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

172-PT

PROFICIENCY TESTING 2015

BRUCELLOSIS (BRU)

Detection of BRU-specific antibodies in:

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

(ii) bovine milk by ELISA

CODA-CERVA-UCCLE

DATE BEGIN PT: 21 SEPTEMBER 2015

DATE REPORT: 22 DECEMBER 2015

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 'PT2015BRUSERNS1', 'PT2015BRUSERNS2' and 'PT2015BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2015BRUSERPS1', 'PT2015BRUSERPS2' and 'PT2015BRUSERPS3'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2015BRUSERNS1, PT2015BRUSERNS2, PT2015BRUSERPS2 and PT2015BRUSERPS3, and 4 aliquots of the reference serum samples PT2015BRUSERNS3 and PT2015BRUSERPS1. 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 PT2015BRUSERNS1 and PT2015BRUSERNS2 were derived from BRU-free farms. The reference serum sample PT2015BRUSERNS3 was a sample taken at abattoir and obtained from a BRU-free farm, whereas the reference serum sample PT2015BRUSERPS3 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 PT2015BRUSERPS1 and PT2015BRUSERPS2 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 PT2015BRUSERNS1, PT2015BRUSERNS2 and PT2015BRUSERNS3 were considered as negative sera, and the reference serum samples PT2015BRUSERPS1, PT2015BRUSERPS2 and PT2015BRUSERPS3 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 'PT2015BRUSERNM1' and 'PT2015BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2015BRUSERPM1', 'PT2015BRUSERPM2', 'PT2015BRUSERPM3' and 'PT2015BRUSERPM4'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2015BRUSERNM1, PT2015BRUSERPM1, PT2015BRUSERPM3 and PT2015BRUSERPM4, and 4 aliquots of the reference milk samples PT2015BRUSERNM2 and PT2015BRUSERPM2. 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 PT2015BRUSERNM1 and PT2015BRUSERNM2 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, PT2015BRUSERPM1 was spiked with serum 1275 in a 1/200 dilution and PT2015BRUSERPM2 was spiked with serum 3667 in a 1/1000 dilution, respectively, whereas PT2015BRUSERPM3 and PT2015BRUSERPM4 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 PT2015BRUSERNM1 and PT2015BRUSERNM2 were considered as negative milk samples, and the reference samples PT2015BRUSERPM1, PT2015BRUSERPM2, PT2015BRUSERPM3 and PT2015BRUSERPM4 as variably positive milk samples in BRU antibody ELISA.

After aliquoting the different reference milk samples, a homogeneity check was performed on 10 aliquots of each reference milk sample using the BRU antibody ELISA test kit from IDEXX Montpellier SAS, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Consequently, all reference milk samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BRU-specific antibodies in bovine milk. In addition, 3 aliquots of each reference milk sample were tested after the PT in order to confirm their stability and status (post-verification) using the BRU antibody ELISA test kit from IDEXX Montpellier SAS.

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

III.3.1. Reference serum samples

III.3.1.1. Classification of results

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

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

III.3.1.2. Level of agreement

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

III.3.1.3. Threshold for qualification

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

III.3.2. Reference milk samples

III.3.2.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

III.3.2.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference milk samples used for this PT.

III.3.2.3. Threshold for qualification

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

IV. Results

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

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

LAB1, LAB4, LAB5 and LAB6 participated in both the PT serum and the PT milk and hence received 40 aliquots: 20 aliquots of reference serum samples and 20 aliquots of reference milk samples. In contrast, LAB2 and LAB3 only participated in the PT serum, whereas LAB7 only participated in the PT milk. These 4 participating laboratories hence received either 20 aliquots of reference serum samples or 20 aliquots of reference milk samples. An overview of the different serological tests performed by the laboratories participating to the PT serum can be found in Table 1. The reference serum samples were sent lyophilized, whereas the reference milk samples were sent frozen (dry ice) to each of the participating laboratories by national courier or international courier on 21th of September 2015 (220 aliquots in total). All participants acknowledged receipt of the samples on the same day except LAB 4 which acknowledged receipt of the samples still frozen on 23th September 2015. Analyses were performed between 21th and 25th of September 2015 for serum and between 22th and 25th of September 2015 for milk (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 25th of September and 5th of October 2015 (Table 1). Hereby, all laboratories except LAB1 respected the deadline of the 2st of October 2015 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 CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of analysis serum			Start of analysis milk	Submission of the results (Excel file)
		SAW-EDTA	ELISA	RBT		
LAB1	21/09/2015	22/09/2015	22/09/2015	23/09/2015	25/09/2015	05/10/2015
LAB2	21/09/2015	21/09/2015	22/09/2015	21/09/2015	NA	29/09/2015
LAB 3	21/09/2015	21/09/2015	22/09/2015	NA	NA	28/09/2015
LAB4	23/09/2015	NA	25/09/2015	NA	25/09/2015	25/09/2015
LAB5	21/09/2015	NA	NA	22/09/2015	23/09/2015	01/10/2015
LAB6	21/09/2015	NA	NA	23/09/2015	22/09/2015	01/10/2015
LAB7	21/09/2015	NA	NA	NA	25/09/2015	30/09/2015

