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172-PT

PROFICIENCY TESTING 2016

Bovine Viral Diarrhea Virus (BVDV)

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

CODA-CERVA-UCCLE

DATE BEGIN PT: 21 NOVEMBER 2016

DATE REPORT: 2 MARCH 2017

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 BVDV-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 BVDV 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 7 reference serum samples of bovine origin, either free from detectable BVDV-specific antibodies (n=2; coded 'PT2016BVDAbSERNS1' and 'PT2016BVDAbSERNS2') or containing detectable BVDV-specific antibodies (n=5; coded 'PT2016BVDAbSERPS1', 'PT2016BVDAbSERPS2', 'PT2016BVDAbSERPS3', 'PT2016BVDAbSERPS4' and 'PT2016BVDAbSERPS5'), were used. In total, 160 aliquots were distributed to 8 laboratories. All laboratories received 20 aliquots: 2 aliquots of the reference serum samples 'PT2016BVDAbSERPS1', 'PT2016BVDAbSERPS2', 'PT2016BVDAbSERPS3' and 'PT2016BVDAbSERPS4', 3 aliquots of the reference serum sample 'PT2016BVDAbSERPS5', 4 aliquots of the reference serum sample 'PT2016BVDAbSERNS1' and 5 aliquots of the reference serum sample 'PT2016BVDAbSERNS2'. 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 herds of origin, (ii) the results obtained with the Virus Neutralization Test (VNT) for BVD type 1 and/or type 2 and (iii) the results obtained during pre-verification, hereby using the MONOSCREEN AB BVD ELISA kit [ELISA for serodiagnosis of BVDV Blocking test for blood sera and plasma (E0) (batch CBVDB15L07)] from BioX.

The reference serum samples PT2016BVDAbSERNS1 and PT2016BVDAbSERNS2 were obtained from two animals from a BVDV-free unvaccinated herd.

The reference serum samples PT2016BVDAbSERPS1, PT2016BVDAbSERPS2, PT2016BVDAbSERPS3, and PT2016BVDAbSERPS4 were obtained from 4 seropositive animals from different herds and had a BVD-type1 VNT titer of 1/640, 1/60, 1/60, 1/60 respectively. The reference serum sample PT2016BVDAbSERPS5 was obtained after dilution 1/160 in a negative serum sample of a strong seropositive sample from an animal experimentally infected with a BVD type2. The BVD-type2 VNT titer for this dilution was between 1/10 and 1/20 and the BVD-type1 VNT was negative after dilution.

Taken together, the reference serum samples PT2016BVDAbSERNS1 and PT2016BVDAbSERNS2 were considered as negative sera and the reference serum samples PT2016BVDAbSERPS1, PT2016BVDAbSERPS2, PT2016BVDAbSERPS3, PT2016BVDAbSERPS4 and PT2016BVDAbSERPS5 as positive sera in BVDV antibody ELISA. However, for PT2016BVDAbSERPS2, positive, negative and non-interpretable (doubtful) ELISA results were accepted given that the true individual status (infected or vaccinated) of the animal was unknown. For PT2016BVDAbSERPS5, positive and non-interpretable (doubtful) ELISA results were accepted (weak positive sample for BVD-type2) but negative results were rejected (see discussion section V.).

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the MONOSCREEN AB BVD ELISA kit from BioX, 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 BVDV-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 MONOSCREEN AB BVD ELISA kit from BioX .

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 8 laboratories by national or international courier on 21th of November 2016 (160 aliquots in total). LAB2, LAB3, LAB4 and LAB5 acknowledged receipt of the samples on the same day, whereas LAB7 and LAB8 acknowledged receipt of the samples on 22th of November 2016 and LAB1 and LAB6 acknowledged receipt of the samples on 23th of November 2016. LAB8 informed us that he could not participate in the proficiency test and send back the samples. LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB7 performed their analyses between 22th and the 30th of November 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 24th of November and 14th of December 2016 (Table 1). All participants except LAB4 respected the deadline of 9th of December 2016 for submission of the results.

