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172-PT

PROFICIENCY TESTING 2013

Enzootic Bovine Leukosis (EBL)

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

OPERATIONAL UNIT
COORDINATION OF VETERINARY DIAGNOSIS
EPIDEMIOLOGY AND RISK ASSESSMENT
(CVD-ERA)

DATE BEGIN PT: 21 OCTOBER 2013
DATE REPORT: 25 FEBRUARY 2014

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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 ‘PT2013EBLSERNS1’ and ‘PT2013EBLSERNS2’) or containing detectable EBL-specific antibodies (n=4; coded ‘PT2013EBLSERPS1’, ‘PT2013EBLSERPS2’, ‘PT2013EBLSERPS3’ and ‘PT2013EBLSERPS4’), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2013EBLSERPS1, PT2013EBLSERPS2, PT2013EBLSERPS3 and PT2013EBLSERPS4, and 4 aliquots of the reference serum samples PT2013EBLSERNS1 and PT2013EBLSERNS2. 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 PT2013EBLSERNS1 and PT2013EBLSERNS2 were obtained from EBL-free animals. In contrast, the reference serum samples PT2013EBLSERPS1 and PT2013EBLSERPS4 were derived from 2 different animals that were experimentally infected with EBL. Hereby, the reference serum sample PT2013EBLSERPS1 was a 1/2 dilution of the original serum. The reference serum samples PT2013EBLSERPS2 and PT2013EBLSERPS3 were derived from an animal that was EBL antibody positive upon birth and stayed EBL antibody positive during its entire life. Hereby, reference serum sample PT2013EBLSERPS3 was a 1/2 dilution of the original serum (=PT2013EBLSERPS2). For each reference serum sample, the same qualitative result was obtained with both test methods used. Taken together, the reference serum samples PT2013EBLSERNS1 and PT2013EBLSERNS2 were considered as negative sera, and the reference serum samples PT2013EBLSERPS1, PT2013EBLSERPS2, PT2013EBLSERPS3 and PT2013EBLSERPS4 as variably 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.

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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 operational unit CVD-ERA of CODA-CERVA.

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 6 participating laboratories by national or international courier on 21st of October 2013 (120 aliquots in total). LAB3, LAB4, LAB5 and LAB6 acknowledged receipt of the samples on the same day, whereas LAB1 and LAB2 acknowledged receipt of the samples on 22nd and 23rd of October 2013, respectively. Analyses were performed between 22nd of October and 5th of November 2013 (Table 1).

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results were submitted to the operational unit CVD-ERA between 24th of October and 8th of November 2013. All participants hereby respected the deadline of 8th of November 2013 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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	22/10/2013	28/10/2013	29/10/2013
LAB2	23/10/2013	05/11/2013	08/11/2013
LAB3	21/10/2013	23/10/2013	24/10/2013
LAB4	21/10/2013	04/11/2013	05/11/2013 (*)
LAB5	21/10/2013	23/10/2013	07/11/2013
LAB6	21/10/2013	22/10/2013	24/10/2013

Legend: (*) LAB4 sent a corrected version on 13/11/2013

IV.3. Compliance with the procedure

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

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IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that 4 out of 6 participating laboratories (LAB2, LAB3, LAB4 and LAB6) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement), whereas LAB1 misclassified 5 aliquots (75% of agreement) and LAB5 misclassified 1 aliquot (95% of agreement) of reference serum samples (Table 2).
A quantitative data analysis (including box plots) is shown for educational purposes in Annex 1 and Annex 2.

Table 2. Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by CODA-CERVA. 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**
failure	5 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)
success	**15 (75.0)**	20 (100.0)	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB2, LAB3, LAB4 and LAB6 since these participants correctly identified all reference serum samples. In contrast, LAB1 misclassified all 3 aliquots of the positive reference serum sample PT2013EBLSERPS3 (3x NI instead of POS) and 2 out of 3 aliquots of the positive reference serum sample PT2013EBLSERPS4 (2x NI instead of POS), whereas LAB5 misclassified 1 out of 3 aliquots of the positive reference serum sample PT2013EBLSERPS3 (NI instead of POS).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 3.

