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081-PT

PROFICIENCY TESTING 2018

BRUCELLOSIS (BRU)

ISOLATION OF BRUCELLA SPP. FROM ORGANS

AND

DETECTION OF BRU-SPECIFIC ANTIBODIES IN BOVINE MILK BY ELISA

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS

SCIENSANO

DATE BEGIN PT: 17 SEPTEMBER 2018

DATE REPORT: 9 JANUARI 2019

THIS REPORT REPLACES AND CANCELS THE PREVIOUS REPORT PT2018BRUBAC+SER

Reason : error in the deadline for submission of the results by the participants

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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 Brucella spp. in organs and the absence or presence of BRU-specific antibodies in bovine milk samples by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined organ samples must be analyzed by means of a Brucella isolation test and/or predefined reference milk samples must be tested by means of a BRU antibody ELISA test. The procedures for the isolation tests and/or BRU antibody ELISA test must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference organ samples

Ten reference organ samples of bovine origin either free from Brucella spp (n=5; coded 'PT2018BRUBACNO1', 'PT2018BRUBACNO2', 'PT2018BRUBACNO3', 'PT2018BRUBACNO4' and 'PT2018BRUBACNO5') or containing Brucella spp (n=5; coded 'PT2018BRUBACPO1', 'PT2018BRUBACPO2', 'PT2018BRUBACPO3', 'PT2018BRUBACPO4' and 'PT2018BRUBACPO5') were used. In total, 30 aliquots were distributed to 3 participating laboratories. All participants received 10 aliquots : one aliquot of each reference organ sample. The identification numbers of the reference organ samples were randomized for all participants (Table 4).

For each reference organ sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference organ samples was determined based on (i) the historical background of the samples and (ii) the results obtained during pre-verification, hereby using the in-house isolation procedure (SOP/BAC/ANA/04).

The reference organ samples were obtained from bovine lymphatic ganglions free of Brucellosis (PT2018BRUBACNO1) that were spiked either with bacterial contaminants (PT2018BRUBACNO2, PT2018BRUBACNO3) or bacteria phylogenically close to *Brucella spp.* (PT2018BRUBACNO4, PT2018BRUBACNO5) or *Brucella abortus* biovar 3 (PT2018BRUBACPO1, PT2018BRUBACPO3, PT2018BRUBACPO5) or *Brucella suis* biovar 2 (PT2018BRUBACPO2, PT2018BRUBACPO4). Taken together, the reference samples PT2018BRUBACNO1, PT2018BRUBACNO2, PT2018BRUBACNO3, PT2018BRUBACNO4 and PT2018BRUBACNO5 were considered as negative and the reference samples PT2018BRUBACPO1, PT2018BRUBACPO2, PT2018BRUBACPO3, PT2018BRUBACPO4 and PT2018BRUBACPO5 as positive samples.

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 'PT2018BRUSERNM1' and 'PT2018BRUSERNM2') or containing detectable BRU-specific antibodies (n=4; coded 'PT2018BRUSERPM1', 'PT2018BRUSERPM2', 'PT2018BRUSERPM3' and 'PT2018BRUSERPM4'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 2 aliquots of the reference milk samples PT2018BRUSERNM1 and PT2018BRUSERNM2 and 4 aliquots of the reference milk sample PT2018BRUSERPM1, PT2018BRUSERPM2, PT2018BRUSERPM3 and PT2018BRUSERPM4. The identification numbers of the reference milk samples were randomized for all participants (Table 5).

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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. The reference milk samples PT2018BRUSERNM1 and PT2018BRUSERNM2 were derived from 2 different batches of commercial whole milk, whereas the other reference milk samples were commercial whole milk samples spiked with serum containing BRU-specific antibodies. More specifically, PT2018BRUSERPM1 was spiked with serum 3467 in a 1/14000 dilution and PT2018BRUSERPM2 was spiked with serum 3467 in a 1/6400 dilution, respectively, whereas PT2018BRUSERPM3 was spiked with serum 3667 in a 1/800 and PT2018BRUSERPM4 was spiked with serum 3667 in a 1/1000 dilution. Serums 3467, 3667 were obtained from animals that were experimentally infected with the *Brucella abortus* strain W99. Taken together, the reference samples PT2018BRUSERNM1 and PT2017BRUSERNM2 were considered as negative milk samples, and the reference samples PT2018BRUSERPM1, PT2018BRUSERPM2, PT2018BRUSERPM3 and PT2018BRUSERPM4 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, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Before the PT, only 3 aliquots of each reference milk sample were tested to confirm their stability and status (pre-verification) using the BRU antibody ELISA test kit from IDEXX. 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, one aliquot of each reference milk sample was tested after the PT in order to confirm their stability and status (post-verification) using the BRU antibody ELISA test kit from IDEXX.