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	23/11/2016	24/11/2016	02/12/2016
LAB2	21/11/2016	22/11/2016	08/12/2016
LAB3	21/11/2016	29/11/2016	08/12/2016
LAB4	21/11/2016	24/11/2016	14/12/2016
LAB5	21/11/2016	30/11/2016	05/12/2016
LAB6	23/11/2016	29/11/2016	29/11/2016
LAB7	22/11/2016	23/11/2016	24/11/2016

IV.3. Compliance with the procedure

All 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 (Table 2) showed that :

- 3 participating laboratories (LAB2, LAB4, LAB5) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement),
- 2 participating laboratories (LAB1 and LAB3) misclassified 1 sample and hence obtained 95% of agreement,
- 2 participating laboratories (LAB6 and LAB7) misclassified 3 samples and hence obtained 85% of agreement.

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 BVDV 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	1 (5.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	<u>3 (15.0)</u>	<u>3 (15.0)</u>
success	19 (95.0)	20 (100.0)	19 (95.0)	20 (100.0)	20 (100.0)	<u>17 (85.0)</u>	<u>17 (85.0)</u>

IV.4.2. Variability among participating laboratories

LAB1 and LAB3 misclassified 1 aliquot of PT2016BVDAbSERPS5. LAB6 and LAB7 misclassified the 3 aliquots of PT2016BVDAbSERPS5. All these aliquots were reported as negative instead of positive or non-interpretable (doubtful).

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 BVDV reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI : non interpretable (doubtful).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016BVDAbSERNS2	NEG	NEG	1
2	1	2	PT2016BVDAbSERNS1	NEG	NEG	1
3	1	3	PT2016BVDAbSERPS1	POS	POS	1
4	1	4	PT2016BVDAbSERPS3	POS	POS	1
5	1	5	PT2016BVDAbSERNS2	NEG	NEG	1
6	1	6	PT2016BVDAbSERNS2	NEG	NEG	1
7	1	7	PT2016BVDAbSERPS5	POS	NI	1
8	1	8	PT2016BVDAbSERPS2	POS	POS	1
9	1	9	PT2016BVDAbSERNS1	NEG	NEG	1
10	1	10	PT2016BVDAbSERPS5	POS	NI	1
11	1	11	PT2016BVDAbSERPS4	POS	POS	1
12	1	12	PT2016BVDAbSERPS5	POS	NEG	0
13	1	13	PT2016BVDAbSERNS2	NEG	NEG	1
14	1	14	PT2016BVDAbSERPS4	POS	POS	1
15	1	15	PT2016BVDAbSERPS3	POS	POS	1
16	1	16	PT2016BVDAbSERPS1	POS	POS	1
17	1	17	PT2016BVDAbSERNS1	NEG	NEG	1
18	1	18	PT2016BVDAbSERNS2	NEG	NEG	1
19	1	19	PT2016BVDAbSERPS2	POS	POS	1
20	1	20	PT2016BVDAbSERNS1	NEG	NEG	1
21	2	1	PT2016BVDAbSERNS2	NEG	NEG	1
22	2	2	PT2016BVDAbSERPS4	POS	POS	1
23	2	3	PT2016BVDAbSERNS1	NEG	NEG	1
24	2	4	PT2016BVDAbSERPS5	POS	POS	1
25	2	5	PT2016BVDAbSERPS1	POS	POS	1
26	2	6	PT2016BVDAbSERNS1	NEG	NEG	1
27	2	7	PT2016BVDAbSERPS2	POS	POS	1
28	2	8	PT2016BVDAbSERPS3	POS	POS	1
29	2	9	PT2016BVDAbSERPS5	POS	POS	1
30	2	10	PT2016BVDAbSERNS2	NEG	NEG	1
31	2	11	PT2016BVDAbSERPS5	POS	POS	1
32	2	12	PT2016BVDAbSERPS4	POS	POS	1
33	2	13	PT2016BVDAbSERNS1	NEG	NEG	1
34	2	14	PT2016BVDAbSERPS3	POS	POS	1
35	2	15	PT2016BVDAbSERNS2	NEG	NEG	1
36	2	16	PT2016BVDAbSERPS1	POS	POS	1
37	2	17	PT2016BVDAbSERPS2	POS	POS	1
38	2	18	PT2016BVDAbSERNS2	NEG	NEG	1
39	2	19	PT2016BVDAbSERNS1	NEG	NEG	1
40	2	20	PT2016BVDAbSERNS2	NEG	NEG	1



