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081-PT

PROFICIENCY TESTING 2019

BRUCELLOSIS (BRU)

DETECTION OF BRU-SPECIFIC ANTIBODIES IN:
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)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE START PT: 23 SEPTEMBER 2019

DATE REPORT: 19 DECEMBER 2019

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 bovine serum by SAW-EDTA and/or RBT and/or 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. The procedures for these tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 6 reference serum samples of bovine origin, either free from detectable BRU-specific antibodies (n=3; coded 'PT2019BRUSERNS1', 'PT2019BRUSERNS2' and 'PT2019BRUSERNS3') or containing detectable BRU-specific antibodies (n=3; coded 'PT2019BRUSERPS1', 'PT2019BRUSERPS2' and 'PT2019BRUSERPS3'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2019BRUSERNS1, PT2019BRUSERPS1, PT2019BRUSERPS2 and PT2019BRUSERPS3 and 4 aliquots of the reference serum samples PT2019BRUSERNS2 and PT2019BRUSERNS3. The identification numbers of the reference serum samples were randomized for all participants (Table 5, Table 6 and Table 7).

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 PT2019BRUSERNS1, PT2019BRUSERNS2 and PT2019BRUSERNS3 were samples taken at abattoir and obtained from a BRU-free farm, whereas the reference serum sample PT2019BRUSERPS1 was a serum obtained from an animal that was experimentally infected with the *Brucella abortus* strain W99 (serum 1174).The reference serum sample PT2019BRUSERPS2 was a 1/22,5 dilution of serum obtained from an animal experimentally infected with the *Brucella abortus* strain W99 (serum 3467) and the reference serum sample PT2019BRUSERPS3 was a 1/16 dilution of serum obtained from a BRU-positive farm during a BRU breakdown in December 2010 in Belgium (resp.serum 1909). For each reference serum sample, the same qualitative result was obtained with all test methods used. Taken together, the reference serum samples PT2019BRUSERNS1, PT2019BRUSERNS2 and PT2019BRUSERNS3 were considered as negative sera, and the reference serum samples PT2019BRUSERPS1, PT2019BRUSERPS2 and PT2019BRUSERPS3 as positive sera for BRU-specific antibodies.

After aliquoting and lyophilisation of the different reference serum samples, a homogeneity check was performed on 5 aliquots of reference sera PT2019BRUSERNS2, PT2019BRUSERNS3 and on 10 aliquots of reference sera PT2019BRUSERNS1, PT2019BRUSERPS1, PT2019BRUSERPS2 and PT2019BRUSERPS3 sample using SAW-EDTA, RBT and an in-house BRU antibody ELISA test, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample with each test method used. Before the PT, only 3 aliquots of each reference serum sample were tested to confirm their stability and status (pre-verification) using SAW-EDTA, RBT and an in-house BRU antibody ELISA test. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BRU-specific antibodies in bovine serum. In addition, one

aliquot of each reference serum sample was tested after the PT in order to confirm their stability and status (post-verification) using SAW-EDTA, RBT and an in-house BRU antibody ELISA test.

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

III.3.1. Classification of results

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

III.3.2. Level of agreement

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

III.3.3. 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.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

The 20 reference serum samples were sent lyophilized to each of the 6 participating laboratories by national or international courier on 23th of September 2019. LAB2, LAB3, LAB4 and LAB6 acknowledged receipt of the samples on the same day whereas LAB1 and LAB5 received the samples on 25th of September 2019. Analyses were performed between 24th of September and 4th of October 2019 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 27st of September and 4th of November 2019 (Table 1). All participating laboratories, except LAB3 respected the deadline of 11th of October 2019 for submission of the results.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Participating laboratory	Reference samples received	Start of analysis ELISA	Start of analysis RBT	Start of analysis SAW-EDTA	Submission of the results (Excel file)
LAB1	25/09/2019	30/09/2019	01/10/2019	30/09/2019	10/10/2019
LAB2	23/09/2019	02/10/2019	30/09/2019	30/09/2019	08/10/2019
LAB3	23/09/2019	01/10/2019	01/10/2019	02/10/2019	**04/11/2019**
LAB4	23/09/2019	04/10/2019	27/09/2019	26/09/2019	10/10/2019

