAB3 did not provide information about the used antigen. The 4 laboratories that performed ELISA used BRU antibody ELISA kits from 2 different commercial kit producers (Synbiotics: batches 12SBRU3OCB27 and 12SBRU3OCB28; IDEXX Montpellier SAS: batch 1253) and 1 in-house developed BRU antibody ELISA kit. Hereby, LAB1 analyzed the reference serum samples with ELISA kits from 2 different producers. LAB1.1, LAB4 and LAB5 used a BRU antibody ELISA kit from the same producer. In addition, LAB1.1 and LAB5 used the same batch.

For the detection of BRU-specific antibodies in reference milk samples, the 4 participating laboratories provided qualitative results that were in full agreement with the true 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 1041 (LAB1) and batch 1121 (LAB2, LAB3 and LAB6).

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)

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 SAS 9.2. (summary statistics) and R (box plots) were used. All quantitative data analyses were performed on normalized data, namely the percentages S/P ratio calculated as follows: [(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.

I. Box plots

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

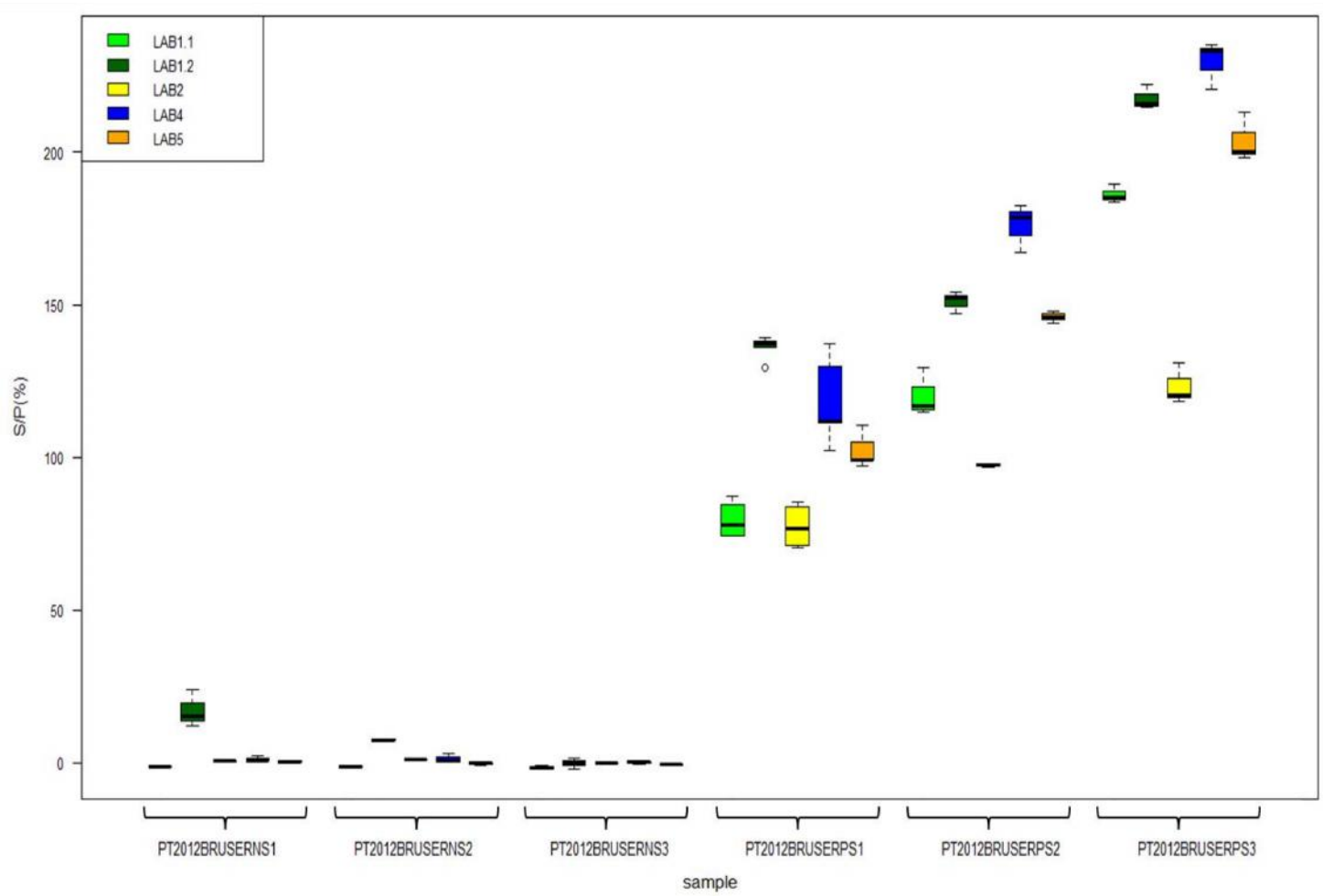

Figure 1. Box plots showing the percentage S/P ratio per reference serum sample and per participating (sub)laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values are not shown since BRU antibody ELISA kits from different producers were used. LAB1.1, LAB4 and LAB5 used a BRU antibody ELISA kit from the same producer. Hereby, LAB1.1 and LAB5 used the same batch.