Legend: NA = not applicable

IV.3. Compliance with the procedure

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).

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

	LABNR		
	1	**2**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)

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

	LABNR			
	1	**2**	**5**	**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)

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

	LABNR			
	1	**2**	**3**	**4**
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.4.1.2. Reference milk samples

Four out of five participating laboratories (LAB1, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the assigned status of the reference milk samples and hence achieved 100% of agreement. In contrast, LAB4 misclassified 3 aliquots (85% of agreement) of reference milk samples (Table 5).

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

	LABNR				
	1	**4**	**5**	**6**	**7**
failure	0 (0.0)	3 (15.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	17 (85.0)	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

No variability between LAB1, LAB5, LAB6 and LAB7 could be observed since these participants correctly identified all reference milk samples. In contrast, LAB4 misclassified 1 out of 4 aliquots of the reference milk sample PT2015BRUSERPM2 (NEG instead of POS) and 2 out of 3 aliquots of the reference milk sample PT2015BRUSERPM4 (2x NEG instead of POS).

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

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BRUSERNS1	NEG	NEG	1
2	1	2	PT2015BRUSERNS2	NEG	NEG	1
3	1	3	PT2015BRUSERPS3	>=100	>=100	1
4	1	4	PT2015BRUSERPS1	50	50	1
5	1	5	PT2015BRUSERNS3	NEG	NEG	1
6	1	6	PT2015BRUSERNS2	NEG	NEG	1
7	1	7	PT2015BRUSERPS2	>=100	>=100	1
8	1	8	PT2015BRUSERNS1	NEG	NEG	1
9	1	9	PT2015BRUSERPS1	50	50	1
10	1	10	PT2015BRUSERNS3	NEG	NEG	1
11	1	11	PT2015BRUSERPS3	>=100	>=100	1
12	1	12	PT2015BRUSERNS3	NEG	NEG	1
13	1	13	PT2015BRUSERNS2	NEG	NEG	1
14	1	14	PT2015BRUSERNS3	NEG	NEG	1
15	1	15	PT2015BRUSERNS1	NEG	NEG	1
16	1	16	PT2015BRUSERPS2	>=100	>=100	1
17	1	17	PT2015BRUSERPS3	>=100	>=100	1
18	1	18	PT2015BRUSERPS1	50	50	1
19	1	19	PT2015BRUSERPS2	>=100	>=100	1
20	1	20	PT2015BRUSERPS1	50	50	1
21	2	1	PT2015BRUSERPS1	50	50	1
22	2	2	PT2015BRUSERNS3	NEG	NEG	1
23	2	3	PT2015BRUSERNS2	NEG	NEG	1
24	2	4	PT2015BRUSERNS3	NEG	NEG	1
25	2	5	PT2015BRUSERNS1	NEG	NEG	1
26	2	6	PT2015BRUSERPS3	>=100	>=100	1
27	2	7	PT2015BRUSERPS2	>=100	>=100	1
28	2	8	PT2015BRUSERNS2	NEG	NEG	1
29	2	9	PT2015BRUSERPS3	>=100	>=100	1
30	2	10	PT2015BRUSERNS1	NEG	NEG	1
31	2	11	PT2015BRUSERNS3	NEG	NEG	1
32	2	12	PT2015BRUSERNS3	NEG	NEG	1
33	2	13	PT2015BRUSERPS1	50	50	1
34	2	14	PT2015BRUSERPS2	>=100	>=100	1
35	2	15	PT2015BRUSERPS1	50	50	1
36	2	16	PT2015BRUSERPS3	>=100	>=100	1
37	2	17	PT2015BRUSERNS2	NEG	NEG	1
38	2	18	PT2015BRUSERPS2	>=100	>=100	1
39	2	19	PT2015BRUSERNS1	NEG	NEG	1
40	2	20	PT2015BRUSERPS1	50	50	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2015BRUSERNS1	NEG	NEG	1
42	3	2	PT2015BRUSERNS2	NEG	NEG	1
43	3	3	PT2015BRUSERPS3	>=100	>=100	1
44	3	4	PT2015BRUSERPS1	50	50	1
45	3	5	PT2015BRUSERNS3	NEG	NEG	1
46	3	6	PT2015BRUSERNS2	NEG	NEG	1
47	3	7	PT2015BRUSERPS2	>=100	>=100	1
48	3	8	PT2015BRUSERNS1	NEG	NEG	1
49	3	9	PT2015BRUSERPS1	50	50	1
50	3	10	PT2015BRUSERNS3	NEG	NEG	1
51	3	11	PT2015BRUSERPS3	>=100	>=100	1
52	3	12	PT2015BRUSERNS3	NEG	NEG	1
53	3	13	PT2015BRUSERNS2	NEG	NEG	1
54	3	14	PT2015BRUSERNS3	NEG	NEG	1
55	3	15	PT2015BRUSERNS1	NEG	NEG	1
56	3	16	PT2015BRUSERPS2	>=100	>=100	1
57	3	17	PT2015BRUSERPS3	>=100	>=100	1
58	3	18	PT2015BRUSERPS1	50	50	1
59	3	19	PT2015BRUSERPS2	>=100	>=100	1
60	3	20	PT2015BRUSERPS1	50	50	1