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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 (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013EBLSERPS2	POS	POS	1
2	1	2	PT2013EBLSERNS1	NEG	NEG	1
3	1	3	PT2013EBLSERPS1	POS	POS	1
4	1	4	PT2013EBLSERPS3	**POS**	**NI**	**0**
5	1	5	PT2013EBLSERNS2	NEG	NEG	1
6	1	6	PT2013EBLSERPS4	**POS**	**NI**	**0**
7	1	7	PT2013EBLSERPS2	POS	POS	1
8	1	8	PT2013EBLSERNS1	NEG	NEG	1
9	1	9	PT2013EBLSERNS2	NEG	NEG	1
10	1	10	PT2013EBLSERPS1	POS	POS	1
11	1	11	PT2013EBLSERPS4	POS	POS	1
12	1	12	PT2013EBLSERNS2	NEG	NEG	1
13	1	13	PT2013EBLSERPS3	**POS**	**NI**	**0**
14	1	14	PT2013EBLSERNS1	NEG	NEG	1
15	1	15	PT2013EBLSERPS4	**POS**	**NI**	**0**
16	1	16	PT2013EBLSERPS2	POS	POS	1
17	1	17	PT2013EBLSERNS1	NEG	NEG	1
18	1	18	PT2013EBLSERPS3	**POS**	**NI**	**0**
19	1	19	PT2013EBLSERPS1	POS	POS	1
20	1	20	PT2013EBLSERNS2	NEG	NEG	1
21	2	1	PT2013EBLSERNS2	NEG	NEG	1
22	2	2	PT2013EBLSERPS4	POS	POS	1
23	2	3	PT2013EBLSERPS2	POS	POS	1
24	2	4	PT2013EBLSERNS1	NEG	NEG	1
25	2	5	PT2013EBLSERNS2	NEG	NEG	1
26	2	6	PT2013EBLSERPS1	POS	POS	1
27	2	7	PT2013EBLSERPS4	POS	POS	1
28	2	8	PT2013EBLSERNS2	NEG	NEG	1
29	2	9	PT2013EBLSERPS3	POS	POS	1
30	2	10	PT2013EBLSERNS1	NEG	NEG	1
31	2	11	PT2013EBLSERPS4	POS	POS	1
32	2	12	PT2013EBLSERPS2	POS	POS	1
33	2	13	PT2013EBLSERNS1	NEG	NEG	1
34	2	14	PT2013EBLSERPS3	POS	POS	1
35	2	15	PT2013EBLSERPS1	POS	POS	1
36	2	16	PT2013EBLSERNS2	NEG	NEG	1
37	2	17	PT2013EBLSERPS2	POS	POS	1
38	2	18	PT2013EBLSERNS1	NEG	NEG	1
39	2	19	PT2013EBLSERPS1	POS	POS	1
40	2	20	PT2013EBLSERPS3	POS	POS	1

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(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013EBLSERPS2	POS	POS	1
42	3	2	PT2013EBLSERNS1	NEG	NEG	1
43	3	3	PT2013EBLSERPS1	POS	POS	1
44	3	4	PT2013EBLSERPS3	POS	POS	1
45	3	5	PT2013EBLSERNS2	NEG	NEG	1
46	3	6	PT2013EBLSERPS4	POS	POS	1
47	3	7	PT2013EBLSERPS2	POS	POS	1
48	3	8	PT2013EBLSERNS1	NEG	NEG	1
49	3	9	PT2013EBLSERNS2	NEG	NEG	1
50	3	10	PT2013EBLSERPS1	POS	POS	1
51	3	11	PT2013EBLSERPS4	POS	POS	1
52	3	12	PT2013EBLSERNS2	NEG	NEG	1
53	3	13	PT2013EBLSERPS3	POS	POS	1
54	3	14	PT2013EBLSERNS1	NEG	NEG	1
55	3	15	PT2013EBLSERPS4	POS	POS	1
56	3	16	PT2013EBLSERPS2	POS	POS	1
57	3	17	PT2013EBLSERNS1	NEG	NEG	1
58	3	18	PT2013EBLSERPS3	POS	POS	1
59	3	19	PT2013EBLSERPS1	POS	POS	1
60	3	20	PT2013EBLSERNS2	NEG	NEG	1
61	4	1	PT2013EBLSERNS2	NEG	NEG	1
62	4	2	PT2013EBLSERPS4	POS	POS	1
63	4	3	PT2013EBLSERPS2	POS	POS	1
64	4	4	PT2013EBLSERNS1	NEG	NEG	1
65	4	5	PT2013EBLSERNS2	NEG	NEG	1
66	4	6	PT2013EBLSERPS1	POS	POS	1
67	4	7	PT2013EBLSERPS4	POS	POS	1
68	4	8	PT2013EBLSERNS2	NEG	NEG	1
69	4	9	PT2013EBLSERPS3	POS	POS	1
70	4	10	PT2013EBLSERNS1	NEG	NEG	1
71	4	11	PT2013EBLSERPS4	POS	POS	1
72	4	12	PT2013EBLSERPS2	POS	POS	1
73	4	13	PT2013EBLSERNS1	NEG	NEG	1
74	4	14	PT2013EBLSERPS3	POS	POS	1
75	4	15	PT2013EBLSERPS1	POS	POS	1
76	4	16	PT2013EBLSERNS2	NEG	NEG	1
77	4	17	PT2013EBLSERPS2	POS	POS	1
78	4	18	PT2013EBLSERNS1	NEG	NEG	1
79	4	19	PT2013EBLSERPS1	POS	POS	1
80	4	20	PT2013EBLSERPS3	POS	POS	1