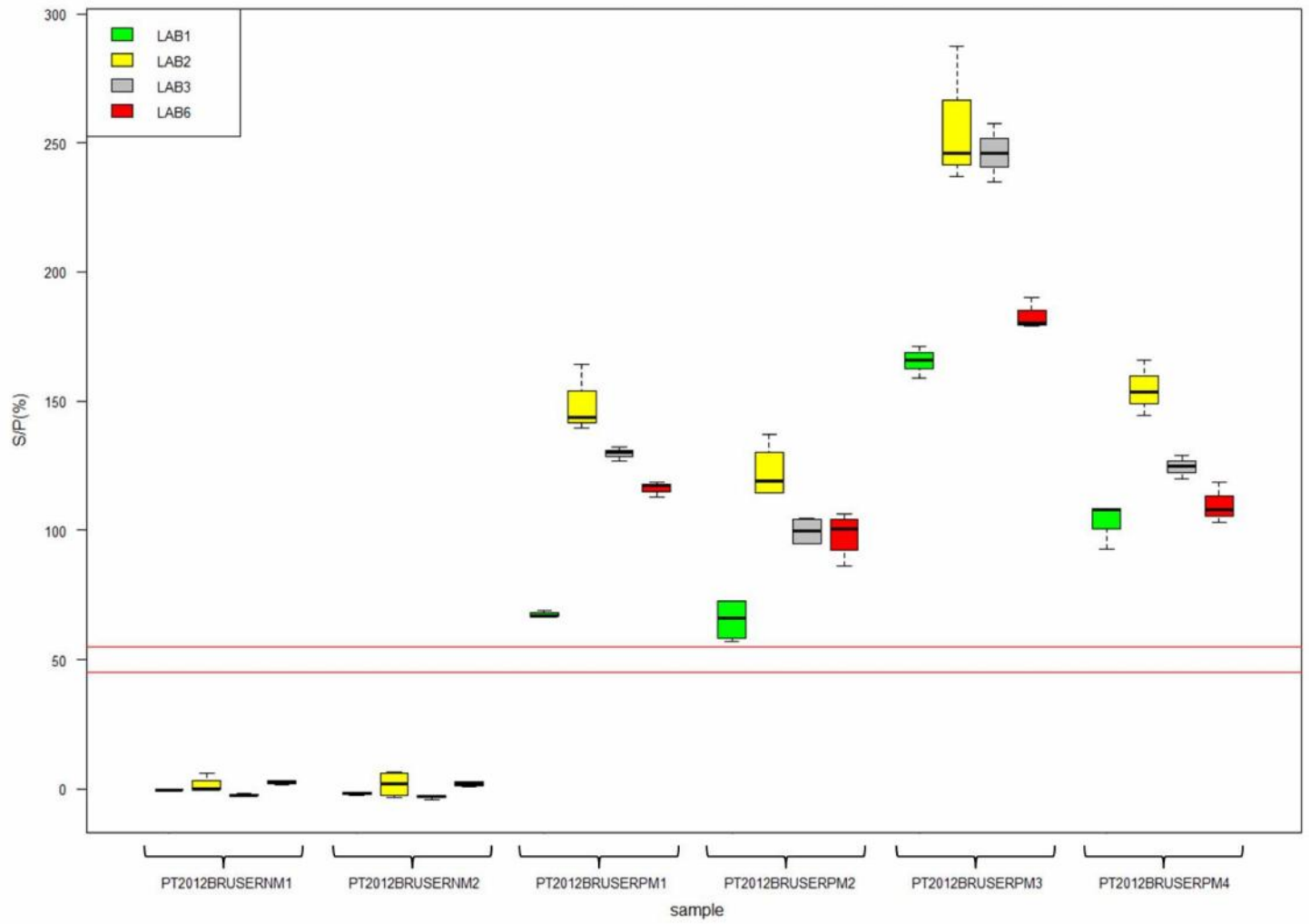

Figure 2. Box plots showing the percentage S/P ratio per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. All participating laboratories used the BRU antibody ELISA kit from IDEXX Montpellier SAS. In addition, LAB2, LAB3 and LAB6 used the same batch. 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-lab variability (reproducibility) and within-lab 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 based on percentages S/P ratio per reference serum sample and per participating (sub)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 1,57 for the PT serum (p=5) and 1,42 for the PT milk (p=4). For the PT serum, the maximum value for Mandel's k-statistic are 1,62 for the reference serum samples PT2012BRUSERNS1, PT2012BRUSERNS2, PT2012BRUSERNS3, PT2012BRUSERPS2 and PT2012BRUSERPS3 (p=5 and n=3), and 1,46 for the reference serum sample PT2012BRUSERPS1 (p=5 and n=5). For the PT milk, the maximum value for Mandel's k-statistic are 1,59 for the reference milk samples PT2012BRUSERNM1, PT2012BRUSERPM1, PT2012BRUSERPM3 and PT2012BRUSERPM4 (p=4 and n=3) and 1,50 for the reference milk samples PT2012BRUSERNM2 and PT2012BRUSERPM2 (p=4 and n=4).

For the detection of BRU-specific antibodies in serum, only 2 out of 5 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples: LAB4 and LAB5. The other participants showed a slightly increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB1.1 for the negative reference serum sample PT2012BRUSERNS3 (h=-1,63), LAB1.2 for the negative reference serum samples PT2012BRUSERNS1 (h=1,78) and PT2012BRUSERNS2 (h=1,71) and LAB2 for the positive reference serum sample PT2012BRUSERPS3 (h=-1,65). LAB1.1, LAB4 and LAB5 used a BRU antibody ELISA kit from the same producer. Hereby, LAB1.1 and LAB5 also used the same batch.