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BRUSERNS1	NEG	NEG	1
2	1	2	PT2015BRUSERNS2	NEG	NEG	1
3	1	3	PT2015BRUSERPS3	POS	POS	1
4	1	4	PT2015BRUSERPS1	POS	POS	1
5	1	5	PT2015BRUSERNS3	NEG	NEG	1
6	1	6	PT2015BRUSERNS2	NEG	NEG	1
7	1	7	PT2015BRUSERPS2	POS	POS	1
8	1	8	PT2015BRUSERNS1	NEG	NEG	1
9	1	9	PT2015BRUSERPS1	POS	POS	1
10	1	10	PT2015BRUSERNS3	NEG	NEG	1
11	1	11	PT2015BRUSERPS3	POS	POS	1
12	1	12	PT2015BRUSERNS3	NEG	NEG	1
13	1	13	PT2015BRUSERNS2	NEG	NEG	1
14	1	14	PT2015BRUSERNS3	NEG	NEG	1
15	1	15	PT2015BRUSERNS1	NEG	NEG	1
16	1	16	PT2015BRUSERPS2	POS	POS	1
17	1	17	PT2015BRUSERPS3	POS	POS	1
18	1	18	PT2015BRUSERPS1	POS	POS	1
19	1	19	PT2015BRUSERPS2	POS	POS	1
20	1	20	PT2015BRUSERPS1	POS	POS	1
21	2	1	PT2015BRUSERPS1	POS	POS	1
22	2	2	PT2015BRUSERNS3	NEG	NEG	1
23	2	3	PT2015BRUSERNS2	NEG	NEG	1
24	2	4	PT2015BRUSERNS3	NEG	NEG	1
25	2	5	PT2015BRUSERNS1	NEG	NEG	1
26	2	6	PT2015BRUSERPS3	POS	POS	1
27	2	7	PT2015BRUSERPS2	POS	POS	1
28	2	8	PT2015BRUSERNS2	NEG	NEG	1
29	2	9	PT2015BRUSERPS3	POS	POS	1
30	2	10	PT2015BRUSERNS1	NEG	NEG	1
31	2	11	PT2015BRUSERNS3	NEG	NEG	1
32	2	12	PT2015BRUSERNS3	NEG	NEG	1
33	2	13	PT2015BRUSERPS1	POS	POS	1
34	2	14	PT2015BRUSERPS2	POS	POS	1
35	2	15	PT2015BRUSERPS1	POS	POS	1
36	2	16	PT2015BRUSERPS3	POS	POS	1
37	2	17	PT2015BRUSERNS2	NEG	NEG	1
38	2	18	PT2015BRUSERPS2	POS	POS	1
39	2	19	PT2015BRUSERNS1	NEG	NEG	1
