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081-PT

PROFICIENCY TESTING 2018

ENZOOTIC BOVINE LEUKOSIS (EBL)

DETECTION OF EBL-SPECIFIC ANTIBODIES IN BOVINE SERUM BY ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE BEGIN PT: 18 JUNE 2018

DATE REPORT: 5 SEPTEMBER 2018

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 '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 EBL-specific antibodies in bovine serum by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be analyzed by means of an ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 9 reference serum samples of bovine origin, either free from detectable EBL-specific antibodies (n=2; coded 'PT2018EBLSERNS1' and 'PT2018EBLSERNS2') or containing detectable EBL-specific antibodies (n=7; coded 'PT2018EBLSERPS1', 'PT2018EBLSERPS2', 'PT2018EBLSERPS3', 'PT2018EBLSERPS4', 'PT2018EBLSERPS5', 'PT2018EBLSERPS6' and 'PT2018EBLSERPS7'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 1 aliquot of the reference serum samples PT2018EBLSERNS1, PT2018EBLSERPS1 and PT2018EBLSERPS3, 2 aliquots of the reference serum samples PT2018EBLSERPS7 and 3 aliquots of the reference serum samples PT2018EBLSERNS2, PT2018EBLSERPS2, PT2018EBLSERPS4, PT2018EBLSERPS5 and PT2018EBLSERPS6. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 3).

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 based on (i) the historical background of the animals and (ii) the results obtained during pre-verification, hereby using an immunodiffusion assay and the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics Europe (Zoetis).
The reference serum samples PT2018EBLSERNS1 and PT2018EBLSERNS2 were obtained from the field (EBL negative herds). The reference serum samples PT2018EBLSERPS1, PT2018EBLSERPS2, PT2018EBLSERPS3, PT2018EBLSERPS4 were derived from 3 different animals that were experimentally infected with EBL. The reference serum samples PT2018EBLSERPS5, PT2018EBLSERPS6 and PT2018EBLSERPS7 were derived from one naturally intected animal. The reference serum samples PT2018EBLSERPS1 and PT2018EBLSERPS2 were respectively 1/2 and 1/32 dilutions of the same positive serum in a negative field sample obtained from an EBL negative herd. The reference serum sample PT2018EBLSERPS3 was a 1/2 dilution of a second positive sera in the same negative field sample obtained from an EBL negative herd. The reference serum sample PT2018EBLSERPS4 is an undiluted serum from a third positive animal. The reference serum samples PT2018EBLSERPS5, PT2018EBLSERPS6 and PT2018EBLSERPS7 were respectively 1/25, 1/50 and 1/75 dilutions of the same serum originated from the fourth positive animal in a negative field sample obtained from an EBL negative herd.
Taken together, the reference serum samples PT2018EBLSERNS1 and PT2018EBLSERNS2 were considered as negative sera, and the reference serum samples PT2018EBLSERPS1, PT2018EBLSERPS2, PT2018EBLSERPS3, PT2018EBLSERPS4, PT2018EBLSERPS5, PT2018EBLSERPS6 and PT2018EBLSERPS7 as positive sera in EBL antibody ELISA. The reference serum sample PT2018EBLSERPS7 is a weak positive sera in EBL antibody ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics Europe (Zoetis), hereby obtaining 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 laboratories to correctly identify the absence or presence of EBL-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 the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics Europe (Zoetis).

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 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 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

The 20 aliquots of reference serum samples were sent frozen (dry ice) to each of the 7 participating laboratories by national or international courier on 18th of June 2018 (140 aliquots in total). LAB1, LAB2, LAB5 and LAB6 acknowledged receipt of the samples on the same day, whereas LAB3 and LAB7 acknowledged receipt of the samples on 20th of June 2018 and LAB4 acknowledged receipt of the samples on 21th of June 2018. Analyses were performed between 18th of June and 2nd of July 2018 (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 22th of June 2018 and 9th of July 2018 (Table 1). All participants, except LAB3, respected the deadline of 6th of July 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	Start of analysis	Submission of the results (Excel file)
LAB1	18/06/2018	19/06/2018	27/06/2018
LAB2	18/06/2018	28/06/2018	29/06/2018
LAB3	20/06/2018	02/07/2018	**09/07/2018**
LAB4	21/06/2018	29/06/2018	06/07/2018

LAB5	18/06/2018	18/06/2018	04/07/2018
LAB6	18/06/2018	21/06/2018	22/06/2018
LAB7	20/06/2018	26/06/2018	02/07/2018

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 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).

A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the EBL 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**	**6**	**7**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between all participating laboratories 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 3.

