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172-PT

PROFICIENCY TESTING 2016

Enzootic Bovine Leukosis (EBL)

Detection of EBL-specific antibodies in bovine serum by Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 18 APRIL 2016

DATE REPORT: 5 JULY 2016

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 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 tested by means of an EBL antibody 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 6 reference serum samples of bovine origin, either free from detectable EBL-specific antibodies (n=2; coded 'PT2016EBLSERNS1' and 'PT2016EBLSERNS2') or containing detectable EBL-specific antibodies (n=4; coded 'PT2016EBLSERPS1', 'PT2016EBLSERPS2', 'PT2016EBLSERPS3' and 'PT2016EBLSERPS4'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2016EBLSERNS1, PT2016EBLSERNS2, PT2016EBLSERPS1 and PT2016EBLSERPS3 and 4 aliquots of the reference serum samples PT2016EBLSERPS2 and PT2016EBLSERPS4. 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.
The reference serum samples PT2016EBLSERNS1 and PT2016EBLSERNS2 were obtained from the field (EBL negative herds). The reference serum samples PT2016EBLSERPS1, 'PT2016EBLSERPS2, PT2016EBLSERPS3 and PT2016EBLSERPS4 were derived from 3 different animals that were experimentally infected with EBL. The reference serum samples PT2016EBLSERPS1 and PT2016EBLSERPS2 were 1/2, respectively 1/32 dilutions in a negative field sample obtained from an EBL negative herd. The reference serum sample PT2016EBLSERPS3 was a 1/2 dilution in the same negative field sample obtained from an EBL negative herd. For each reference serum sample, the same qualitative result was obtained with both test methods used. Taken together, the reference serum samples PT2016EBLSERNS1 and PT2016EBLSERNS2 were considered as negative sera, and the reference serum samples PT2016EBLSERPS1, PT2016EBLSERPS2, PT2016EBLSERPS3 and PT2016EBLSERPS4 as 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, 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.

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 CODA-CERVA-Uccle.

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 April 2016 (140 aliquots in total). LAB1, LAB2, LAB4 and LAB5 acknowledged receipt of the samples on the same day, whereas LAB3 and LAB6 acknowledged receipt of the samples on 19th of April 2016 and LAB7 acknowledged receipt of the samples on 20th of April 2016. Analyses were performed between 18th and the 29th of April 2016 (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 19th of April and 9th of May 2016. All participants hereby respected the deadline of 9th of May 2016 for submission of the results (Table 1).

Table 1. Overview of the dates on which (i) the reference serum 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	Submission of the results (Excel file)
LAB1	18/04/2016	18/04/2016	19/04/2016
LAB2	18/04/2016	26/04/2016	02/05/2016
LAB3	19/04/2016	21/04/2016	04/05/2016
LAB4	18/04/2016	21/04/2016	09/05/2016
LAB5	18/04/2016	29/04/2016	02/05/2016
LAB6	19/04/2016	28/04/2016	02/05/2016
LAB7	20/04/2016	29/04/2016	03/05/2016

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 results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the EBL 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**	**5**	**6**	**7**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