LAB5	25/09/2019	27/09/2019	NA	NA	27/09/2019
LAB6	23/09/2019	NA	24/09/2019	NA	09/10/2019

NA : not applicable

IV.3. Compliance with the procedure

All participating laboratories except LAB3 have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that:

(i) For the detection of BRU-specific antibodies by **SAW-EDTA** in serum : all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2).

(ii) For the detection of BRU-specific antibodies by **RBT** in serum : 4 out of 5 participating laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement), whereas LAB6 misclassified 3 aliquots (85% of agreement) (Table 3).

(iii) For the detection of BRU-specific antibodies by **ELISA** in serum : all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 4).

A quantitative data analysis (box plots) is shown for educational purposes in Annex1.

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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. 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)
success	20 (100)	20 (100)	20 (100)	20 (100)

Table 3. RBT: Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BRU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**2**	**3**	**4**	**6**
failure	0 (0)	0 (0)	0 (0)	0 (0)	**3 (15)**
success	20 (100)	20 (100)	20 (100)	20 (100)	**17 (85)**

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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

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

IV.4.2. Variability among participating laboratories

(i) For the detection of BRU-specific antibodies by **SAW-EDTA** in serum, no variability between the participants could be observed since all participants correctly identified all reference serum samples.

(ii) For the detection of BRU-specific antibodies by **RBT** in serum, no variability between LAB1, LAB2, LAB3 and LAB4 could be observed since these laboratories correctly identified all reference samples. In contrast, LAB6 misclassified the 3 aliquots of the positive reference serum sample PT2019BRUSERPS2 (NEG instead of POS).

(iii) For the detection of BRU-specific antibodies by **ELISA** in serum, no variability between the participants could be observed since all participants correctly identified all reference serum samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 5 (SAW-EDTA), Table 6 (RBT) and Table 7 (ELISA).

Table 5 SAW-EDTA: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BRUSERNS1	NEG	NEG	1
2	1	2	PT2019BRUSERNS3	NEG	NEG	1
3	1	3	PT2019BRUSERPS1	>=100	>=100	1
4	1	4	PT2019BRUSERNS1	NEG	NEG	1
5	1	5	PT2019BRUSERPS2	50	30	1
6	1	6	PT2019BRUSERNS2	NEG	NEG	1
7	1	7	PT2019BRUSERPS3	>=100	>=100	1
8	1	8	PT2019BRUSERNS3	NEG	NEG	1
9	1	9	PT2019BRUSERNS2	NEG	NEG	1
10	1	10	PT2019BRUSERPS3	>=100	50	1
11	1	11	PT2019BRUSERPS1	>=100	>=100	1
12	1	12	PT2019BRUSERNS3	NEG	NEG	1
13	1	13	PT2019BRUSERNS1	NEG	NEG	1
14	1	14	PT2019BRUSERPS2	50	30	1
15	1	15	PT2019BRUSERNS3	NEG	NEG	1
16	1	16	PT2019BRUSERPS3	>=100	>=100	1
17	1	17	PT2019BRUSERNS2	NEG	NEG	1
18	1	18	PT2019BRUSERPS1	>=100	>=100	1
19	1	19	PT2019BRUSERNS2	NEG	NEG	1
20	1	20	PT2019BRUSERPS2	50	30	1
21	2	1	PT2019BRUSERNS2	NEG	NEG	1
22	2	2	PT2019BRUSERNS1	NEG	NEG	1
23	2	3	PT2019BRUSERPS2	50	30	1
24	2	4	PT2019BRUSERNS2	NEG	NEG	1
25	2	5	PT2019BRUSERPS1	>=100	>=100	1
26	2	6	PT2019BRUSERNS1	NEG	NEG	1
27	2	7	PT2019BRUSERNS3	NEG	NEG	1
28	2	8	PT2019BRUSERNS2	NEG	NEG	1
29	2	9	PT2019BRUSERPS2	50	30	1
30	2	10	PT2019BRUSERNS1	NEG	NEG	1
31	2	11	PT2019BRUSERPS3	>=100	50	1
32	2	12	PT2019BRUSERPS2	50	30	1
33	2	13	PT2019BRUSERNS3	NEG	NEG	1
34	2	14	PT2019BRUSERPS1	>=100	50	1
35	2	15	PT2019BRUSERNS2	NEG	NEG	1
36	2	16	PT2019BRUSERPS3	>=100	>=100	1
37	2	17	PT2019BRUSERPS1	>=100	>=100	1
38	2	18	PT2019BRUSERNS3	NEG	NEG	1