40	2	20	PT2015BRUSERPS1	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2015BRUSERNS1	NEG	NEG	1
42	5	2	PT2015BRUSERNS2	NEG	NEG	1
43	5	3	PT2015BRUSERPS3	POS	POS	1
44	5	4	PT2015BRUSERPS1	POS	POS	1
45	5	5	PT2015BRUSERNS3	NEG	NEG	1
46	5	6	PT2015BRUSERNS2	NEG	NEG	1
47	5	7	PT2015BRUSERPS2	POS	POS	1
48	5	8	PT2015BRUSERNS1	NEG	NEG	1
49	5	9	PT2015BRUSERPS1	POS	POS	1
50	5	10	PT2015BRUSERNS3	NEG	NEG	1
51	5	11	PT2015BRUSERPS3	POS	POS	1
52	5	12	PT2015BRUSERNS3	NEG	NEG	1
53	5	13	PT2015BRUSERNS2	NEG	NEG	1
54	5	14	PT2015BRUSERNS3	NEG	NEG	1
55	5	15	PT2015BRUSERNS1	NEG	NEG	1
56	5	16	PT2015BRUSERPS2	POS	POS	1
57	5	17	PT2015BRUSERPS3	POS	POS	1
58	5	18	PT2015BRUSERPS1	POS	POS	1
59	5	19	PT2015BRUSERPS2	POS	POS	1
60	5	20	PT2015BRUSERPS1	POS	POS	1
61	6	1	PT2015BRUSERPS1	POS	POS	1
62	6	2	PT2015BRUSERNS3	NEG	NEG	1
63	6	3	PT2015BRUSERNS2	NEG	NEG	1
64	6	4	PT2015BRUSERNS3	NEG	NEG	1
65	6	5	PT2015BRUSERNS1	NEG	NEG	1
66	6	6	PT2015BRUSERPS3	POS	POS	1
67	6	7	PT2015BRUSERPS2	POS	POS	1
68	6	8	PT2015BRUSERNS2	NEG	NEG	1
69	6	9	PT2015BRUSERPS3	POS	POS	1
70	6	10	PT2015BRUSERNS1	NEG	NEG	1
71	6	11	PT2015BRUSERNS3	NEG	NEG	1
72	6	12	PT2015BRUSERNS3	NEG	NEG	1
73	6	13	PT2015BRUSERPS1	POS	POS	1
74	6	14	PT2015BRUSERPS2	POS	POS	1
75	6	15	PT2015BRUSERPS1	POS	POS	1
76	6	16	PT2015BRUSERPS3	POS	POS	1
77	6	17	PT2015BRUSERNS2	NEG	NEG	1
78	6	18	PT2015BRUSERPS2	POS	POS	1
79	6	19	PT2015BRUSERNS1	NEG	NEG	1
80	6	20	PT2015BRUSERPS1	POS	POS	1