(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2016BVDAbSERNS2	NEG	NEG	1
42	3	2	PT2016BVDAbSERNS1	NEG	NEG	1
43	3	3	PT2016BVDAbSERPS1	POS	POS	1
44	3	4	PT2016BVDAbSERPS3	POS	POS	1
45	3	5	PT2016BVDAbSERNS2	NEG	NEG	1
46	3	6	PT2016BVDAbSERNS2	NEG	NEG	1
47	3	7	PT2016BVDAbSERPS5	POS	NI	1
48	3	8	PT2016BVDAbSERPS2	POS	POS	1
49	3	9	PT2016BVDAbSERNS1	NEG	NEG	1
50	3	10	PT2016BVDAbSERPS5	POS	NEG	0
51	3	11	PT2016BVDAbSERPS4	POS	POS	1
52	3	12	PT2016BVDAbSERPS5	POS	NI	1
53	3	13	PT2016BVDAbSERNS2	NEG	NEG	1
54	3	14	PT2016BVDAbSERPS4	POS	POS	1
55	3	15	PT2016BVDAbSERPS3	POS	POS	1
56	3	16	PT2016BVDAbSERPS1	POS	POS	1
57	3	17	PT2016BVDAbSERNS1	NEG	NEG	1
58	3	18	PT2016BVDAbSERNS2	NEG	NEG	1
59	3	19	PT2016BVDAbSERPS2	POS	POS	1
60	3	20	PT2016BVDAbSERNS1	NEG	NEG	1
61	4	1	PT2016BVDAbSERNS2	NEG	NEG	1
62	4	2	PT2016BVDAbSERPS4	POS	POS	1
63	4	3	PT2016BVDAbSERNS1	NEG	NEG	1
64	4	4	PT2016BVDAbSERPS5	POS	POS	1
65	4	5	PT2016BVDAbSERPS1	POS	POS	1
66	4	6	PT2016BVDAbSERNS1	NEG	NEG	1
67	4	7	PT2016BVDAbSERPS2	POS	POS	1
68	4	8	PT2016BVDAbSERPS3	POS	POS	1
69	4	9	PT2016BVDAbSERPS5	POS	POS	1
70	4	10	PT2016BVDAbSERNS2	NEG	NEG	1
71	4	11	PT2016BVDAbSERPS5	POS	POS	1
72	4	12	PT2016BVDAbSERPS4	POS	POS	1
73	4	13	PT2016BVDAbSERNS1	NEG	NEG	1
74	4	14	PT2016BVDAbSERPS3	POS	POS	1
75	4	15	PT2016BVDAbSERNS2	NEG	NEG	1
76	4	16	PT2016BVDAbSERPS1	POS	POS	1
77	4	17	PT2016BVDAbSERPS2	POS	POS	1
78	4	18	PT2016BVDAbSERNS2	NEG	NEG	1
79	4	19	PT2016BVDAbSERNS1	NEG	NEG	1
80	4	20	PT2016BVDAbSERNS2	NEG	NEG	1