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(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2013EBLSERPS2	POS	POS	1
82	5	2	PT2013EBLSERNS1	NEG	NEG	1
83	5	3	PT2013EBLSERPS1	POS	POS	1
84	5	4	PT2013EBLSERPS3	POS	POS	1
85	5	5	PT2013EBLSERNS2	NEG	NEG	1
86	5	6	PT2013EBLSERPS4	POS	POS	1
87	5	7	PT2013EBLSERPS2	POS	POS	1
88	5	8	PT2013EBLSERNS1	NEG	NEG	1
89	5	9	PT2013EBLSERNS2	NEG	NEG	1
90	5	10	PT2013EBLSERPS1	POS	POS	1
91	5	11	PT2013EBLSERPS4	POS	POS	1
92	5	12	PT2013EBLSERNS2	NEG	NEG	1
93	5	13	PT2013EBLSERPS3	POS	POS	1
94	5	14	PT2013EBLSERNS1	NEG	NEG	1
95	5	15	PT2013EBLSERPS4	POS	POS	1
96	5	16	PT2013EBLSERPS2	POS	POS	1
97	5	17	PT2013EBLSERNS1	NEG	NEG	1
98	5	18	PT2013EBLSERPS3	**POS**	**NI**	**0**
99	5	19	PT2013EBLSERPS1	POS	POS	1
100	5	20	PT2013EBLSERNS2	NEG	NEG	1
101	6	1	PT2013EBLSERNS2	NEG	NEG	1
102	6	2	PT2013EBLSERPS4	POS	POS	1
103	6	3	PT2013EBLSERPS2	POS	POS	1
104	6	4	PT2013EBLSERNS1	NEG	NEG	1
105	6	5	PT2013EBLSERNS2	NEG	NEG	1
106	6	6	PT2013EBLSERPS1	POS	POS	1
107	6	7	PT2013EBLSERPS4	POS	POS	1
108	6	8	PT2013EBLSERNS2	NEG	NEG	1
109	6	9	PT2013EBLSERPS3	POS	POS	1
110	6	10	PT2013EBLSERNS1	NEG	NEG	1
111	6	11	PT2013EBLSERPS4	POS	POS	1
112	6	12	PT2013EBLSERPS2	POS	POS	1
113	6	13	PT2013EBLSERNS1	NEG	NEG	1
114	6	14	PT2013EBLSERPS3	POS	POS	1
115	6	15	PT2013EBLSERPS1	POS	POS	1
116	6	16	PT2013EBLSERNS2	NEG	NEG	1
117	6	17	PT2013EBLSERPS2	POS	POS	1
118	6	18	PT2013EBLSERNS1	NEG	NEG	1
119	6	19	PT2013EBLSERPS1	POS	POS	1
120	6	20	PT2013EBLSERPS3	POS	POS	1

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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, 4 out of 6 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement): LAB2, LAB3, LAB4 and LAB6. In contrast, LAB1 misclassified 3 aliquots of the positive reference serum sample PT2013EBLSERPS3 and 2 aliquots of the positive reference serum sample PT2013EBLSERPS4 (75% of agreement), whereas LAB5 misclassified 1 aliquot of the positive reference serum sample PT2013EBLSERPS3 (95% of agreement) (Table 2 and Table 3).