39	2	19	PT2019BRUSERPS3	>=100	50	1
40	2	20	PT2019BRUSERNS3	NEG	NEG	1
41	3	1	PT2019BRUSERNS1	NEG	NEG	1
42	3	2	PT2019BRUSERNS3	NEG	NEG	1
43	3	3	PT2019BRUSERPS1	>=100	>=100	1
44	3	4	PT2019BRUSERNS1	NEG	NEG	1
45	3	5	PT2019BRUSERPS2	50	50	1
46	3	6	PT2019BRUSERNS2	NEG	NEG	1
47	3	7	PT2019BRUSERPS3	>=100	>=100	1
48	3	8	PT2019BRUSERNS3	NEG	NEG	1
49	3	9	PT2019BRUSERNS2	NEG	NEG	1
50	3	10	PT2019BRUSERPS3	>=100	>=100	1
51	3	11	PT2019BRUSERPS1	>=100	>=100	1
52	3	12	PT2019BRUSERNS3	NEG	NEG	1
53	3	13	PT2019BRUSERNS1	NEG	NEG	1
54	3	14	PT2019BRUSERPS2	50	50	1
55	3	15	PT2019BRUSERNS3	NEG	NEG	1
56	3	16	PT2019BRUSERPS3	>=100	>=100	1
57	3	17	PT2019BRUSERNS2	NEG	NEG	1
58	3	18	PT2019BRUSERPS1	>=100	>=100	1
59	3	19	PT2019BRUSERNS2	NEG	NEG	1
60	3	20	PT2019BRUSERPS2	50	50	1
61	4	1	PT2019BRUSERNS2	NEG	NEG	1
62	4	2	PT2019BRUSERNS1	NEG	NEG	1
63	4	3	PT2019BRUSERPS2	50	50	1
64	4	4	PT2019BRUSERNS2	NEG	NEG	1
65	4	5	PT2019BRUSERPS1	>=100	>=100	1
66	4	6	PT2019BRUSERNS1	NEG	NEG	1
67	4	7	PT2019BRUSERNS3	NEG	NEG	1
68	4	8	PT2019BRUSERNS2	NEG	NEG	1
69	4	9	PT2019BRUSERPS2	50	50	1
70	4	10	PT2019BRUSERNS1	NEG	NEG	1
71	4	11	PT2019BRUSERPS3	>=100	>=100	1
72	4	12	PT2019BRUSERPS2	50	50	1
73	4	13	PT2019BRUSERNS3	NEG	NEG	1
74	4	14	PT2019BRUSERPS1	>=100	>=100	1
75	4	15	PT2019BRUSERNS2	NEG	NEG	1
76	4	16	PT2019BRUSERPS3	>=100	>=100	1
77	4	17	PT2019BRUSERPS1	>=100	>=100	1
78	4	18	PT2019BRUSERNS3	NEG	NEG	1
79	4	19	PT2019BRUSERPS3	>=100	>=100	1
80	4	20	PT2019BRUSERNS3	NEG	NEG	1