(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2016BVDAbSERNS2	NEG	NEG	1
82	5	2	PT2016BVDAbSERNS1	NEG	NEG	1
83	5	3	PT2016BVDAbSERPS1	POS	POS	1
84	5	4	PT2016BVDAbSERPS3	POS	POS	1
85	5	5	PT2016BVDAbSERNS2	NEG	NEG	1
86	5	6	PT2016BVDAbSERNS2	NEG	NEG	1
87	5	7	PT2016BVDAbSERPS5	POS	POS	1
88	5	8	PT2016BVDAbSERPS2	POS	POS	1
89	5	9	PT2016BVDAbSERNS1	NEG	NEG	1
90	5	10	PT2016BVDAbSERPS5	POS	POS	1
91	5	11	PT2016BVDAbSERPS4	POS	POS	1
92	5	12	PT2016BVDAbSERPS5	POS	POS	1
93	5	13	PT2016BVDAbSERNS2	NEG	NEG	1
94	5	14	PT2016BVDAbSERPS4	POS	POS	1
95	5	15	PT2016BVDAbSERPS3	POS	POS	1
96	5	16	PT2016BVDAbSERPS1	POS	POS	1
97	5	17	PT2016BVDAbSERNS1	NEG	NEG	1
98	5	18	PT2016BVDAbSERNS2	NEG	NEG	1
99	5	19	PT2016BVDAbSERPS2	POS	POS	1
100	5	20	PT2016BVDAbSERNS1	NEG	NEG	1
101	6	1	PT2016BVDAbSERNS2	NEG	NEG	1
102	6	2	PT2016BVDAbSERPS4	POS	POS	1
103	6	3	PT2016BVDAbSERNS1	NEG	NEG	1
104	6	4	PT2016BVDAbSERPS5	POS	NEG	0
105	6	5	PT2016BVDAbSERPS1	POS	POS	1
106	6	6	PT2016BVDAbSERNS1	NEG	NEG	1
107	6	7	PT2016BVDAbSERPS2	POS	NI	1
108	6	8	PT2016BVDAbSERPS3	POS	POS	1
109	6	9	PT2016BVDAbSERPS5	POS	NEG	0
110	6	10	PT2016BVDAbSERNS2	NEG	NEG	1
111	6	11	PT2016BVDAbSERPS5	POS	NEG	0
112	6	12	PT2016BVDAbSERPS4	POS	POS	1
113	6	13	PT2016BVDAbSERNS1	NEG	NEG	1
114	6	14	PT2016BVDAbSERPS3	POS	POS	1
115	6	15	PT2016BVDAbSERNS2	NEG	NEG	1
116	6	16	PT2016BVDAbSERPS1	POS	POS	1
117	6	17	PT2016BVDAbSERPS2	POS	NI	1
118	6	18	PT2016BVDAbSERNS2	NEG	NEG	1
119	6	19	PT2016BVDAbSERNS1	NEG	NEG	1
120	6	20	PT2016BVDAbSERNS2	NEG	NEG	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2016BVDAbSERNS2	NEG	NEG	1
122	7	2	PT2016BVDAbSERNS1	NEG	NEG	1
123	7	3	PT2016BVDAbSERPS1	POS	POS	1
124	7	4	PT2016BVDAbSERPS3	POS	POS	1
125	7	5	PT2016BVDAbSERNS2	NEG	NEG	1
126	7	6	PT2016BVDAbSERNS2	NEG	NEG	1
127	7	7	PT2016BVDAbSERPS5	POS	NEG	0
128	7	8	PT2016BVDAbSERPS2	POS	NEG	1
129	7	9	PT2016BVDAbSERNS1	NEG	NEG	1
130	7	10	PT2016BVDAbSERPS5	POS	NEG	0
131	7	11	PT2016BVDAbSERPS4	POS	POS	1
132	7	12	PT2016BVDAbSERPS5	POS	NEG	0
133	7	13	PT2016BVDAbSERNS2	NEG	NEG	1
134	7	14	PT2016BVDAbSERPS4	POS	POS	1
135	7	15	PT2016BVDAbSERPS3	POS	POS	1
136	7	16	PT2016BVDAbSERPS1	POS	POS	1
137	7	17	PT2016BVDAbSERNS1	NEG	NEG	1
138	7	18	PT2016BVDAbSERNS2	NEG	NEG	1
139	7	19	PT2016BVDAbSERPS2	POS	NEG	1
140	7	20	PT2016BVDAbSERNS1	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 BVDV-specific antibodies by ELISA.