Table 3. 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 EBL 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	PT2018EBLSERPS2	POS	POS	1
2	1	2	PT2018EBLSERNS2	NEG	NEG	1
3	1	3	PT2018EBLSERPS5	POS	POS	1
4	1	4	PT2018EBLSERPS6	POS	POS	1
5	1	5	PT2018EBLSERNS1	NEG	NEG	1
6	1	6	PT2018EBLSERPS4	POS	POS	1
7	1	7	PT2018EBLSERPS2	POS	POS	1
8	1	8	PT2018EBLSERPS7	POS	POS	1
9	1	9	PT2018EBLSERPS6	POS	POS	1
10	1	10	PT2018EBLSERNS2	NEG	NEG	1
11	1	11	PT2018EBLSERPS4	POS	POS	1
12	1	12	PT2018EBLSERPS6	POS	POS	1
13	1	13	PT2018EBLSERNS2	NEG	NEG	1
14	1	14	PT2018EBLSERPS5	POS	POS	1
15	1	15	PT2018EBLSERPS2	POS	POS	1
16	1	16	PT2018EBLSERPS3	POS	POS	1
17	1	17	PT2018EBLSERPS1	POS	POS	1
18	1	18	PT2018EBLSERPS4	POS	POS	1
19	1	19	PT2018EBLSERPS5	POS	POS	1
20	1	20	PT2018EBLSERPS7	POS	POS	1
21	2	1	PT2018EBLSERNS2	NEG	NEG	1
22	2	2	PT2018EBLSERPS6	POS	POS	1
23	2	3	PT2018EBLSERPS2	POS	POS	1
24	2	4	PT2018EBLSERNS1	NEG	NEG	1
25	2	5	PT2018EBLSERPS4	POS	POS	1
26	2	6	PT2018EBLSERPS2	POS	POS	1
27	2	7	PT2018EBLSERPS5	POS	POS	1
28	2	8	PT2018EBLSERNS2	NEG	NEG	1
29	2	9	PT2018EBLSERPS5	POS	POS	1
30	2	10	PT2018EBLSERPS4	POS	POS	1
31	2	11	PT2018EBLSERNS2	NEG	NEG	1
32	2	12	PT2018EBLSERPS7	POS	POS	1
33	2	13	PT2018EBLSERPS3	POS	POS	1
34	2	14	PT2018EBLSERPS6	POS	POS	1
35	2	15	PT2018EBLSERPS5	POS	POS	1
36	2	16	PT2018EBLSERPS1	POS	POS	1
37	2	17	PT2018EBLSERPS7	POS	POS	1
38	2	18	PT2018EBLSERPS2	POS	POS	1
39	2	19	PT2018EBLSERPS6	POS	POS	1
40	2	20	PT2018EBLSERPS4	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2018EBLSERPS2	POS	POS	1
42	3	2	PT2018EBLSERNS2	NEG	NEG	1
43	3	3	PT2018EBLSERPS5	POS	POS	1
44	3	4	PT2018EBLSERPS6	POS	POS	1
45	3	5	PT2018EBLSERNS1	NEG	NEG	1
46	3	6	PT2018EBLSERPS4	POS	POS	1
47	3	7	PT2018EBLSERPS2	POS	POS	1
48	3	8	PT2018EBLSERPS7	POS	POS	1
49	3	9	PT2018EBLSERPS6	POS	POS	1
50	3	10	PT2018EBLSERNS2	NEG	NEG	1
51	3	11	PT2018EBLSERPS4	POS	POS	1
52	3	12	PT2018EBLSERPS6	POS	POS	1
53	3	13	PT2018EBLSERNS2	NEG	NEG	1
54	3	14	PT2018EBLSERPS5	POS	POS	1
55	3	15	PT2018EBLSERPS2	POS	POS	1
56	3	16	PT2018EBLSERPS3	POS	POS	1
57	3	17	PT2018EBLSERPS1	POS	POS	1
58	3	18	PT2018EBLSERPS4	POS	POS	1
59	3	19	PT2018EBLSERPS5	POS	POS	1
60	3	20	PT2018EBLSERPS7	POS	POS	1
61	4	1	PT2018EBLSERNS2	NEG	NEG	1
62	4	2	PT2018EBLSERPS6	POS	POS	1
63	4	3	PT2018EBLSERPS2	POS	POS	1
64	4	4	PT2018EBLSERNS1	NEG	NEG	1
65	4	5	PT2018EBLSERPS4	POS	POS	1
66	4	6	PT2018EBLSERPS2	POS	POS	1
67	4	7	PT2018EBLSERPS5	POS	POS	1
68	4	8	PT2018EBLSERNS2	NEG	NEG	1
69	4	9	PT2018EBLSERPS5	POS	POS	1
70	4	10	PT2018EBLSERPS4	POS	POS	1
71	4	11	PT2018EBLSERNS2	NEG	NEG	1
72	4	12	PT2018EBLSERPS7	POS	POS	1
73	4	13	PT2018EBLSERPS3	POS	POS	1