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 identification of the reference serum samples (SAMPLE), the positions of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by the EBL reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016EBLSERPS1	POS	POS	1
2	1	2	PT2016EBLSERPS2	POS	POS	1
3	1	3	PT2016EBLSERNS1	NEG	NEG	1
4	1	4	PT2016EBLSERNS2	NEG	NEG	1
5	1	5	PT2016EBLSERPS3	POS	POS	1
6	1	6	PT2016EBLSERPS1	POS	POS	1
7	1	7	PT2016EBLSERPS4	POS	POS	1
8	1	8	PT2016EBLSERPS3	POS	POS	1
9	1	9	PT2016EBLSERPS2	POS	POS	1
10	1	10	PT2016EBLSERNS1	NEG	NEG	1
11	1	11	PT2016EBLSERPS3	POS	POS	1
12	1	12	PT2016EBLSERPS4	POS	POS	1
13	1	13	PT2016EBLSERNS2	NEG	NEG	1
14	1	14	PT2016EBLSERPS4	POS	POS	1
15	1	15	PT2016EBLSERPS4	POS	POS	1
16	1	16	PT2016EBLSERPS2	POS	POS	1
17	1	17	PT2016EBLSERPS1	POS	POS	1
18	1	18	PT2016EBLSERPS2	POS	POS	1
19	1	19	PT2016EBLSERNS2	NEG	NEG	1
20	1	20	PT2016EBLSERNS1	NEG	NEG	1
21	2	1	PT2016EBLSERPS2	POS	POS	1
22	2	2	PT2016EBLSERNS1	NEG	NEG	1
23	2	3	PT2016EBLSERPS3	POS	POS	1
24	2	4	PT2016EBLSERPS4	POS	POS	1
25	2	5	PT2016EBLSERNS2	NEG	NEG	1
26	2	6	PT2016EBLSERPS2	POS	POS	1
27	2	7	PT2016EBLSERNS1	NEG	NEG	1
28	2	8	PT2016EBLSERNS2	NEG	NEG	1
29	2	9	PT2016EBLSERPS1	POS	POS	1
30	2	10	PT2016EBLSERPS4	POS	POS	1
31	2	11	PT2016EBLSERPS4	POS	POS	1
32	2	12	PT2016EBLSERPS3	POS	POS	1
33	2	13	PT2016EBLSERPS2	POS	POS	1
34	2	14	PT2016EBLSERPS1	POS	POS	1
35	2	15	PT2016EBLSERPS4	POS	POS	1
36	2	16	PT2016EBLSERNS1	NEG	NEG	1
37	2	17	PT2016EBLSERPS2	POS	POS	1
38	2	18	PT2016EBLSERNS2	NEG	NEG	1
39	2	19	PT2016EBLSERPS3	POS	POS	1
40	2	20	PT2016EBLSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2016EBLSERPS1	POS	POS	1
42	3	2	PT2016EBLSERPS2	POS	POS	1
43	3	3	PT2016EBLSERNS1	NEG	NEG	1
44	3	4	PT2016EBLSERNS2	NEG	NEG	1
45	3	5	PT2016EBLSERPS3	POS	POS	1
46	3	6	PT2016EBLSERPS1	POS	POS	1
47	3	7	PT2016EBLSERPS4	POS	POS	1
48	3	8	PT2016EBLSERPS3	POS	POS	1
49	3	9	PT2016EBLSERPS2	POS	POS	1
50	3	10	PT2016EBLSERNS1	NEG	NEG	1
51	3	11	PT2016EBLSERPS3	POS	POS	1
52	3	12	PT2016EBLSERPS4	POS	POS	1
53	3	13	PT2016EBLSERNS2	NEG	NEG	1
54	3	14	PT2016EBLSERPS4	POS	POS	1
55	3	15	PT2016EBLSERPS4	POS	POS	1
56	3	16	PT2016EBLSERPS2	POS	POS	1
57	3	17	PT2016EBLSERPS1	POS	POS	1
58	3	18	PT2016EBLSERPS2	POS	POS	1
59	3	19	PT2016EBLSERNS2	NEG	NEG	1
60	3	20	PT2016EBLSERNS1	NEG	NEG	1
61	4	1	PT2016EBLSERPS2	POS	POS	1
62	4	2	PT2016EBLSERNS1	NEG	NEG	1
63	4	3	PT2016EBLSERPS3	POS	POS	1
64	4	4	PT2016EBLSERPS4	POS	POS	1
65	4	5	PT2016EBLSERNS2	NEG	NEG	1
66	4	6	PT2016EBLSERPS2	POS	POS	1
67	4	7	PT2016EBLSERNS1	NEG	NEG	1
68	4	8	PT2016EBLSERNS2	NEG	NEG	1
69	4	9	PT2016EBLSERPS1	POS	POS	1
70	4	10	PT2016EBLSERPS4	POS	POS	1
71	4	11	PT2016EBLSERPS4	POS	POS	1
72	4	12	PT2016EBLSERPS3	POS	POS	1
73	4	13	PT2016EBLSERPS2	POS	POS	1
74	4	14	PT2016EBLSERPS1	POS	POS	1
75	4	15	PT2016EBLSERPS4	POS	POS	1
76	4	16	PT2016EBLSERNS1	NEG	NEG	1
77	4	17	PT2016EBLSERPS2	POS	POS	1