Furthermore, 3 out of 5 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples: LAB1.1, LAB2 and LAB5. The other participants showed an increased value for Mandel's k-statistic for 2 reference serum samples: LAB1.2 for the negative reference serum samples PT2012BRUSERNS1 (k=2,21) and PT2012BRUSERNS3 (k=2,07), and LAB4 for the negative reference serum sample PT2012BRUSERNS2 (k=2,15) and the positive reference serum sample PT2012BRUSERPS1 (k=1,76).

For the detection of BRU-specific antibodies in milk, all participating laboratories obtained a satisfactory between-laboratory consistency for all reference milk samples. Interestingly, all participants used a BRU antibody ELISA kit from the same producer. In addition, LAB2, LAB3 and LAB6 used the same batch.
Furthermore, 3 out of 4 participating laboratories obtained a satisfactory within-laboratory consistency for all reference milk samples: LAB1, LAB3 and LAB6. In contrast, LAB2 showed an increased value for Mandel's k-statistic for 4 out of 6 reference milk samples: PT2012BRUSERNM1 (k=1,91), PT2012BRUSERNM2 (k=1,93), PT2012BRUSERPM1 (k=1,91) and PT2012BRUSERPM3 (k=1,77).

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 (in this case the percentage S/P ratio) 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.

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 both the negative and positive reference serum samples. The quantitative results obtained by LAB1 using BRU antibody ELISA kits from 2 different producers (LAB1.1 and LAB1.2) were significantly different for the negative, but not for the positive reference serum samples. Indeed, LAB1.2 reported percentages S/P ratio that were significantly higher than those reported by LAB1.1 and the other participants for the negative reference serum samples. For the positive reference serum samples, LAB2 reported S/P ratios that were significantly lower than those reported by LAB1.2 and LAB4.