Table 6 RBT: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BRUSERNS1	NEG	NEG	1
2	1	2	PT2019BRUSERNS3	NEG	NEG	1
3	1	3	PT2019BRUSERPS1	POS	POS	1
4	1	4	PT2019BRUSERNS1	NEG	NEG	1
5	1	5	PT2019BRUSERPS2	POS	POS	1
6	1	6	PT2019BRUSERNS2	NEG	NEG	1
7	1	7	PT2019BRUSERPS3	POS	POS	1
8	1	8	PT2019BRUSERNS3	NEG	NEG	1
9	1	9	PT2019BRUSERNS2	NEG	NEG	1
10	1	10	PT2019BRUSERPS3	POS	POS	1
11	1	11	PT2019BRUSERPS1	POS	POS	1

12	1	12	PT2019BRUSERNS3	NEG	NEG	1
13	1	13	PT2019BRUSERNS1	NEG	NEG	1
14	1	14	PT2019BRUSERPS2	POS	POS	1
15	1	15	PT2019BRUSERNS3	NEG	NEG	1
16	1	16	PT2019BRUSERPS3	POS	POS	1
17	1	17	PT2019BRUSERNS2	NEG	NEG	1
18	1	18	PT2019BRUSERPS1	POS	POS	1
19	1	19	PT2019BRUSERNS2	NEG	NEG	1
20	1	20	PT2019BRUSERPS2	POS	POS	1
21	2	1	PT2019BRUSERNS2	NEG	NEG	1
22	2	2	PT2019BRUSERNS1	NEG	NEG	1
23	2	3	PT2019BRUSERPS2	POS	POS	1
24	2	4	PT2019BRUSERNS2	NEG	NEG	1
25	2	5	PT2019BRUSERPS1	POS	POS	1
26	2	6	PT2019BRUSERNS1	NEG	NEG	1
27	2	7	PT2019BRUSERNS3	NEG	NEG	1
28	2	8	PT2019BRUSERNS2	NEG	NEG	1
29	2	9	PT2019BRUSERPS2	POS	POS	1
30	2	10	PT2019BRUSERNS1	NEG	NEG	1
31	2	11	PT2019BRUSERPS3	POS	POS	1
32	2	12	PT2019BRUSERPS2	POS	POS	1
33	2	13	PT2019BRUSERNS3	NEG	NEG	1
34	2	14	PT2019BRUSERPS1	POS	POS	1
35	2	15	PT2019BRUSERNS2	NEG	NEG	1
36	2	16	PT2019BRUSERPS3	POS	POS	1
37	2	17	PT2019BRUSERPS1	POS	POS	1
38	2	18	PT2019BRUSERNS3	NEG	NEG	1
39	2	19	PT2019BRUSERPS3	POS	POS	1
40	2	20	PT2019BRUSERNS3	NEG	NEG	1
41	3	1	PT2019BRUSERNS1	NEG	NEG	1
42	3	2	PT2019BRUSERNS3	NEG	NEG	1
43	3	3	PT2019BRUSERPS1	POS	POS	1
44	3	4	PT2019BRUSERNS1	NEG	NEG	1
45	3	5	PT2019BRUSERPS2	POS	POS	1
46	3	6	PT2019BRUSERNS2	NEG	NEG	1
47	3	7	PT2019BRUSERPS3	POS	POS	1
48	3	8	PT2019BRUSERNS3	NEG	NEG	1
49	3	9	PT2019BRUSERNS2	NEG	NEG	1
50	3	10	PT2019BRUSERPS3	POS	POS	1
51	3	11	PT2019BRUSERPS1	POS	POS	1
52	3	12	PT2019BRUSERNS3	NEG	NEG	1
53	3	13	PT2019BRUSERNS1	NEG	NEG	1
54	3	14	PT2019BRUSERPS2	POS	POS	1
55	3	15	PT2019BRUSERNS3	NEG	NEG	1
56	3	16	PT2019BRUSERPS3	POS	POS	1
57	3	17	PT2019BRUSERNS2	NEG	NEG	1
58	3	18	PT2019BRUSERPS1	POS	POS	1
59	3	19	PT2019BRUSERNS2	NEG	NEG	1
60	3	20	PT2019BRUSERPS2	POS	POS	1
61	4	1	PT2019BRUSERNS2	NEG	NEG	1
62	4	2	PT2019BRUSERNS1	NEG	NEG	1
63	4	3	PT2019BRUSERPS2	POS	POS	1
64	4	4	PT2019BRUSERNS2	NEG	NEG	1
65	4	5	PT2019BRUSERPS1	POS	POS	1
66	4	6	PT2019BRUSERNS1	NEG	NEG	1
67	4	7	PT2019BRUSERNS3	NEG	NEG	1
68	4	8	PT2019BRUSERNS2	NEG	NEG	1
69	4	9	PT2019BRUSERPS2	POS	POS	1
70	4	10	PT2019BRUSERNS1	NEG	NEG	1