For the detection of BVDV-specific antibodies in reference serum samples, three out of seven participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement).

LAB1 and LAB3 misclassified 1 out of the 3 aliquots of the sample PT2016BVDAbSERPS5 (negative instead of positive or non-interpretable); the 2 other aliquots of this sample were reported as non-interpretable. LAB6 and LAB7 misclassified the 3 aliquots of the sample PT2016BVDAbSERPS5 (negative instead of positive or non-interpretable). This reference sample was obtained after dilution of a strong positive sample from an animal experimentally infected with BVD-type2. Although this is not the most common circulating BVDV genotype in Belgium and in Europe, it is considered that Antibody ELISAs should be able to detect antibodies specific both for BVD-type1 and BVD-type2 in order to be used in an official monitoring programme.

It was observed that LAB6 and LAB7 gave a non-interpretable (NI) and negative result respectively for the 2 aliquots of the sample PT2016BVDAbSERPS2. Both laboratories used an anti-p80 (or anti-NS3) competitive ELISA. It is well known that such ELISAs do not consistently provide a positive result with samples obtained from uninfected animals vaccinated against BVDV with an inactivated vaccine. In contrast, other participating laboratories used a total antibody ELISA or an anti-E0 competitive ELISA and gave positive results for this sample. As the true status of the animal was unknown (vaccinated or infected), all results were accepted (positive, non-interpretable, negative) for this sample.

LAB1 and LAB3 used the BVDV Total Ab indirect ELISA kit from IDEXX (batch: F991), LAB7 the BVDV p80 Ab competition ELISA kit from IDEXX (batch: 6053), LAB6 the SERELISA BVD p80 Ab Mono Blocking ELISA kit from Synbiotics (batch: 16ZEAK001) whereas the other participants used the Mono Screen Ab BVDV ELISA kit from BioX (LAB2 batch: CBVDB15L07, LAB4 batch: CBVDB15L29 and LAB5 batch: CBVDB15L16).

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 BVDV reference laboratory of CODA-CERVA-Uccle (see III.3.3.). Consequently, 5 out of 7 participating laboratories achieved a satisfactory performance for the detection of BVDV-specific antibodies by ELISA. LAB6 and LAB7 did not achieve a satisfactory performance for the detection of BVDV-specific antibodies in serum samples.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement
et du travail (ANSES) (Niort, France)

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

Lavetan NV (Turnhout, Belgium)

Synbiotics Europe (Lyon, France)

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: $[\text{OD sample} - \text{mean (OD negative kit controls)}] / \text{mean (OD positive kit controls)} - \text{mean (OD negative kit controls)}] * 100$.

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 $P25 = \text{minimum}$ and $P75 = \text{maximum}$ when the number data is 2. LAB1 and LAB3 used the BVDV Total Ab indirect ELISA kit from IDEXX (batch: F991), LAB7 the BVDV p80 Ab competition ELISA kit from IDEXX (batch: 6053), LAB6 the SERELISA BVD p80 Ab Mono Blocking ELISA kit from Synbiotics (batch: 16ZEAK001) whereas the other participants used the Mono Screen Ab BVDV ELISA kit from BioX (LAB2 batch: CBVDB15L07, LAB4 batch: CBVDB15L29 and LAB5 batch: CBVDB15L16). Cut-off values for the BVDV Total Ab indirect ELISA kit from IDEXX (20-30%), the BVDV p80 Ab competition ELISA kit from IDEXX (50%), the SERELISA BVD p80 Ab Mono Blocking ELISA kit from Synbiotics (30-50%) and the Mono Screen Ab BVDV ELISA kit from BioX (50%) are shown by horizontal lines.

