CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE
GROESELENBERG 99 – B 1180 BRUSSELS (UKKEL)
TEL: +32 (0)2 379 04 11
FAX : + 32 (0)2 379 06 70
HTTP: // WWW.CODA-CERVA.BE

172-PT

PROFICIENCY TESTING 2016

Bovine Viral Diarrhea Virus (BVDV)

Detection of BVDV-specific antigens in bovine Serum and Ear notch samples by Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR) and/or Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 18 APRIL 2016

DATE REPORT: 28 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 BVDV-specific antigens in bovine serum and ear notch samples by RT-qPCR and/or antigen ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum and ear notch samples must be tested by means of a BVDV RT-qPCR and/or a BVDV antigen ELISA. The procedures for these tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

LAB1, LAB2 and LAB3 received 40 aliquots, namely 10 aliquots of the matrix serum and ear notch samples to perform BVDV RT-qPCR and BVDV antigen ELISA. LAB4 received 20 aliquots, namely 10 aliquots of the matrix serum and ear notch samples to perform BVDV antigen ELISA. LAB5 and LAB6 received 20 aliquots, namely 10 aliquots of the matrix serum and ear notch samples to perform BVDV RT- qPCR. LAB7, LAB8 and LAB9 received 10 aliquots of the matrix ear notch samples to perform BVDV RT- qPCR. Each matrix was sent in a different block/bag with reference samples (position 1-10).

III.2.1. Reference serum samples

Replicates of 10 reference serum samples of bovine origin, either free from detectable BVDV-specific antigens (n=2 per method; coded 'PT2016BVDVIRNE1 ELSER or PCRSER' and 'PT2016BVDVIRNE2 ELSER or PCRSER') or containing detectable BVDV-specific antigens (n=3 per method; coded 'PT2016BVDVIRPE1 ELSER or PCRSER', 'PT2016BVDVIRPE2 ELSER or PCRSER', 'PT2016BVDVIRPE3 ELSER or PCRSER'), were used.
In total, 90 aliquots of reference serum samples were distributed to 6 participating laboratories.The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 6 and Table 8).

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 by an in-house developed BVDV RT-qPCR assays and the BVDV antigen test kit/serum plus ELISA from IDEXX (pre-verification).

The reference serum samples PT2016BVDVIRNE1 ELSER, PT2016BVDVIRNE2 ELSER, PT2016BVDVIRNE1 PCRSER and PT2016BVDVIRNE2 PCRSER were obtained from 4 different BVDV-free animals from the field. The reference samples PT2016BVDVIRPE1 ELSER and PT2016BVDVIRPE3 ELSER were obtained from 2 different calves that were classified as immunotolerant persistently (BVDV-2) infected (IPI) animals. The reference sample PT2016BVDVIRPE2 ELSER was obtained from a calf that was classified as an immunotolerant persistently (BVDV-1) infected (IPI) animal. This sample was a 1/50 dilution in serum from a BVDV-free animal from the field. The reference samples PT2016BVDVIRPE1 PCRSER, PT2016BVDVIRPE2 PCRSER and PT2016BVDVIRPE3 PCRSER were obtained from 3 different calves that were classified as immunotolerant persistently (BVDV-1) infected (IPI) animals. The reference sample PT2016BVDVIRPE3 PCRSER was a 1/1000 dilution in serum from a BVDV-free animal from the field. For each reference serum sample, the same qualitative result were obtained with the in-house developed BVDV RT-qPCR assays and the BVDV antigen ELISA kit from IDEXX, except for the PT2016BVDVIRPE3 PCRSER which was negative in the BVDV antigen ELISA kit from IDEXX but positive in the in-house developed BVDV RT-qPCR assay.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 alliquots of the reference serum samples using an in-house developed BVDV RT-qPCR (PCRSER reference serum samples) and the BVDV antigen ELISA kit from IDEXX (ELSER reference serum samples), hereby obtaining the same qualitative result for all 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 antigens in bovine serum. In addition, all reference serum samples were tested three times after the PT in order to confirm their stability and status (post-verification) using the in-house developed BVDV RT-qPCR (PCRSER reference serum samples) and the BVDV antigen ELISA kit from IDEXX (ELSER reference serum samples).

III.2.2. Reference ear notch samples

Replicates of 5 reference ear notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=2; coded 'PT2016BVDVIRNE1 ERNS' and 'PT2016BVDVIRNE2 ERNS') or containing detectable BVDV-specific antigens (n=3; coded 'PT2016BVDVIRPE1 ERNS', 'PT2016BVDVIRPE2 ERNS' and 'PT2016BVDVIRPE3 ERNS'), were used.
In total, 120 aliquots of reference ear notch samples were distributed to 9 participating laboratories (Table 7 and Table 9).