71	4	11	PT2019BRUSERPS3	POS	POS	1
72	4	12	PT2019BRUSERPS2	POS	POS	1
73	4	13	PT2019BRUSERNS3	NEG	NEG	1
74	4	14	PT2019BRUSERPS1	POS	POS	1
75	4	15	PT2019BRUSERNS2	NEG	NEG	1
76	4	16	PT2019BRUSERPS3	POS	POS	1
77	4	17	PT2019BRUSERPS1	POS	POS	1
78	4	18	PT2019BRUSERNS3	NEG	NEG	1
79	4	19	PT2019BRUSERPS3	POS	POS	1
80	4	20	PT2019BRUSERNS3	NEG	NEG	1
81	6	1	PT2019BRUSERNS2	NEG	NEG	1
82	6	2	PT2019BRUSERNS1	NEG	NEG	1
83	6	3	PT2019BRUSERPS2	**POS**	**NEG**	**0**
84	6	4	PT2019BRUSERNS2	NEG	NEG	1
85	6	5	PT2019BRUSERPS1	POS	POS	1
86	6	6	PT2019BRUSERNS1	NEG	NEG	1
87	6	7	PT2019BRUSERNS3	NEG	NEG	1
88	6	8	PT2019BRUSERNS2	NEG	NEG	1
89	6	9	PT2019BRUSERPS2	**POS**	**NEG**	**0**
90	6	10	PT2019BRUSERNS1	NEG	NEG	1
91	6	11	PT2019BRUSERPS3	POS	POS	1
92	6	12	PT2019BRUSERPS2	**POS**	**NEG**	**0**
93	6	13	PT2019BRUSERNS3	NEG	NEG	1
94	6	14	PT2019BRUSERPS1	POS	POS	1
95	6	15	PT2019BRUSERNS2	NEG	NEG	1
96	6	16	PT2019BRUSERPS3	POS	POS	1
97	6	17	PT2019BRUSERPS1	POS	POS	1
98	6	18	PT2019BRUSERNS3	NEG	NEG	1
99	6	19	PT2019BRUSERPS3	POS	POS	1
100	6	20	PT2019BRUSERNS3	NEG	NEG	1