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* 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. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 10 aliquots of reference organ samples and the 20 aliquots of reference milk samples used for each PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 aliquots of reference organ samples and the 20 aliquots of reference milk samples used is at least 90%.

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

LAB1 and LAB2 participated in both the PT organ and the PT milk and hence received 30 aliquots: 10 aliquots of reference organ samples and 20 aliquots of reference milk samples. In contrast, LAB3, LAB4 and LAB5 only participated in the PT milk and hence received 20 aliquots of reference milk samples. LAB6 only participated in the PT organ and hence received 10 aliquots of reference organ samples.
Frozen organ samples (30 aliquots in total) and lyophilized reference milk samples (100 aliquots in total) were sent to the 6 participating laboratories by national or international courier on the 17th of September 2018 except LAB2 to which the samples were sent on 18th of September. LAB1, LAB3, LAB5 and LAB6 acknowledged receipt of the samples on 17th of September 2018, whereas LAB2 and LAB4 acknowledged receipt of the samples on 18th of September 2018. Analyses were performed between 17th and 28th of September 2018 (Table 1).

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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 20th of September and 16th of October 2018 (Table 1). All participants except LAB1 respected the deadline of 5th of **October** 2018 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.

Laboratory	Reference samples received	Dates Performing serological tests	Dates Performing bacteriological tests	Submission of the results (Excel file)
LAB1	17/09/2018	19/09/2018	17/09/2018	**16/10/2018**
LAB2	18/09/2018	19/09/2018	18/09/2018	3-4/10/2018
LAB3	17/09/2018	18/09/2018	NA	20/09/2018
LAB4	18/09/2018	28/09/2018	NA	05/10/2018
LAB5	17/09/2018	20/09/2018	NA	27/09/2018
LAB6	17/09/2018	NA	17/09/2018	04/10/2018

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories 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 Brucella in **organ**, all 3 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference organ samples and thus achieved 100% of agreement (Table 2).

(ii) For the detection of BRU-specific antibodies in **milk**, the 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples and thus achieved 100% of agreement (Table 3).
A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

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Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference **organ** samples assigned by the BRU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 10 aliquots of reference **organ** samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**6**
failure	0 (0)	0 (0)	0 (0)
success	10 (100)	10 (100)	10 (100)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference **milk** 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 **milk** 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

Variability in the qualitative laboratory results of the participating laboratories was not observed with any of the reference organ and milk samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 4 for the PT organ and in Table 5 for the PT milk.