78	4	18	PT2016EBLSERNS2	NEG	NEG	1
79	4	19	PT2016EBLSERPS3	POS	POS	1
80	4	20	PT2016EBLSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2016EBLSERPS1	POS	POS	1
82	5	2	PT2016EBLSERPS2	POS	POS	1
83	5	3	PT2016EBLSERNS1	NEG	NEG	1
84	5	4	PT2016EBLSERNS2	NEG	NEG	1
85	5	5	PT2016EBLSERPS3	POS	POS	1
86	5	6	PT2016EBLSERPS1	POS	POS	1
87	5	7	PT2016EBLSERPS4	POS	POS	1
88	5	8	PT2016EBLSERPS3	POS	POS	1
89	5	9	PT2016EBLSERPS2	POS	POS	1
90	5	10	PT2016EBLSERNS1	NEG	NEG	1
91	5	11	PT2016EBLSERPS3	POS	POS	1
92	5	12	PT2016EBLSERPS4	POS	POS	1
93	5	13	PT2016EBLSERNS2	NEG	NEG	1
94	5	14	PT2016EBLSERPS4	POS	POS	1
95	5	15	PT2016EBLSERPS4	POS	POS	1
96	5	16	PT2016EBLSERPS2	POS	POS	1
97	5	17	PT2016EBLSERPS1	POS	POS	1
98	5	18	PT2016EBLSERPS2	POS	POS	1
99	5	19	PT2016EBLSERNS2	NEG	NEG	1
100	5	20	PT2016EBLSERNS1	NEG	NEG	1
101	6	1	PT2016EBLSERPS2	POS	POS	1
102	6	2	PT2016EBLSERNS1	NEG	NEG	1
103	6	3	PT2016EBLSERPS3	POS	POS	1
104	6	4	PT2016EBLSERPS4	POS	POS	1
105	6	5	PT2016EBLSERNS2	NEG	NEG	1
106	6	6	PT2016EBLSERPS2	POS	POS	1
107	6	7	PT2016EBLSERNS1	NEG	NEG	1
108	6	8	PT2016EBLSERNS2	NEG	NEG	1
109	6	9	PT2016EBLSERPS1	POS	POS	1
110	6	10	PT2016EBLSERPS4	POS	POS	1
111	6	11	PT2016EBLSERPS4	POS	POS	1
112	6	12	PT2016EBLSERPS3	POS	POS	1
113	6	13	PT2016EBLSERPS2	POS	POS	1
114	6	14	PT2016EBLSERPS1	POS	POS	1
115	6	15	PT2016EBLSERPS4	POS	POS	1
116	6	16	PT2016EBLSERNS1	NEG	NEG	1
117	6	17	PT2016EBLSERPS2	POS	POS	1
118	6	18	PT2016EBLSERNS2	NEG	NEG	1
119	6	19	PT2016EBLSERPS3	POS	POS	1
120	6	20	PT2016EBLSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2016EBLSERPS1	POS	POS	1
122	7	2	PT2016EBLSERPS2	POS	POS	1
123	7	3	PT2016EBLSERNS1	NEG	NEG	1
124	7	4	PT2016EBLSERNS2	NEG	NEG	1
125	7	5	PT2016EBLSERPS3	POS	POS	1
126	7	6	PT2016EBLSERPS1	POS	POS	1
127	7	7	PT2016EBLSERPS4	POS	POS	1
128	7	8	PT2016EBLSERPS3	POS	POS	1
129	7	9	PT2016EBLSERPS2	POS	POS	1
130	7	10	PT2016EBLSERNS1	NEG	NEG	1
131	7	11	PT2016EBLSERPS3	POS	POS	1
132	7	12	PT2016EBLSERPS4	POS	POS	1
133	7	13	PT2016EBLSERNS2	NEG	NEG	1
134	7	14	PT2016EBLSERPS4	POS	POS	1
135	7	15	PT2016EBLSERPS4	POS	POS	1
136	7	16	PT2016EBLSERPS2	POS	POS	1
137	7	17	PT2016EBLSERPS1	POS	POS	1
138	7	18	PT2016EBLSERPS2	POS	POS	1
139	7	19	PT2016EBLSERNS2	NEG	NEG	1
140	7	20	PT2016EBLSERNS1	NEG	NEG	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 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).
LAB3 used the Leukosis serum screening kit from IDEXX (batch: 5086), LAB7 used a home-made EBL Ab ELISA kit (batch: 06/04/2016), whereas the other participants used the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics (LAB1, LAB2, LAB5 and LAB6 batch: 13SBLV1128 and LAB4 batch : 13 SBLV 133). Hereby, all laboratories except LAB3 performed the long incubation protocol for the conjugate (LAB2 did not provide information about the used incubation protocol for the conjugate).