74	4	14	PT2018EBLSERPS6	POS	POS	1
75	4	15	PT2018EBLSERPS5	POS	POS	1
76	4	16	PT2018EBLSERPS1	POS	POS	1
77	4	17	PT2018EBLSERPS7	POS	POS	1
78	4	18	PT2018EBLSERPS2	POS	POS	1
79	4	19	PT2018EBLSERPS6	POS	POS	1
80	4	20	PT2018EBLSERPS4	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2018EBLSERPS2	POS	POS	1
82	5	2	PT2018EBLSERNS2	NEG	NEG	1
83	5	3	PT2018EBLSERPS5	POS	POS	1
84	5	4	PT2018EBLSERPS6	POS	POS	1
85	5	5	PT2018EBLSERNS1	NEG	NEG	1
86	5	6	PT2018EBLSERPS4	POS	POS	1
87	5	7	PT2018EBLSERPS2	POS	POS	1
88	5	8	PT2018EBLSERPS7	POS	POS	1
89	5	9	PT2018EBLSERPS6	POS	POS	1
90	5	10	PT2018EBLSERNS2	NEG	NEG	1
91	5	11	PT2018EBLSERPS4	POS	POS	1
92	5	12	PT2018EBLSERPS6	POS	POS	1
93	5	13	PT2018EBLSERNS2	NEG	NEG	1
94	5	14	PT2018EBLSERPS5	POS	POS	1
95	5	15	PT2018EBLSERPS2	POS	POS	1
96	5	16	PT2018EBLSERPS3	POS	POS	1
97	5	17	PT2018EBLSERPS1	POS	POS	1
98	5	18	PT2018EBLSERPS4	POS	POS	1
99	5	19	PT2018EBLSERPS5	POS	POS	1
100	5	20	PT2018EBLSERPS7	POS	POS	1
101	6	1	PT2018EBLSERNS2	NEG	NEG	1
102	6	2	PT2018EBLSERPS6	POS	POS	1
103	6	3	PT2018EBLSERPS2	POS	POS	1
104	6	4	PT2018EBLSERNS1	NEG	NEG	1
105	6	5	PT2018EBLSERPS4	POS	POS	1
106	6	6	PT2018EBLSERPS2	POS	POS	1
107	6	7	PT2018EBLSERPS5	POS	POS	1
108	6	8	PT2018EBLSERNS2	NEG	NEG	1
109	6	9	PT2018EBLSERPS5	POS	POS	1
110	6	10	PT2018EBLSERPS4	POS	POS	1
111	6	11	PT2018EBLSERNS2	NEG	NEG	1
112	6	12	PT2018EBLSERPS7	POS	POS	1
113	6	13	PT2018EBLSERPS3	POS	POS	1
114	6	14	PT2018EBLSERPS6	POS	POS	1
115	6	15	PT2018EBLSERPS5	POS	POS	1
116	6	16	PT2018EBLSERPS1	POS	POS	1
117	6	17	PT2018EBLSERPS7	POS	POS	1
118	6	18	PT2018EBLSERPS2	POS	POS	1
119	6	19	PT2018EBLSERPS6	POS	POS	1
120	6	20	PT2018EBLSERPS4	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2018EBLSERPS2	POS	POS	1
122	7	2	PT2018EBLSERNS2	NEG	NEG	1
123	7	3	PT2018EBLSERPS5	POS	POS	1
124	7	4	PT2018EBLSERPS6	POS	POS	1
125	7	5	PT2018EBLSERNS1	NEG	NEG	1
126	7	6	PT2018EBLSERPS4	POS	POS	1
127	7	7	PT2018EBLSERPS2	POS	POS	1
128	7	8	PT2018EBLSERPS7	POS	POS	1
129	7	9	PT2018EBLSERPS6	POS	POS	1
130	7	10	PT2018EBLSERNS2	NEG	NEG	1
131	7	11	PT2018EBLSERPS4	POS	POS	1
132	7	12	PT2018EBLSERPS6	POS	POS	1
133	7	13	PT2018EBLSERNS2	NEG	NEG	1
134	7	14	PT2018EBLSERPS5	POS	POS	1
135	7	15	PT2018EBLSERPS2	POS	POS	1
136	7	16	PT2018EBLSERPS3	POS	POS	1
137	7	17	PT2018EBLSERPS1	POS	POS	1
138	7	18	PT2018EBLSERPS4	POS	POS	1
139	7	19	PT2018EBLSERPS5	POS	POS	1
140	7	20	PT2018EBLSERPS7	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performance of the participating laboratories when analyzing reference serum samples of bovine origin for the detection of EBL-specific antibodies by ELISA.