Table 7 ELISA: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the BRU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BRUSERNS1	NEG	NEG	1
2	1	2	PT2019BRUSERNS3	NEG	NEG	1
3	1	3	PT2019BRUSERPS1	POS	POS	1
4	1	4	PT2019BRUSERNS1	NEG	NEG	1
5	1	5	PT2019BRUSERPS2	POS	POS	1
6	1	6	PT2019BRUSERNS2	NEG	NEG	1
7	1	7	PT2019BRUSERPS3	POS	POS	1
8	1	8	PT2019BRUSERNS3	NEG	NEG	1
9	1	9	PT2019BRUSERNS2	NEG	NEG	1
10	1	10	PT2019BRUSERPS3	POS	POS	1
11	1	11	PT2019BRUSERPS1	POS	POS	1
12	1	12	PT2019BRUSERNS3	NEG	NEG	1
13	1	13	PT2019BRUSERNS1	NEG	NEG	1
14	1	14	PT2019BRUSERPS2	POS	POS	1
15	1	15	PT2019BRUSERNS3	NEG	NEG	1
16	1	16	PT2019BRUSERPS3	POS	POS	1
17	1	17	PT2019BRUSERNS2	NEG	NEG	1
18	1	18	PT2019BRUSERPS1	POS	POS	1
19	1	19	PT2019BRUSERNS2	NEG	NEG	1
20	1	20	PT2019BRUSERPS2	POS	POS	1
21	2	1	PT2019BRUSERNS2	NEG	NEG	1
22	2	2	PT2019BRUSERNS1	NEG	NEG	1
23	2	3	PT2019BRUSERPS2	POS	POS	1

24	2	4	PT2019BRUSERNS2	NEG	NEG	1
25	2	5	PT2019BRUSERPS1	POS	POS	1
26	2	6	PT2019BRUSERNS1	NEG	NEG	1
27	2	7	PT2019BRUSERNS3	NEG	NEG	1
28	2	8	PT2019BRUSERNS2	NEG	NEG	1
29	2	9	PT2019BRUSERPS2	POS	POS	1
30	2	10	PT2019BRUSERNS1	NEG	NEG	1
31	2	11	PT2019BRUSERPS3	POS	POS	1
32	2	12	PT2019BRUSERPS2	POS	POS	1
33	2	13	PT2019BRUSERNS3	NEG	NEG	1
34	2	14	PT2019BRUSERPS1	POS	POS	1
35	2	15	PT2019BRUSERNS2	NEG	NEG	1
36	2	16	PT2019BRUSERPS3	POS	POS	1
37	2	17	PT2019BRUSERPS1	POS	POS	1
38	2	18	PT2019BRUSERNS3	NEG	NEG	1
39	2	19	PT2019BRUSERPS3	POS	POS	1
40	2	20	PT2019BRUSERNS3	NEG	NEG	1
41	3	1	PT2019BRUSERNS1	NEG	NEG	1
42	3	2	PT2019BRUSERNS3	NEG	NEG	1
43	3	3	PT2019BRUSERPS1	POS	POS	1
44	3	4	PT2019BRUSERNS1	NEG	NEG	1
45	3	5	PT2019BRUSERPS2	POS	POS	1
46	3	6	PT2019BRUSERNS2	NEG	NEG	1
47	3	7	PT2019BRUSERPS3	POS	POS	1
48	3	8	PT2019BRUSERNS3	NEG	NEG	1
49	3	9	PT2019BRUSERNS2	NEG	NEG	1
50	3	10	PT2019BRUSERPS3	POS	POS	1
51	3	11	PT2019BRUSERPS1	POS	POS	1
52	3	12	PT2019BRUSERNS3	NEG	NEG	1
53	3	13	PT2019BRUSERNS1	NEG	NEG	1
54	3	14	PT2019BRUSERPS2	POS	POS	1
55	3	15	PT2019BRUSERNS3	NEG	NEG	1
56	3	16	PT2019BRUSERPS3	POS	POS	1
57	3	17	PT2019BRUSERNS2	NEG	NEG	1
58	3	18	PT2019BRUSERPS1	POS	POS	1
59	3	19	PT2019BRUSERNS2	NEG	NEG	1
60	3	20	PT2019BRUSERPS2	POS	POS	1
61	4	1	PT2019BRUSERNS2	NEG	NEG	1
62	4	2	PT2019BRUSERNS1	NEG	NEG	1
63	4	3	PT2019BRUSERPS2	POS	POS	1
64	4	4	PT2019BRUSERNS2	NEG	NEG	1
65	4	5	PT2019BRUSERPS1	POS	POS	1
66	4	6	PT2019BRUSERNS1	NEG	NEG	1
67	4	7	PT2019BRUSERNS3	NEG	NEG	1
68	4	8	PT2019BRUSERNS2	NEG	NEG	1
69	4	9	PT2019BRUSERPS2	POS	POS	1
70	4	10	PT2019BRUSERNS1	NEG	NEG	1
71	4	11	PT2019BRUSERPS3	POS	POS	1
72	4	12	PT2019BRUSERPS2	POS	POS	1
73	4	13	PT2019BRUSERNS3	NEG	NEG	1
74	4	14	PT2019BRUSERPS1	POS	POS	1
75	4	15	PT2019BRUSERNS2	NEG	NEG	1
76	4	16	PT2019BRUSERPS3	POS	POS	1
77	4	17	PT2019BRUSERPS1	POS	POS	1
78	4	18	PT2019BRUSERNS3	NEG	NEG	1
79	4	19	PT2019BRUSERPS3	POS	POS	1
80	4	20	PT2019BRUSERNS3	NEG	NEG	1
81	5	1	PT2019BRUSERNS1	NEG	NEG	1
82	5	2	PT2019BRUSERNS3	NEG	NEG	1