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 both the negative and positive reference milk samples. LAB3 reported percentages S/P ratio that were significantly lower than those reported by LAB2 and LAB6 for the negative reference milk samples, whereas LAB1 reported percentages S/P ratio that were significantly lower than those reported by LAB2 for the positive reference milk samples.

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
PT2012BRUSERNS1	1.1	3	0,01	-1,32	3,61	0,65	2,73	4,64	3,75	-0,64	0,04	-8,29
PT2012BRUSERNS1	1.2	3	36,44	17,22	3,61	0,65	2,73	4,64	3,75	1,78	2,21	35,06
PT2012BRUSERNS1	2	3	0,01	0,49	3,61	0,65	2,73	4,64	3,75	-0,41	0,04	24,83
PT2012BRUSERNS1	4	3	0,73	1,16	3,61	0,65	2,73	4,64	3,75	-0,32	0,31	73,41
PT2012BRUSERNS1	5	3	0,05	0,51	3,61	0,65	2,73	4,64	3,75	-0,41	0,08	43,30
PT2012BRUSERNS2	1.1	3	0,01	-1,09	1,75	0,83	0,76	1,85	1,69	-0,83	0,12	-8,41
PT2012BRUSERNS2	1.2	3	0,03	7,57	1,75	0,83	0,76	1,85	1,69	1,71	0,23	2,25
PT2012BRUSERNS2	2	3	0,02	1,13	1,75	0,83	0,76	1,85	1,69	-0,18	0,19	12,76
PT2012BRUSERNS2	4	3	2,64	1,41	1,75	0,83	0,76	1,85	1,69	-0,10	2,15	115,44
PT2012BRUSERNS2	5	3	0,16	-0,26	1,75	0,83	0,76	1,85	1,69	-0,59	0,52	-154,10
PT2012BRUSERNS3	1.1	3	0,35	-1,61	-0,45	0,07	0,88	0,92	0,25	-1,63	0,67	-36,89
PT2012BRUSERNS3	1.2	3	3,36	-0,11	-0,45	0,07	0,88	0,92	0,25	0,48	2,07	-1617,03
PT2012BRUSERNS3	2	3	0,01	0,07	-0,45	0,07	0,88	0,92	0,25	0,74	0,12	144,53
PT2012BRUSERNS3	4	3	0,15	0,05	-0,45	0,07	0,88	0,92	0,25	0,70	0,44	842,37
PT2012BRUSERNS3	5	3	0,03	-0,66	-0,45	0,07	0,88	0,92	0,25	-0,29	0,18	-24,62
PT2012BRUSERPS1	1.1	5	35,99	79,66	102,74	0,69	8,23	14,89	12,41	-0,92	0,73	7,53
PT2012BRUSERPS1	1.2	5	14,46	135,94	102,74	0,69	8,23	14,89	12,41	1,32	0,46	2,80
PT2012BRUSERPS1	2	5	47,82	77,52	102,74	0,69	8,23	14,89	12,41	-1,01	0,84	8,92
PT2012BRUSERPS1	4	5	210,18	118,46	102,74	0,69	8,23	14,89	12,41	0,63	1,76	12,24
PT2012BRUSERPS1	5	5	30,55	102,14	102,74	0,69	8,23	14,89	12,41	-0,02	0,67	5,41
PT2012BRUSERPS2	1.1	3	62,86	120,26	138,14	0,89	5,40	15,94	15,00	-0,59	1,47	6,59
PT2012BRUSERPS2	1.2	3	13,98	151,08	138,14	0,89	5,40	15,94	15,00	0,43	0,69	2,47
PT2012BRUSERPS2	2	3	0,45	97,44	138,14	0,89	5,40	15,94	15,00	-1,35	0,12	0,69
PT2012BRUSERPS2	4	3	65,00	176,03	138,14	0,89	5,40	15,94	15,00	1,26	1,49	4,58
PT2012BRUSERPS2	5	3	3,65	145,88	138,14	0,89	5,40	15,94	15,00	0,26	0,35	1,31
PT2012BRUSERPS3	1.1	3	8,91	186,03	192,06	0,92	6,25	21,71	20,79	-0,14	0,48	1,60
PT2012BRUSERPS3	1.2	3	15,92	217,55	192,06	0,92	6,25	21,71	20,79	0,61	0,64	1,83