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Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference organ samples (SAMPLE), the external identification of the reference organ 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	PT2018BRUBACNO5	NEG	NEG	1
2	1	2	PT2018BRUBACPO1	POS	POS	1
3	1	3	PT2018BRUBACPO4	POS	POS	1
4	1	4	PT2018BRUBACNO1	NEG	NEG	1
5	1	5	PT2018BRUBACPO3	POS	POS	1
6	1	6	PT2018BRUBACNO3	NEG	NEG	1
7	1	7	PT2018BRUBACPO2	POS	POS	1
8	1	8	PT2018BRUBACNO2	NEG	NEG	1
9	1	9	PT2018BRUBACNO4	NEG	NEG	1
10	1	10	PT2018BRUBACPO5	POS	POS	1
11	2	1	PT2018BRUBACNO2	NEG	NEG	1
12	2	2	PT2018BRUBACNO4	NEG	NEG	1
13	2	3	PT2018BRUBACPO2	POS	POS	1
14	2	4	PT2018BRUBACPO5	POS	POS	1
15	2	5	PT2018BRUBACNO3	NEG	NEG	1
16	2	6	PT2018BRUBACPO4	POS	POS	1
17	2	7	PT2018BRUBACPO1	POS	POS	1
18	2	8	PT2018BRUBACNO1	NEG	NEG	1
19	2	9	PT2018BRUBACPO3	POS	POS	1
20	2	10	PT2018BRUBACNO5	NEG	NEG	1
21	6	1	PT2018BRUBACNO5	NEG	NEG	1
22	6	2	PT2018BRUBACPO1	POS	POS	1
23	6	3	PT2018BRUBACPO4	POS	POS	1
24	6	4	PT2018BRUBACNO1	NEG	NEG	1
25	6	5	PT2018BRUBACPO3	POS	POS	1
26	6	6	PT2018BRUBACNO3	NEG	NEG	1
27	6	7	PT2018BRUBACPO2	POS	POS	1
28	6	8	PT2018BRUBACNO2	NEG	NEG	1
29	6	9	PT2018BRUBACNO4	NEG	NEG	1
30	6	10	PT2018BRUBACPO5	POS	POS	1

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Table 5. 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2018BRUSERPM2	POS	POS	1
2	1	2	PT2018BRUSERPM2	POS	POS	1
3	1	3	PT2018BRUSERNM1	NEG	NEG	1
4	1	4	PT2018BRUSERPM2	POS	POS	1
5	1	5	PT2018BRUSERPM3	POS	POS	1
6	1	6	PT2018BRUSERPM1	POS	POS	1
7	1	7	PT2018BRUSERNM1	NEG	NEG	1
8	1	8	PT2018BRUSERPM2	POS	POS	1
9	1	9	PT2018BRUSERPM4	POS	POS	1
10	1	10	PT2018BRUSERNM2	NEG	NEG	1
11	1	11	PT2018BRUSERPM3	POS	POS	1
12	1	12	PT2018BRUSERPM1	POS	POS	1
13	1	13	PT2018BRUSERPM4	POS	POS	1
14	1	14	PT2018BRUSERPM3	POS	POS	1
15	1	15	PT2018BRUSERPM4	POS	POS	1
16	1	16	PT2018BRUSERNM2	NEG	NEG	1
17	1	17	PT2018BRUSERPM4	POS	POS	1
18	1	18	PT2018BRUSERPM1	POS	POS	1
19	1	19	PT2018BRUSERPM3	POS	POS	1
20	1	20	PT2018BRUSERPM1	POS	POS	1
21	2	1	PT2018BRUSERPM1	POS	POS	1
22	2	2	PT2018BRUSERPM2	POS	POS	1
23	2	3	PT2018BRUSERPM1	POS	POS	1
24	2	4	PT2018BRUSERPM3	POS	POS	1
25	2	5	PT2018BRUSERPM2	POS	POS	1
26	2	6	PT2018BRUSERNM2	NEG	NEG	1
27	2	7	PT2018BRUSERPM3	POS	POS	1
28	2	8	PT2018BRUSERPM4	POS	POS	1
29	2	9	PT2018BRUSERNM1	NEG	NEG	1
30	2	10	PT2018BRUSERPM1	POS	POS	1
31	2	11	PT2018BRUSERPM2	POS	POS	1
32	2	12	PT2018BRUSERNM1	NEG	NEG	1
33	2	13	PT2018BRUSERPM3	POS	POS	1
34	2	14	PT2018BRUSERNM2	NEG	NEG	1
35	2	15	PT2018BRUSERPM4	POS	POS	1
36	2	16	PT2018BRUSERPM2	POS	POS	1
37	2	17	PT2018BRUSERPM3	POS	POS	1
38	2	18	PT2018BRUSERPM1	POS	POS	1
39	2	19	PT2018BRUSERPM4	POS	POS	1
40	2	20	PT2018BRUSERPM4	POS	POS	1