83	5	3	PT2019BRUSERPS1	POS	POS	1
84	5	4	PT2019BRUSERNS1	NEG	NEG	1
85	5	5	PT2019BRUSERPS2	POS	POS	1
86	5	6	PT2019BRUSERNS2	NEG	NEG	1
87	5	7	PT2019BRUSERPS3	POS	POS	1
88	5	8	PT2019BRUSERNS3	NEG	NEG	1
89	5	9	PT2019BRUSERNS2	NEG	NEG	1
90	5	10	PT2019BRUSERPS3	POS	POS	1
91	5	11	PT2019BRUSERPS1	POS	POS	1
92	5	12	PT2019BRUSERNS3	NEG	NEG	1
93	5	13	PT2019BRUSERNS1	NEG	NEG	1
94	5	14	PT2019BRUSERPS2	POS	POS	1
95	5	15	PT2019BRUSERNS3	NEG	NEG	1
96	5	16	PT2019BRUSERPS3	POS	POS	1
97	5	17	PT2019BRUSERNS2	NEG	NEG	1
98	5	18	PT2019BRUSERPS1	POS	POS	1
99	5	19	PT2019BRUSERNS2	NEG	NEG	1
100	5	20	PT2019BRUSERPS2	POS	POS	1

V. Discussion

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

For the detection of BRU-specific antibodies by SAW-EDTA in reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2 and Table 5).
The 4 participating laboratories that performed SAW-EDTA used SAW antigen from two different producers. LAB2, LAB3 and LAB4 used the SAW antigen from Zoetis (batch : 17ZBAI001) and LAB1 used the SAW antigen from IDEXX (batch 421).

For the detection of BRU-specific antibodies by RBT in reference serum samples, 4 out of 5 participating laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB6 misclassified the 3 aliquots of the positive reference serum sample PT2019BRUSERPS2 (85% of agreement) (Table 3 and Table 6).
The 5 participating laboratories that performed RBT used a RBT antigen from two different producers. LAB1, LAB2, LAB3 and LAB4 used the RBT antigen from IDEXX (batches: 418, 427, 409-100 and 406-100) and LAB6 used the RBT antigen from Zoetis (batch: 18ZBAB015).

For the detection of BRU-specific antibodies by ELISA in reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 4 and Table 7).
The 5 participating laboratories that performed ELISA used ELISA kits from different producers, LAB3 used an home made developed BRU antibody ELISA kit, whereas LAB2, LAB4 and LAB5 used a commercially BRU antibody ELISA kit from Zoetis (batch : 18ZEAJ008) and LAB1 used a commercially BRU antibody ELISA kit from IDEXX (batch 8020).