LAB2 used an in-house developed EBL antibody ELISA kit, whereas the other participants used the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics Europe (2 batches: 12SBLV1117, 12SBLV1118). Hereby, LAB3, LAB4, LAB5 and LAB6 used the same batch and at least LAB1, LAB3, LAB4 and LAB6 performed the long incubation protocol for the conjugate (LAB5 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 (see III.3.3.). Consequently, 5 out of 6 participants achieved a satisfactory performance for the detection of EBL-specific antibodies in reference serum samples by ELISA. Hereby, LAB1 did not reach the required 90% of agreement.

Head CVD-ERA
Yves Van der Stede

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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)
State Veterinary Institute Zvolen (Zvolen, Slovakia)
Synbiotics Europe (Lyon, France)
Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)

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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 programs R (box plots) and SAS 9.2 (summary statistics). 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.

I. Box plots

Box plots of the percentages blocking per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

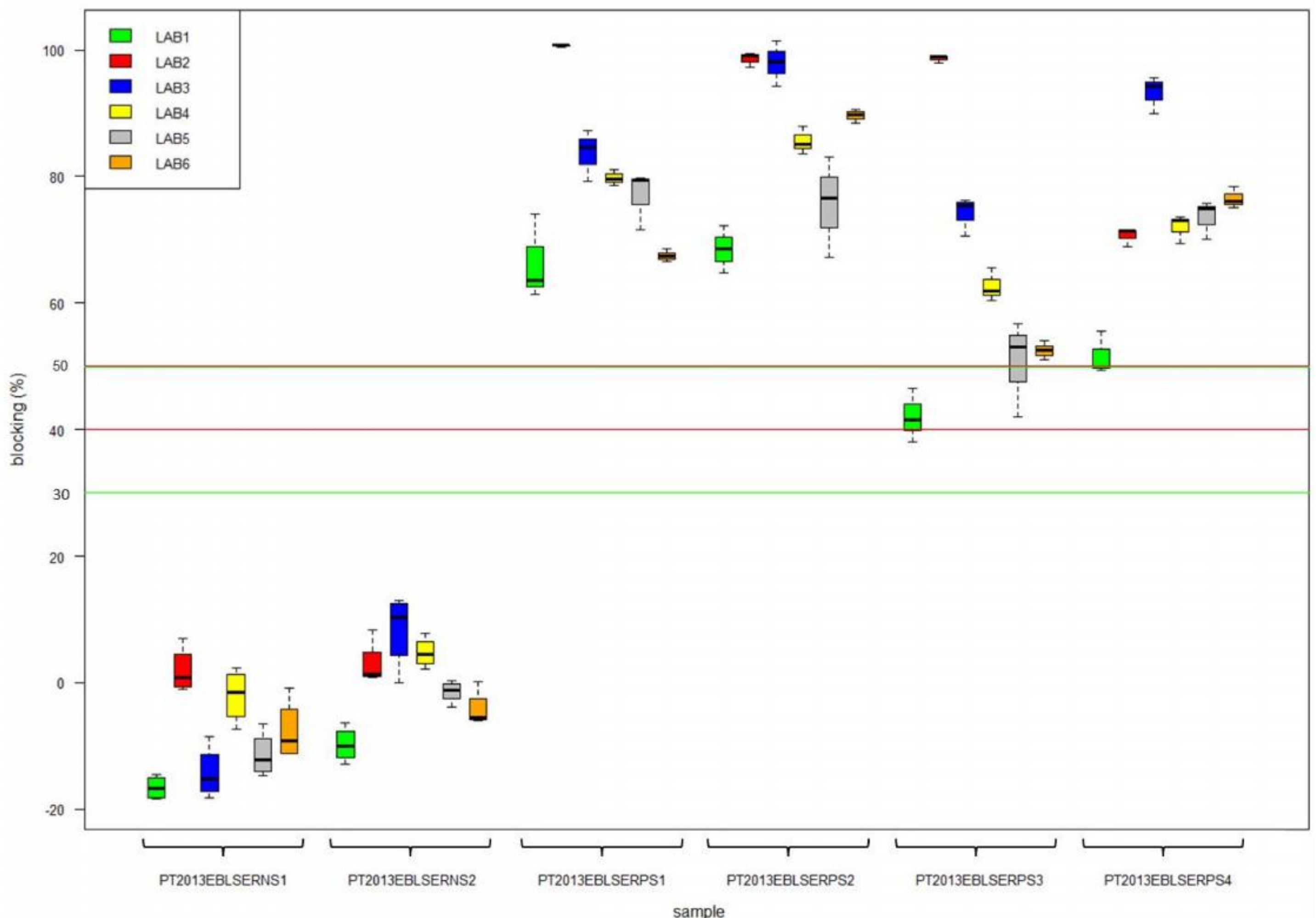

Figure 1. Box plots showing the percentage blocking per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. LAB2 used an in-house developed EBL antibody ELISA kit, whereas the other participants used the same commercial ELISA kit. Hereby, LAB3, LAB4, LAB5 and LAB6 used the same batch and at least LAB1, LAB3, LAB4 and LAB6 performed the long incubation protocol for the conjugate. Cut-off values for the in-house developed EBL antibody ELISA kit (40-50%) and the EBL antibody ELISA kit from Synbiotics Europe (30-50%) are shown in red and green, respectively.

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II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-laboratory variability (reproducibility) and within-laboratory variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated per reference serum sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,40	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic for this PT is 1,66 (p=6), whereas the maximum value for Mandel's k-statistic is 1,64 for the reference serum samples PT2013EBLSERPS1, PT2013EBLSERPS2, PT2013EBLSERPS3 and PT2013EBLSERPS4 (p=6 and n=3) and 1,54 for the reference serum samples PT2013EBLSERNS1 and PT2013EBLSERNS2 (p=6 and n=4).