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BRUSERNS1	NEG	NEG	1
2	1	2	PT2015BRUSERNS2	NEG	NEG	1
3	1	3	PT2015BRUSERPS3	POS	POS	1
4	1	4	PT2015BRUSERPS1	POS	POS	1
5	1	5	PT2015BRUSERNS3	NEG	NEG	1
6	1	6	PT2015BRUSERNS2	NEG	NEG	1
7	1	7	PT2015BRUSERPS2	POS	POS	1
8	1	8	PT2015BRUSERNS1	NEG	NEG	1
9	1	9	PT2015BRUSERPS1	POS	POS	1
10	1	10	PT2015BRUSERNS3	NEG	NEG	1
11	1	11	PT2015BRUSERPS3	POS	POS	1
12	1	12	PT2015BRUSERNS3	NEG	NEG	1
13	1	13	PT2015BRUSERNS2	NEG	NEG	1
14	1	14	PT2015BRUSERNS3	NEG	NEG	1
15	1	15	PT2015BRUSERNS1	NEG	NEG	1
16	1	16	PT2015BRUSERPS2	POS	POS	1
17	1	17	PT2015BRUSERPS3	POS	POS	1
18	1	18	PT2015BRUSERPS1	POS	POS	1
19	1	19	PT2015BRUSERPS2	POS	POS	1
20	1	20	PT2015BRUSERPS1	POS	POS	1
21	2	1	PT2015BRUSERPS1	POS	POS	1
22	2	2	PT2015BRUSERNS3	NEG	NEG	1
23	2	3	PT2015BRUSERNS2	NEG	NEG	1
24	2	4	PT2015BRUSERNS3	NEG	NEG	1
25	2	5	PT2015BRUSERNS1	NEG	NEG	1
26	2	6	PT2015BRUSERPS3	POS	POS	1
27	2	7	PT2015BRUSERPS2	POS	POS	1
28	2	8	PT2015BRUSERNS2	NEG	NEG	1
29	2	9	PT2015BRUSERPS3	POS	POS	1
30	2	10	PT2015BRUSERNS1	NEG	NEG	1
31	2	11	PT2015BRUSERNS3	NEG	NEG	1
32	2	12	PT2015BRUSERNS3	NEG	NEG	1
33	2	13	PT2015BRUSERPS1	POS	POS	1
34	2	14	PT2015BRUSERPS2	POS	POS	1
35	2	15	PT2015BRUSERPS1	POS	POS	1
36	2	16	PT2015BRUSERPS3	POS	POS	1
37	2	17	PT2015BRUSERNS2	NEG	NEG	1
38	2	18	PT2015BRUSERPS2	POS	POS	1
39	2	19	PT2015BRUSERNS1	NEG	NEG	1
40	2	20	PT2015BRUSERPS1	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2015BRUSERNS1	NEG	NEG	1
42	3	2	PT2015BRUSERNS2	NEG	NEG	1
43	3	3	PT2015BRUSERPS3	POS	POS	1
44	3	4	PT2015BRUSERPS1	POS	POS	1
45	3	5	PT2015BRUSERNS3	NEG	NEG	1
46	3	6	PT2015BRUSERNS2	NEG	NEG	1
47	3	7	PT2015BRUSERPS2	POS	POS	1
48	3	8	PT2015BRUSERNS1	NEG	NEG	1
49	3	9	PT2015BRUSERPS1	POS	POS	1
50	3	10	PT2015BRUSERNS3	NEG	NEG	1
51	3	11	PT2015BRUSERPS3	POS	POS	1
52	3	12	PT2015BRUSERNS3	NEG	NEG	1
53	3	13	PT2015BRUSERNS2	NEG	NEG	1
54	3	14	PT2015BRUSERNS3	NEG	NEG	1
55	3	15	PT2015BRUSERNS1	NEG	NEG	1
56	3	16	PT2015BRUSERPS2	POS	POS	1
57	3	17	PT2015BRUSERPS3	POS	POS	1
58	3	18	PT2015BRUSERPS1	POS	POS	1
59	3	19	PT2015BRUSERPS2	POS	POS	1
60	3	20	PT2015BRUSERPS1	POS	POS	1
61	4	1	PT2015BRUSERPS1	POS	POS	1
62	4	2	PT2015BRUSERNS3	NEG	NEG	1
63	4	3	PT2015BRUSERNS2	NEG	NEG	1
64	4	4	PT2015BRUSERNS3	NEG	NEG	1
65	4	5	PT2015BRUSERNS1	NEG	NEG	1
66	4	6	PT2015BRUSERPS3	POS	POS	1
67	4	7	PT2015BRUSERPS2	POS	POS	1
68	4	8	PT2015BRUSERNS2	NEG	NEG	1
69	4	9	PT2015BRUSERPS3	POS	POS	1
70	4	10	PT2015BRUSERNS1	NEG	NEG	1
71	4	11	PT2015BRUSERNS3	NEG	NEG	1
72	4	12	PT2015BRUSERNS3	NEG	NEG	1
73	4	13	PT2015BRUSERPS1	POS	POS	1
74	4	14	PT2015BRUSERPS2	POS	POS	1
75	4	15	PT2015BRUSERPS1	POS	POS	1
76	4	16	PT2015BRUSERPS3	POS	POS	1
77	4	17	PT2015BRUSERNS2	NEG	NEG	1
78	4	18	PT2015BRUSERPS2	POS	POS	1
79	4	19	PT2015BRUSERNS1	NEG	NEG	1
80	4	20	PT2015BRUSERPS1	POS	POS	1