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 CODA-CERVA-Uccle (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)

Synbiotics Europe (Lyon, France)

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

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

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

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. All quantitative data analyses were performed on normalized data, namely the percentages blocking calculated according to the instructions of the PT provider: [(mean $OD_{Negative\ Kit\ Controls}$ - OD_{Sample}) / (mean $OD_{Negative\ Kit\ Controls}$ - mean $OD_{Positive\ Kit\ Controls}$)] * 100.

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

Box plots of the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

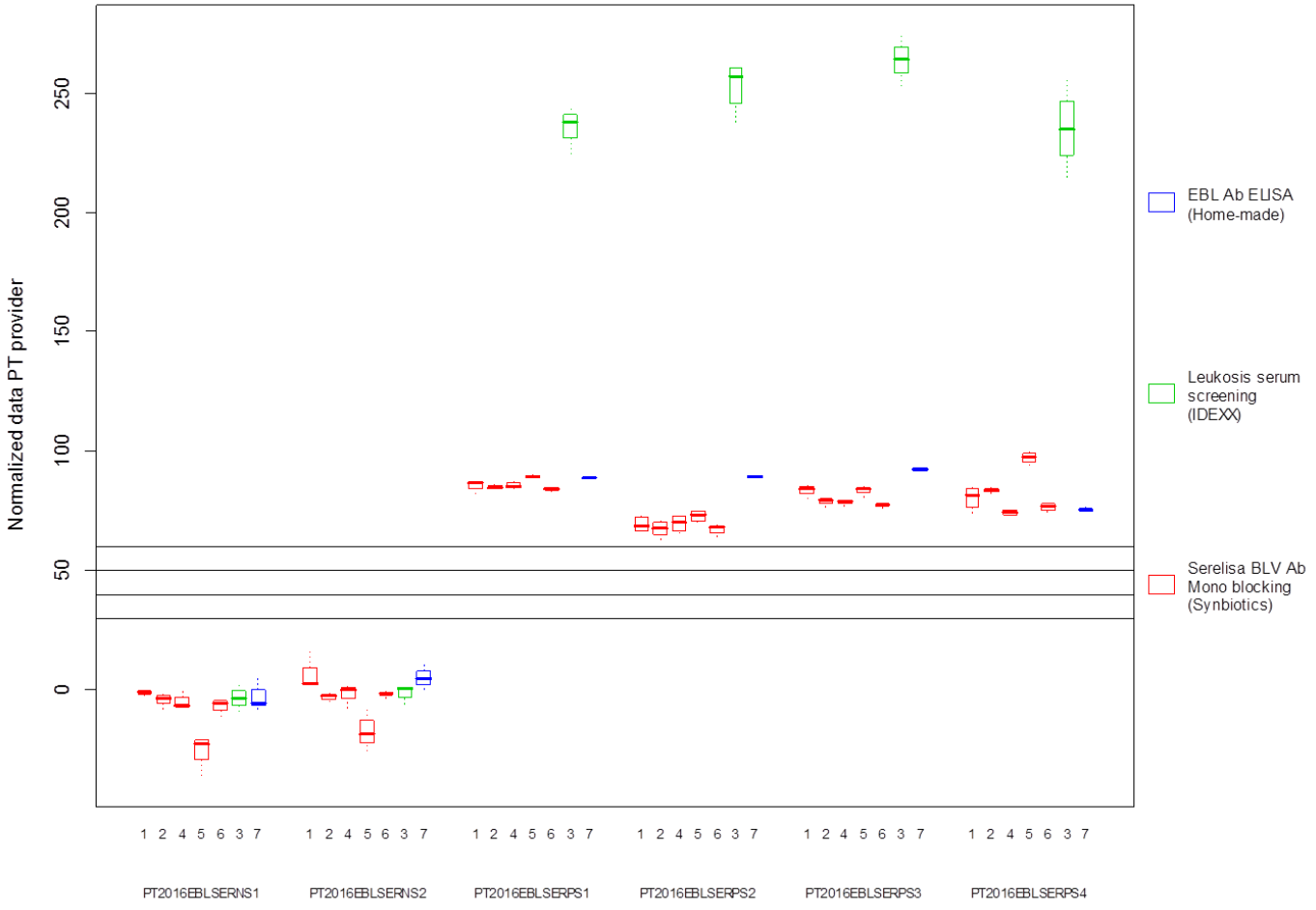

Figure 1. Box plots showing the percentage blocking calculated according to the instructions of the PT provider per reference serum sample and per participating laboratory.
Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively 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.
LAB1, LAB2, LAB4, LAB5 and LAB6 used the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics (red box plots) (LAB1, LAB2, LAB5 and LAB6 batch: 13SBLV1128 and LAB4 batch: 13 SBLV 133). LAB3 used the Leukosis serum screening kit from IDEXX batch: 5086 (green box plots). LAB7 used a home-made EBL Ab ELISA kit batch: 06/04/2016 (blue box plots).
Cut-off values for the Leukosis serum screening kit from IDEXX (60%), the home-made EBL Ab ELISA kit (40-50%) and the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics (30-50%) are shown by horizontal lines.