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(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018BRUSERPM2	POS	POS	1
42	3	2	PT2018BRUSERPM2	POS	POS	1
43	3	3	PT2018BRUSERNM1	NEG	NEG	1
44	3	4	PT2018BRUSERPM2	POS	POS	1
45	3	5	PT2018BRUSERPM3	POS	POS	1
46	3	6	PT2018BRUSERPM1	POS	POS	1
47	3	7	PT2018BRUSERNM1	NEG	NEG	1
48	3	8	PT2018BRUSERPM2	POS	POS	1
49	3	9	PT2018BRUSERPM4	POS	POS	1
50	3	10	PT2018BRUSERNM2	NEG	NEG	1
51	3	11	PT2018BRUSERPM3	POS	POS	1
52	3	12	PT2018BRUSERPM1	POS	POS	1
53	3	13	PT2018BRUSERPM4	POS	POS	1
54	3	14	PT2018BRUSERPM3	POS	POS	1
55	3	15	PT2018BRUSERPM4	POS	POS	1
56	3	16	PT2018BRUSERNM2	NEG	NEG	1
57	3	17	PT2018BRUSERPM4	POS	POS	1
58	3	18	PT2018BRUSERPM1	POS	POS	1
59	3	19	PT2018BRUSERPM3	POS	POS	1
60	3	20	PT2018BRUSERPM1	POS	POS	1
61	4	1	PT2018BRUSERPM1	POS	POS	1
62	4	2	PT2018BRUSERPM2	POS	POS	1
63	4	3	PT2018BRUSERPM1	POS	POS	1
64	4	4	PT2018BRUSERPM3	POS	POS	1
65	4	5	PT2018BRUSERPM2	POS	POS	1
66	4	6	PT2018BRUSERNM2	NEG	NEG	1
67	4	7	PT2018BRUSERPM3	POS	POS	1
68	4	8	PT2018BRUSERPM4	POS	POS	1
69	4	9	PT2018BRUSERNM1	NEG	NEG	1
70	4	10	PT2018BRUSERPM1	POS	POS	1
71	4	11	PT2018BRUSERPM2	POS	POS	1
72	4	12	PT2018BRUSERNM1	NEG	NEG	1
73	4	13	PT2018BRUSERPM3	POS	POS	1
74	4	14	PT2018BRUSERNM2	NEG	NEG	1
75	4	15	PT2018BRUSERPM4	POS	POS	1
76	4	16	PT2018BRUSERPM2	POS	POS	1
77	4	17	PT2018BRUSERPM3	POS	POS	1
78	4	18	PT2018BRUSERPM1	POS	POS	1
79	4	19	PT2018BRUSERPM4	POS	POS	1
80	4	20	PT2018BRUSERPM4	POS	POS	1

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(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2018BRUSERPM2	POS	POS	1
82	5	2	PT2018BRUSERPM2	POS	POS	1
83	5	3	PT2018BRUSERNM1	NEG	NEG	1
84	5	4	PT2018BRUSERPM2	POS	POS	1
85	5	5	PT2018BRUSERPM3	POS	POS	1
86	5	6	PT2018BRUSERPM1	POS	POS	1
87	5	7	PT2018BRUSERNM1	NEG	NEG	1
88	5	8	PT2018BRUSERPM2	POS	POS	1
89	5	9	PT2018BRUSERPM4	POS	POS	1
90	5	10	PT2018BRUSERNM2	NEG	NEG	1
91	5	11	PT2018BRUSERPM3	POS	POS	1
92	5	12	PT2018BRUSERPM1	POS	POS	1
93	5	13	PT2018BRUSERPM4	POS	POS	1
94	5	14	PT2018BRUSERPM3	POS	POS	1
95	5	15	PT2018BRUSERPM4	POS	POS	1
96	5	16	PT2018BRUSERNM2	NEG	NEG	1
97	5	17	PT2018BRUSERPM4	POS	POS	1
98	5	18	PT2018BRUSERPM1	POS	POS	1
99	5	19	PT2018BRUSERPM3	POS	POS	1
100	5	20	PT2018BRUSERPM1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing organ samples for the detection of Brucella spp. by bacteriological isolation and/or milk samples of bovidae origin for the detection of BRU-specific antibodies by ELISA.

For the detection of Brucella spp. by bacteriological isolation, 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 4).

For the detection of BRU-specific antibodies in milk, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 3 and Table 5).
The participating laboratories used BRU antibody ELISA kits from the same producer but different batches: IDEXX [batches: 6111 (LAB2, LAB4 and LAB5) and 7043 (LAB1 and LAB3)].