Five out of 6 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB2, which showed increased values for Mandel's h-statistic for the positive reference serum samples PT2013EBLSERPS1 (h=1,71) and PT2013EBLSERPS3 (h=1,72). Noteworthy, LAB2 used an in-house developed EBL antibody ELISA kit, whereas the other participants used the SERELISA BLV Ab Mono Blocking ELISA kit from Synbiotics Europe (2 batches).

Three out of 6 participating laboratories (LAB2, LAB4 and LAB6) obtained a satisfactory within-laboratory consistency for all reference serum samples, whereas the other participants showed increased values for Mandel's k-statistic for at least 1 reference serum sample: LAB1 for the positive reference serum sample PT2013EBLSERPS1 (k=1,78), LAB3 for the negative reference serum sample PT2013EBLSERNS2 (k=1,69) and LAB5 for the positive reference serum samples PT2013EBLSERPS2 (k=1,97) and PT2013EBLSERPS3 (k=1,92).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

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III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values as a dependent variable, it was investigated whether statistically significant differences exist (=0,05) between participating laboratories. Comparisons were made at the global level (all reference serum samples were analysed together), status level (all reference serum samples with the same status were analysed together) and sample level (all reference serum samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

No statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.
At the status level, significant differences were observed for both the negative and positive reference serum samples. For the negative reference serum samples, LAB1 reported percentages blocking that were significantly lower than those reported by LAB2 and LAB4. For the positive reference serum samples, LAB1 reported percentages blocking that were significantly lower than those reported by LAB2, LAB3 and LAB4, whereas LAB2 and LAB3 reported percentages blocking that were significantly higher than those reported by LAB6, LAB5 and LAB1.

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Annex 2: Calculations of Mandel's h- and k-statistics (based on % blocking)