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 and at least 90% for RBT and ELISA (see III.3.3.). Consequently, the participants who performed SAW-EDTA and ELISA achieved a satisfactory performance for the detection of BRU-specific antibodies in reference serum samples. For the detection of BRU-specific antibodies in reference serum samples by RBT all participants, except LAB6, achieved a satisfactory performance.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) : Laboratoire de Santé Animale / Unité des Zoonoses Bactériennes (Maisons-Alfort, France)

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

Sciensano (Ukkel, Belgium)

Zoetis France (Lyon, France)

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software R (box plots).

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

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

Only box plots for the antibody ELISA of the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory are shown in Figure 1. The LAB3 data could not be shown on the box plots.

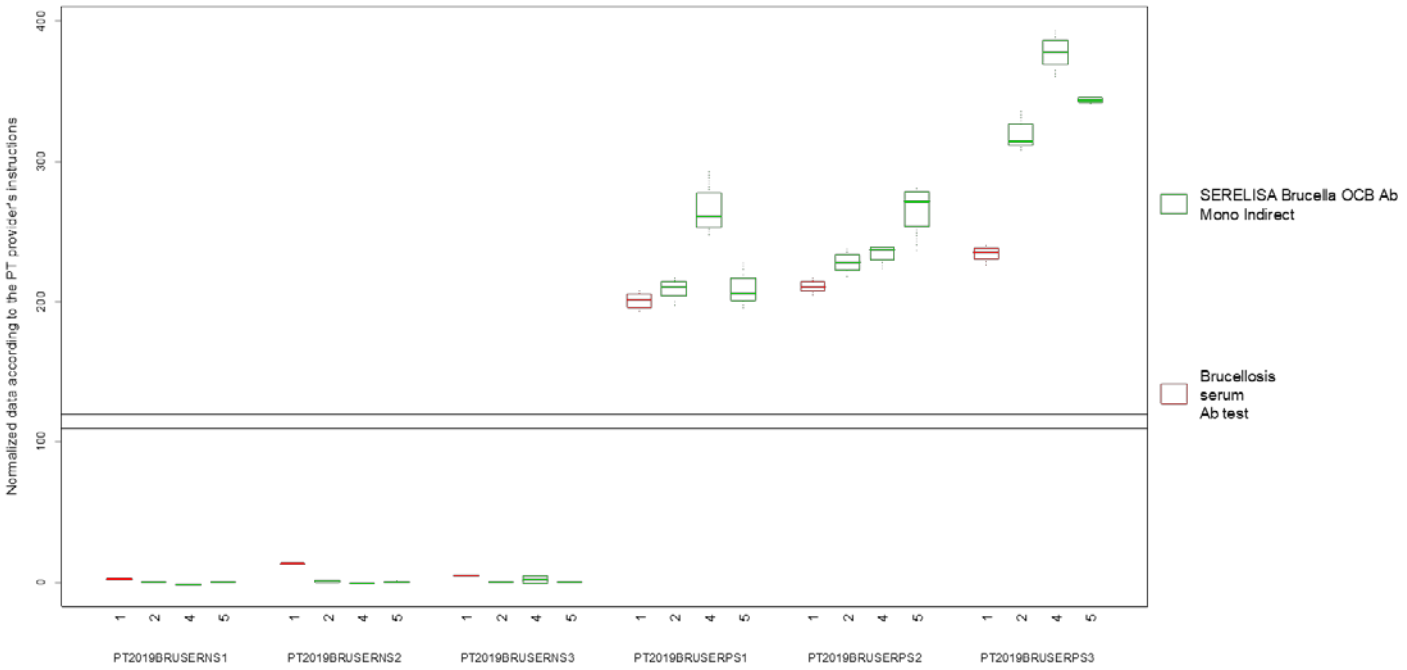

Figure 1. Box plots showing the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory. The LAB3 data could not be shown.
Cut-off values applied by the participating laboratory using IDEXX (110-120) are shown by horizontal lines.