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants in the PT organ and the PT milk achieved a satisfactory performance.

Coordinator proficiency tests

Katia Knapen

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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)

MCC-Vlaanderen (Lier, Belgium)

Sciensano (Ukkel, Belgium)

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Annex 1: Quantitative data analysis (Box plots)

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

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.

Antibody ELISA milk reference samples, box plots of the normalized data values according the PT provider per reference sample and per participating laboratory are shown in Figure 1.

Figure 1 (antibody ELISA milk reference samples)

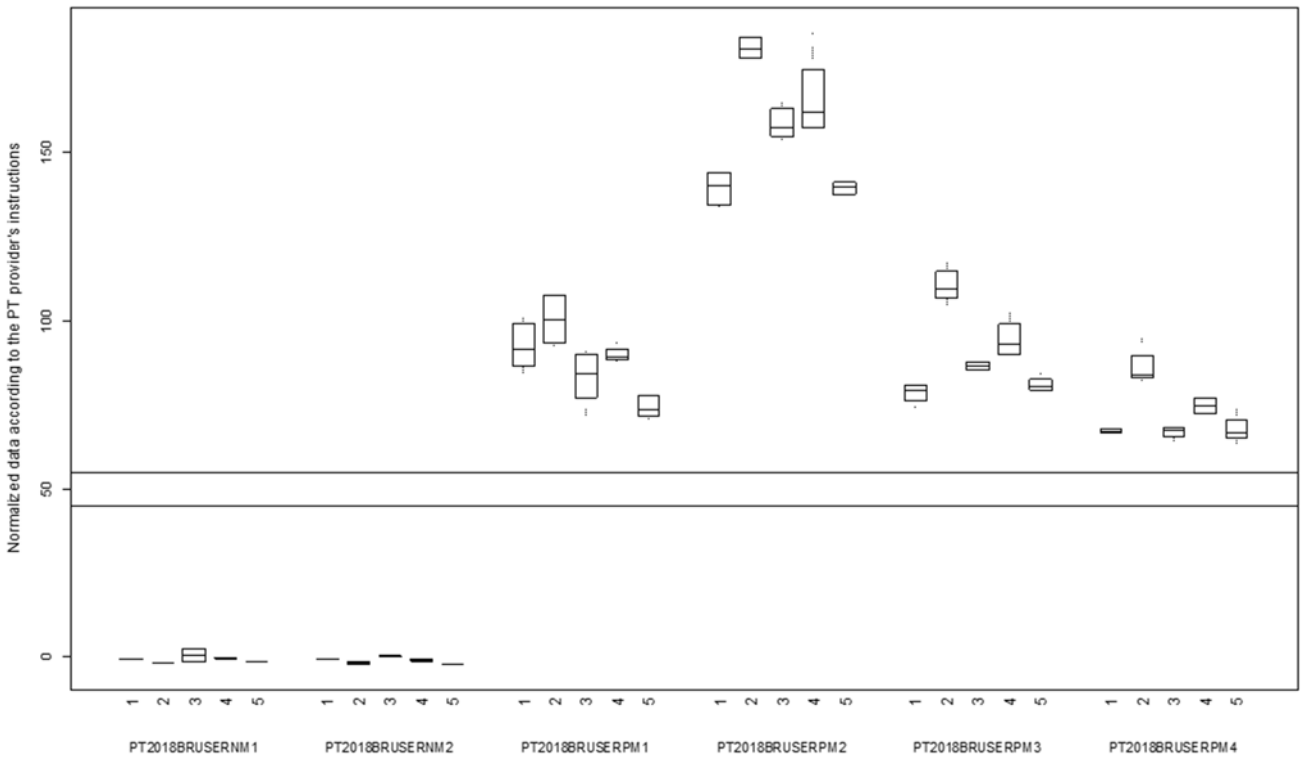

Figure 1. Box plots showing the normalized data values according the PT provider per reference milk and per participating laboratory. All participating laboratories used the BRU antibody ELISA kits from IDEXX (2 batches). Cut-off values (45-55) are shown by a horizontal line.

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