For each reference ear notch sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference ear notch samples was based on (i) the historical background of the animals and (ii) the results obtained by an in-house developed BVDV RT-qPCR assays and the BVDV antigen test kit/serum plus ELISA from IDEXX.

The reference ear notch samples PT2016BVDVIRNE1 ERNS and PT2016BVDVIRNE2 ERNS were obtained from 2 different BVDV-free animals from the field. The reference ear notch samples PT2016BVDVIRPE1 ERNS, PT2016BVDVIRPE2 ERNS and PT2016BVDVIRPE3 ERNS were field samples obtained from 3 different animals that were classified as immunotolerant persistently (BVDV-1) infected (IPI) animals.

For each reference ear notch sample, the same qualitative result was obtained with an in-house developed BVDV RT-qPCR assays and the BVDV antigen ELISA kit from IDEXX.

After aliquoting the different ear notch samples, a homogeneity check was performed on 3 aliquots of each reference ear notch sample using an in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX, hereby obtaining the same qualitative result for all aliquots of the same reference ear notch sample. Consequently, all reference ear notch samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BVDV-specific antigens in bovine ear notch samples. In addition, all reference ear notch samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed BVDV RT-qPCR and the BVDV antigen ELISA 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 (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or failure when the reported result does not match with the assigned status (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for each of the 10 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 10 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

LAB2 and LAB3 participated in the PT serum and ear notch for both RT-qPCR and Antigen ELISA. LAB1 had been registered for both RT-qPCR and Antigen ELISA in the PT serum and ear notch but did'nt participate in the PT serum for RT-qPCR. LAB4 participated in the PT serum and ear notch for Antigen ELISA. LAB5 and LAB6 participated in the PT serum and ear notch for RT-qPCR. LAB7, LAB8 and LAB9 participated in the PT ear notch for RT-qPCR.
The reference serum and ear notch samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 18th of April 2016. LAB1, LAB2, LAB3, LAB4, LAB5, LAB7 and LAB8 acknowledged receipt of the samples on the same day, whereas LAB6 and LAB9 received the samples respectively on 19th and 20th of April 2016. Analyses were performed between 19th of April and 3rd of May 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 26th of April and 9th of May 2016 (Table 1). All participants hereby respected the deadline of 9th of May 2016 for submission of the results.

Table 1. Overview of the laboratories that participated with relation to starting date and submission of results towards the CODA-CERVA-Uccle for the different assays

Participating laboratory	Reference samples received	Start of analysis ELISA	Start of analysis RT-qPCR	Submission of the results (Excel file)
LAB1	18/04/2016	29/04/2016	NR (serum) 03/05/2016 (ear-notch)	09/05/2016
LAB2	18/04/2016	19/04/2016	19/04/2016 (serum) 21/04/2016(ear-notch)	04/05/2016
LAB3	18/04/2016	26/04/2016	21/04/2016 (serum) 19/04/2016(ear-notch)	09/05/2016
LAB4	18/04/2016	19/04/2016	NA	26/04/2016
LAB5	18/04/2016	NA	20/04/2016 (serum) 20/04/2016(ear-notch)	04/05/2016
LAB6	19/04/2016	NA	03/05/2016 (serum) 03/05/2016(ear-notch)	04/05/2016
LAB7	18/04/2016	NA	NA (serum) 20/04/2016(ear-notch)	04/05/2016
LAB8	18/04/2016	NA	NA (serum) 19/04/2016(ear-notch)	04/05/2016
LAB9	20/04/2016	NA	NA (serum) 27/04/2016(ear-notch)	09/05/2016

Legend: NA = not applicable; NR = no results

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 showed that:

(i) For the detection of BVDV-specific antigens by **antigen ELISA** (Table 2 and 3): For the matrix serum, all laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). For the matrix ear notch, all laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the true status of the reference ear notch samples (100% of agreement).

(ii) For the detection of BVDV-specific antigens by **RT-qPCR** (Table 4 and 5): For the matrix serum, all laboratories (LAB2, LAB3, LAB5 and LAB6) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). For the matrix ear notch, 7 out of 8 laboratories (LAB2, LAB3, LAB5, LAB6, LAB7, LAB8 and LAB9) provided qualitative results that were in full agreement with the true status of the reference ear notch samples (100% of agreement). In contrast, LAB1 misclassified 1 aliquot (90% of agreement) of reference ear notch samples.

Table 2. ELISA Serum: Agreement between results generated 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 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	2	3	4
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

Table 3. ELISA Ear notch: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference ear notch samples assigned by the BVDV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 10 aliquots of reference ear notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	2	3	4
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

Table 4. RT-qPCR Serum: Agreement between results generated 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 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	2	3	5	6
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

Table 5. RT-qPCR Ear notch: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference ear notch samples assigned by the BVDV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 10 aliquots of reference ear notch samples. Results are presented as absolute values and percentages (in parentheses).