For the detection of EBL-specific antibodies 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).

LAB1, LAB2, LAB3, LAB5, LAB6, and LAB7 used EBL antibody ELISA kits from 2 different commercial producers : Zoetis and Idexx. LAB4 used a home-made EBL antibody ELISA kit. Also different batches from the same ELISA kit were used. Hereby, Zoetis batch 17ZEAF004 and batch 18ZEAF005.

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 serum samples assigned by the EBL reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of EBL-specific antibodies in reference serum samples by ELISA.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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)

Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE) (Maisons-Alfort, France)

Sciensano (Ukkel, Belgium)

State Veterinary and Food Institute, Veterinyry Institute (Zvolen, Slovakia)

Zoetis France (Lyon, France)

Annex 1: Quantitative data analysis (Box plots)

Besides qualitative data analysis (positive or negative 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.

<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>

The quantitative data analyse was performed on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory (Figure 1). The samples PT2018EBLSERNS1, PT2018EBLSERPS1 and PT2018EBLSERPS3 are not included in the figure because there was only one aliquot in the panel for these samples.

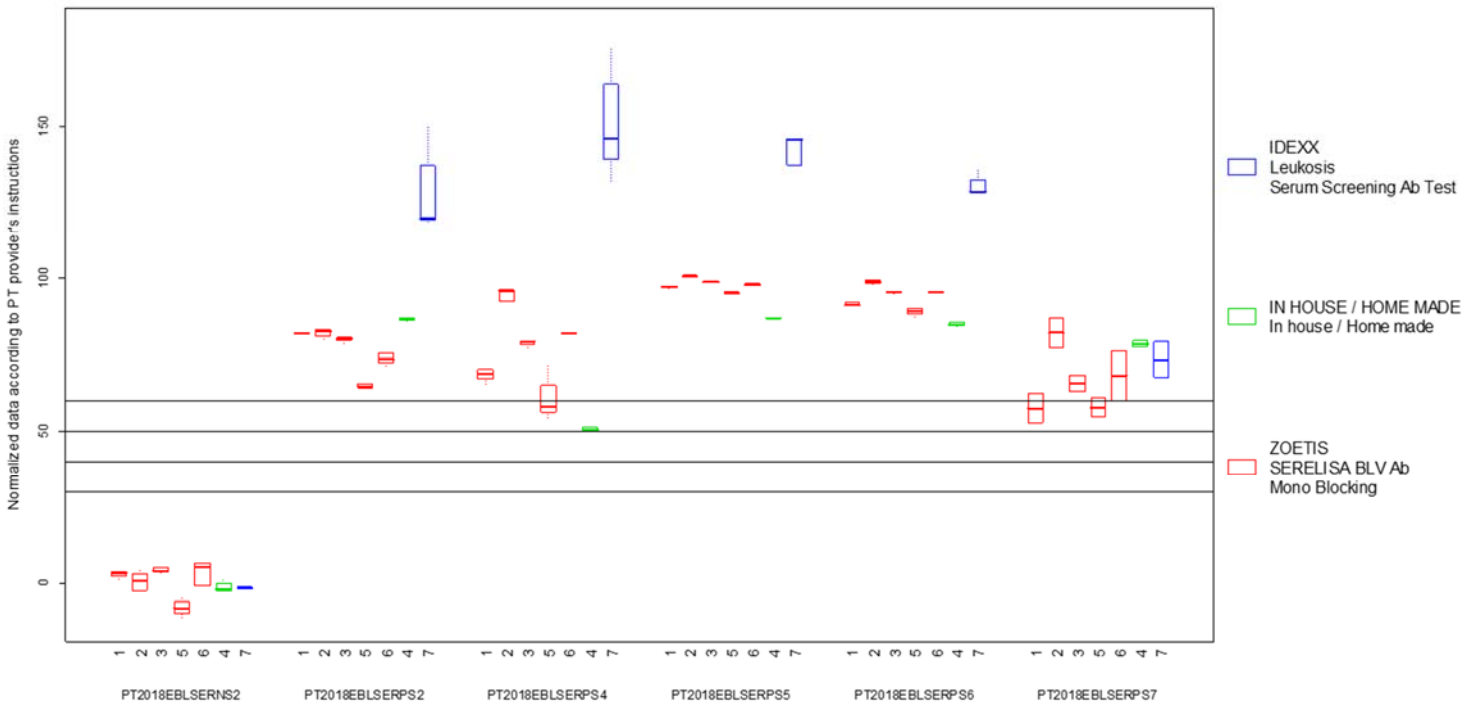

Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory. LAB1, LAB2, LAB3, LAB5, LAB6, and LAB7 used EBL antibody ELISA kits from 2 different commercial producers : Zoetis and Idexx. LAB4 used a home-made EBL antibody ELISA kit. Cut-off values (Zoetis 30-50, Home Made 40-50 and Idexx 60) are shown by horizontal lines.