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012BRUSERPS3	**2**	3	45,82	123,26	192,06	0,92	6,25	21,71	20,79	**-1,65**	1,08	5,49
PT2012BRUSERPS3	4	3	60,53	229,62	192,06	0,92	6,25	21,71	20,79	0,90	1,25	3,39
PT2012BRUSERPS3	5	3	64,03	203,85	192,06	0,92	6,25	21,71	20,79	0,28	1,28	3,93

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised 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
PT2012BRUSERNM1	1	3	0,06	-0,36	0,42	0,25	1,93	2,23	1,11	-0,35	0,12	-66,03
PT2012BRUSERNM1	**2**	3	13,50	1,92	0,42	0,25	1,93	2,23	1,11	0,67	**1,91**	191,60
PT2012BRUSERNM1	3	3	0,47	-2,36	0,42	0,25	1,93	2,23	1,11	-1,25	0,35	-28,87
PT2012BRUSERNM1	6	3	0,82	2,49	0,42	0,25	1,93	2,23	1,11	0,93	0,47	36,35
PT2012BRUSERNM2	1	4	0,24	-1,90	-0,36	0,18	2,63	2,90	1,24	-0,61	0,18	-25,51
PT2012BRUSERNM2	**2**	4	25,71	1,74	-0,36	0,18	2,63	2,90	1,24	0,84	**1,93**	291,54
PT2012BRUSERNM2	3	4	0,46	-3,10	-0,36	0,18	2,63	2,90	1,24	-1,09	0,26	-21,89
PT2012BRUSERNM2	6	4	1,20	1,80	-0,36	0,18	2,63	2,90	1,24	0,86	0,42	60,62
PT2012BRUSERPM1	1	3	1,46	67,54	115,69	0,89	6,92	21,14	19,98	-1,38	0,17	1,79
PT2012BRUSERPM1	**2**	3	174,41	149,21	115,69	0,89	6,92	21,14	19,98	0,96	**1,91**	8,85
PT2012BRUSERPM1	3	3	6,94	129,78	115,69	0,89	6,92	21,14	19,98	0,40	0,38	2,03
PT2012BRUSERPM1	6	3	8,83	116,24	115,69	0,89	6,92	21,14	19,98	0,02	0,43	2,56
PT2012BRUSERPM2	1	4	69,76	65,44	96,48	0,71	8,48	15,79	13,32	-1,32	0,98	12,76
PT2012BRUSERPM2	2	4	114,63	122,44	96,48	0,71	8,48	15,79	13,32	1,11	1,26	8,74
PT2012BRUSERPM2	3	4	29,73	99,69	96,48	0,71	8,48	15,79	13,32	0,14	0,64	5,47

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012BRUSERPM2	6	4	73,80	98,33	96,48	0,71	8,48	15,79	13,32	0,08	1,01	8,74
PT2012BRUSERPM3	1	3	38,33	165,31	212,84	0,74	15,24	29,90	25,73	-1,05	0,41	3,75
PT2012BRUSERPM3	**2**	3	729,32	256,77	212,84	0,74	15,24	29,90	25,73	0,97	**1,77**	10,52
PT2012BRUSERPM3	3	3	125,96	246,21	212,84	0,74	15,24	29,90	25,73	0,73	0,74	4,56
PT2012BRUSERPM3	6	3	36,02	183,08	212,84	0,74	15,24	29,90	25,73	-0,66	0,39	3,28
PT2012BRUSERPM4	1	3	77,22	103,08	123,02	0,71	8,26	15,35	12,94	-0,87	1,06	8,52
PT2012BRUSERPM4	2	3	114,05	154,69	123,02	0,71	8,26	15,35	12,94	1,38	1,29	6,90
PT2012BRUSERPM4	3	3	19,51	124,42	123,02	0,71	8,26	15,35	12,94	0,06	0,53	3,55
PT2012BRUSERPM4	6	3	62,46	109,88	123,02	0,71	8,26	15,35	12,94	-0,57	0,96	7,19

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised 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).