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	K	cv
PT2013EBLSERNS1	1	4	3,62	-16,58	-8,34	0,39	3,84	4,93	3,10	-1,15	0,50	-11,48
PT2013EBLSERNS1	2	4	13,28	1,93	-8,34	0,39	3,84	4,93	3,10	1,43	0,95	189,26
PT2013EBLSERNS1	3	4	17,01	-14,27	-8,34	0,39	3,84	4,93	3,10	-0,82	1,07	-28,90
PT2013EBLSERNS1	4	4	18,35	-2,04	-8,34	0,39	3,84	4,93	3,10	0,88	1,12	-209,79
PT2013EBLSERNS1	5	4	12,95	-11,45	-8,34	0,39	3,84	4,93	3,10	-0,43	0,94	-31,41
PT2013EBLSERNS1	6	4	23,31	-7,64	-8,34	0,39	3,84	4,93	3,10	0,10	1,26	-63,19
PT2013EBLSERNS2	1	4	7,76	-9,78	0,10	0,40	3,51	4,51	2,84	-1,50	0,79	-28,47
PT2013EBLSERNS2	2	4	13,16	2,91	0,10	0,40	3,51	4,51	2,84	0,43	1,03	124,56
PT2013EBLSERNS2	3	4	35,24	8,43	0,10	0,40	3,51	4,51	2,84	1,26	1,69	70,39
PT2013EBLSERNS2	4	4	5,78	4,73	0,10	0,40	3,51	4,51	2,84	0,70	0,69	50,85
PT2013EBLSERNS2	5	4	3,02	-1,42	0,10	0,40	3,51	4,51	2,84	-0,23	0,50	-122,47
PT2013EBLSERNS2	6	4	8,76	-4,24	0,10	0,40	3,51	4,51	2,84	-0,66	0,84	-69,72
PT2013EBLSERPS1	1	3	45,70	66,41	79,14	0,68	3,79	6,70	5,52	-1,02	1,78	10,18
PT2013EBLSERPS1	2	3	0,03	100,64	79,14	0,68	3,79	6,70	5,52	1,71	0,04	0,17
PT2013EBLSERPS1	3	3	16,51	83,64	79,14	0,68	3,79	6,70	5,52	0,36	1,07	4,86
PT2013EBLSERPS1	4	3	1,55	79,76	79,14	0,68	3,79	6,70	5,52	0,05	0,33	1,56
PT2013EBLSERPS1	5	3	21,45	76,91	79,14	0,68	3,79	6,70	5,52	-0,18	1,22	6,02
PT2013EBLSERPS1	6	3	0,97	67,46	79,14	0,68	3,79	6,70	5,52	-0,93	0,26	1,46
PT2013EBLSERPS2	1	3	14,19	68,51	85,98	0,63	4,04	6,65	5,29	-1,45	0,93	5,50
PT2013EBLSERPS2	2	3	1,44	98,58	85,98	0,63	4,04	6,65	5,29	1,05	0,30	1,22
PT2013EBLSERPS2	3	3	12,74	97,94	85,98	0,63	4,04	6,65	5,29	0,99	0,88	3,64
PT2013EBLSERPS2	4	3	4,82	85,55	85,98	0,63	4,04	6,65	5,29	-0,04	0,54	2,57
PT2013EBLSERPS2	5	3	63,20	75,66	85,98	0,63	4,04	6,65	5,29	-0,86	1,97	10,51
PT2013EBLSERPS2	6	3	1,34	89,62	85,98	0,63	4,04	6,65	5,29	0,30	0,29	1,29
PT2013EBLSERPS3	1	3	18,16	42,02	63,41	0,84	3,98	9,92	9,09	-1,05	1,07	10,14
PT2013EBLSERPS3	2	3	0,33	98,56	63,41	0,84	3,98	9,92	9,09	1,72	0,14	0,58

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Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	K	cv
PT2013EBLSERPS3	3	3	9,40	74,12	63,41	0,84	3,98	9,92	9,09	0,52	0,77	4,14
PT2013EBLSERPS3	4	3	6,73	62,65	63,41	0,84	3,98	9,92	9,09	-0,04	0,65	4,14
PT2013EBLSERPS3	**5**	3	58,34	50,59	63,41	0,84	3,98	9,92	9,09	-0,63	**1,92**	15,10
PT2013EBLSERPS3	6	3	2,21	52,52	63,41	0,84	3,98	9,92	9,09	-0,53	0,37	2,83
PT2013EBLSERPS4	1	3	11,43	51,60	72,92	0,84	2,58	6,45	5,92	-1,60	1,31	6,55
PT2013EBLSERPS4	2	3	2,27	70,61	72,92	0,84	2,58	6,45	5,92	-0,17	0,58	2,13
PT2013EBLSERPS4	3	3	9,12	93,23	72,92	0,84	2,58	6,45	5,92	1,53	1,17	3,24
PT2013EBLSERPS4	4	3	5,15	71,95	72,92	0,84	2,58	6,45	5,92	-0,07	0,88	3,15
PT2013EBLSERPS4	5	3	9,12	73,56	72,92	0,84	2,58	6,45	5,92	0,05	1,17	4,11
PT2013EBLSERPS4	6	3	2,72	76,54	72,92	0,84	2,58	6,45	5,92	0,27	0,64	2,15

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalized data (% blocking); **x_i_m** = mean of normalized data (% blocking); **x_g_m** = mean of normalized data (% blocking) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).

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