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BRUSERPM2	POS	POS	1
2	1	2	PT2015BRUSERNM2	NEG	NEG	1
3	1	3	PT2015BRUSERPM1	POS	POS	1
4	1	4	PT2015BRUSERPM2	POS	POS	1
5	1	5	PT2015BRUSERNM2	NEG	NEG	1
6	1	6	PT2015BRUSERNM1	NEG	NEG	1
7	1	7	PT2015BRUSERPM4	POS	POS	1
8	1	8	PT2015BRUSERPM1	POS	POS	1
9	1	9	PT2015BRUSERPM4	POS	POS	1
10	1	10	PT2015BRUSERNM2	NEG	NEG	1
11	1	11	PT2015BRUSERPM2	POS	POS	1
12	1	12	PT2015BRUSERPM3	POS	POS	1
13	1	13	PT2015BRUSERNM1	NEG	NEG	1
14	1	14	PT2015BRUSERPM4	POS	POS	1
15	1	15	PT2015BRUSERPM3	POS	POS	1
16	1	16	PT2015BRUSERPM2	POS	POS	1
17	1	17	PT2015BRUSERNM1	NEG	NEG	1
18	1	18	PT2015BRUSERPM3	POS	POS	1
19	1	19	PT2015BRUSERPM1	POS	POS	1
20	1	20	PT2015BRUSERNM2	NEG	NEG	1
21	4	1	PT2015BRUSERPM3	POS	POS	1
22	4	2	PT2015BRUSERNM1	NEG	NEG	1
23	4	3	PT2015BRUSERNM2	NEG	NEG	1
24	4	4	PT2015BRUSERPM1	POS	POS	1
25	4	5	PT2015BRUSERPM2	**POS**	**NEG**	**0**
26	4	6	PT2015BRUSERPM4	**POS**	**NEG**	**0**
27	4	7	PT2015BRUSERPM3	POS	POS	1
28	4	8	PT2015BRUSERNM1	NEG	NEG	1
29	4	9	PT2015BRUSERPM2	POS	POS	1
30	4	10	PT2015BRUSERPM4	**POS**	**NEG**	**0**
31	4	11	PT2015BRUSERPM1	POS	POS	1
32	4	12	PT2015BRUSERNM2	NEG	NEG	1
33	4	13	PT2015BRUSERPM4	POS	POS	1
34	4	14	PT2015BRUSERPM3	POS	POS	1
35	4	15	PT2015BRUSERNM2	NEG	NEG	1
36	4	16	PT2015BRUSERPM1	POS	POS	1
37	4	17	PT2015BRUSERPM2	POS	POS	1
38	4	18	PT2015BRUSERNM1	NEG	NEG	1
39	4	19	PT2015BRUSERNM2	NEG	NEG	1
40	4	20	PT2015BRUSERPM2	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2015BRUSERPM2	POS	POS	1
42	5	2	PT2015BRUSERNM2	NEG	NEG	1
43	5	3	PT2015BRUSERPM1	POS	POS	1
44	5	4	PT2015BRUSERPM2	POS	POS	1
45	5	5	PT2015BRUSERNM2	NEG	NEG	1
46	5	6	PT2015BRUSERNM1	NEG	NEG	1
47	5	7	PT2015BRUSERPM4	POS	POS	1
48	5	8	PT2015BRUSERPM1	POS	POS	1
49	5	9	PT2015BRUSERPM4	POS	POS	1
50	5	10	PT2015BRUSERNM2	NEG	NEG	1
51	5	11	PT2015BRUSERPM2	POS	POS	1
52	5	12	PT2015BRUSERPM3	POS	POS	1
53	5	13	PT2015BRUSERNM1	NEG	NEG	1
54	5	14	PT2015BRUSERPM4	POS	POS	1
55	5	15	PT2015BRUSERPM3	POS	POS	1
56	5	16	PT2015BRUSERPM2	POS	POS	1
57	5	17	PT2015BRUSERNM1	NEG	NEG	1
58	5	18	PT2015BRUSERPM3	POS	POS	1
59	5	19	PT2015BRUSERPM1	POS	POS	1
60	5	20	PT2015BRUSERNM2	NEG	NEG	1
61	6	1	PT2015BRUSERPM3	POS	POS	1
62	6	2	PT2015BRUSERNM1	NEG	NEG	1
63	6	3	PT2015BRUSERNM2	NEG	NEG	1
64	6	4	PT2015BRUSERPM1	POS	POS	1
65	6	5	PT2015BRUSERPM2	POS	POS	1
66	6	6	PT2015BRUSERPM4	POS	POS	1
67	6	7	PT2015BRUSERPM3	POS	POS	1
68	6	8	PT2015BRUSERNM1	NEG	NEG	1
69	6	9	PT2015BRUSERPM2	POS	POS	1
70	6	10	PT2015BRUSERPM4	POS	POS	1
71	6	11	PT2015BRUSERPM1	POS	POS	1
72	6	12	PT2015BRUSERNM2	NEG	NEG	1
73	6	13	PT2015BRUSERPM4	POS	POS	1
74	6	14	PT2015BRUSERPM3	POS	POS	1
75	6	15	PT2015BRUSERNM2	NEG	NEG	1
76	6	16	PT2015BRUSERPM1	POS	POS	1
77	6	17	PT2015BRUSERPM2	POS	POS	1
78	6	18	PT2015BRUSERNM1	NEG	NEG	1
79	6	19	PT2015BRUSERNM2	NEG	NEG	1
80	6	20	PT2015BRUSERPM2	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	7	1	PT2015BRUSERPM2	POS	POS	1
82	7	2	PT2015BRUSERNM2	NEG	NEG	1
83	7	3	PT2015BRUSERPM1	POS	POS	1
84	7	4	PT2015BRUSERPM2	POS	POS	1
85	7	5	PT2015BRUSERNM2	NEG	NEG	1
86	7	6	PT2015BRUSERNM1	NEG	NEG	1
87	7	7	PT2015BRUSERPM4	POS	POS	1
88	7	8	PT2015BRUSERPM1	POS	POS	1
89	7	9	PT2015BRUSERPM4	POS	POS	1
90	7	10	PT2015BRUSERNM2	NEG	NEG	1
91	7	11	PT2015BRUSERPM2	POS	POS	1
92	7	12	PT2015BRUSERPM3	POS	POS	1
93	7	13	PT2015BRUSERNM1	NEG	NEG	1
94	7	14	PT2015BRUSERPM4	POS	POS	1
95	7	15	PT2015BRUSERPM3	POS	POS	1
96	7	16	PT2015BRUSERPM2	POS	POS	1
97	7	17	PT2015BRUSERNM1	NEG	NEG	1
98	7	18	PT2015BRUSERPM3	POS	POS	1
99	7	19	PT2015BRUSERPM1	POS	POS	1
100	7	20	PT2015BRUSERNM2	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).
Two of the 3 participating laboratories that performed SAW-EDTA used the same batch of the SAW antigen from Synbiotics (batch 15SAW14), LAB1 used the SAW antigen from a different batch (batch 14SAW13). The 4 participating laboratories that performed RBT used a RBT antigen from 2 different producers, namely Synbiotics Europe (1 batch: 14BGT75) and IDEXX (2 batches: 381-100 and 383-100). Hereby, LAB1 and LAB2 used the same antigen. From the 4 participating laboratories that performed ELISA, LAB1 used an home made developed BRU antibody ELISA kit, whereas LAB2, LAB3 and LAB4 used a commercially available BRU antibody ELISA kit from Synbiotics (batch LAB2 and 4: 15SBRU30CB58; batch LAB3 : OCB58).

For the detection of BRU-specific antibodies in reference milk samples, 4 out of the 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement). In contrast, LAB4 misclassified 3 aliquots (85% of agreement) of reference milk samples. For 2 results (LABPOSIT 5 and 10), it could be a wrong interpretation of the raw data (Table 5 and Table 9).
From the 5 participating laboratories that performed ELISA, LAB1, LAB5, LAB6 and LAB7 used the BRU antibody ELISA kit from IDEXX (batch LAB1 : 3248, batch LAB5 and 6: 4039; batch LAB7 : 4121), whereas LAB4 used the BRU antibody ELISA kit from Synbiotics (batch 15LBRU340).

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,for serum all participants achieved a satisfactory performance for the detection of BRU-specific antibodies in reference serum samples by SAW-EDTA and/or RBT and/or ELISA. For milk, 4 of the 5 partipants achivied satisfactory performance for the detection of BRU-specific antibodies in milk.

Coordinator proficiency tests
Katia Knapen

Appendix

Name of the participating laboratories

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

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)

Synbiotics Europe (Synbiotics) (Lyon, France)

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