		LABNR						
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**
failure	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	9 (90.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

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

IV.4.2. Variability among participating laboratories

(i) For the detection of BVDV-specific antigens by **antigen ELISA** no variability between the participating laboratories could be observed since all participants correctly identified all reference serum and ear notch samples.

(ii) For the detection of BVDV-specific antigens by **RT-qPCR in serum**, no variability between the participating laboratories could be observed since all participants correctly identified all reference serum samples.
For the detection of BVDV-specific antigens by **RT-qPCR in ear notch**, no variability between LAB2, LAB3, LAB5, LAB6, LAB7, LAB8 and LAB9 could be observed since these participants correctly identified all reference ear notch samples. In contrast, LAB1 misclassified 1 aliquot of the positive reference ear notch sample PT2016BVDVIRPE2 ERNS. This sample tested negative instead of positive.

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 6 (serum) and Table 7 (ear notch) for antigen ELISA and in Table 8 (serum) and Table 9 (ear notch) for RT-qPCR.

Table 6. Antigen ELISA serum: 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.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016BVDVIRPE1 ELSER	POS	POS	1
2	1	2	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
3	1	3	PT2016BVDVIRPE2 ELSER	POS	POS	1
4	1	4	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
5	1	5	PT2016BVDVIRPE3 ELSER	POS	POS	1
6	1	6	PT2016BVDVIRPE1 ELSER	POS	POS	1
7	1	7	PT2016BVDVIRPE3 ELSER	POS	POS	1
8	1	8	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
9	1	9	PT2016BVDVIRPE2 ELSER	POS	POS	1
10	1	10	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
11	2	1	PT2016BVDVIRPE2 ELSER	POS	POS	1
12	2	2	PT2016BVDVIRPE1 ELSER	POS	POS	1
13	2	3	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
14	2	4	PT2016BVDVIRPE2 ELSER	POS	POS	1
15	2	5	PT2016BVDVIRPE3 ELSER	POS	POS	1
16	2	6	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
17	2	7	PT2016BVDVIRPE3 ELSER	POS	POS	1
18	2	8	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
19	2	9	PT2016BVDVIRPE1 ELSER	POS	POS	1
20	2	10	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
21	3	1	PT2016BVDVIRPE1 ELSER	POS	POS	1
22	3	2	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
23	3	3	PT2016BVDVIRPE2 ELSER	POS	POS	1
24	3	4	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
25	3	5	PT2016BVDVIRPE3 ELSER	POS	POS	1
26	3	6	PT2016BVDVIRPE1 ELSER	POS	POS	1
27	3	7	PT2016BVDVIRPE3 ELSER	POS	POS	1
28	3	8	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
29	3	9	PT2016BVDVIRPE2 ELSER	POS	POS	1
30	3	10	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
31	4	1	PT2016BVDVIRPE2 ELSER	POS	POS	1
32	4	2	PT2016BVDVIRPE1 ELSER	POS	POS	1
33	4	3	PT2016BVDVIRNE1 ELSER	NEG	NEG	1
34	4	4	PT2016BVDVIRPE2 ELSER	POS	POS	1
35	4	5	PT2016BVDVIRPE3 ELSER	POS	POS	1
36	4	6	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
37	4	7	PT2016BVDVIRPE3 ELSER	POS	POS	1
38	4	8	PT2016BVDVIRNE2 ELSER	NEG	NEG	1
39	4	9	PT2016BVDVIRPE1 ELSER	POS	POS	1
40	4	10	PT2016BVDVIRNE1 ELSER	NEG	NEG	1

Table 7. Antigen ELISA ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference ear notch samples (SAMPLE), the positions of the reference ear notch samples as provided for the laboratories (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016BVDVIRPE1 ERNS	POS	POS	1
2	1	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
3	1	3	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
4	1	4	PT2016BVDVIRPE1 ERNS	POS	POS	1
5	1	5	PT2016BVDVIRPE2 ERNS	POS	POS	1
6	1	6	PT2016BVDVIRPE3 ERNS	POS	POS	1
7	1	7	PT2016BVDVIRPE2 ERNS	POS	POS	1
8	1	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
9	1	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
10	1	10	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
11	2	1	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
12	2	2	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
13	2	3	PT2016BVDVIRPE2 ERNS	POS	POS	1
14	2	4	PT2016BVDVIRPE1 ERNS	POS	POS	1
15	2	5	PT2016BVDVIRPE2 ERNS	POS	POS	1
16	2	6	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
17	2	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
18	2	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
19	2	9	PT2016BVDVIRPE3 ERNS	POS	POS	1
20	2	10	PT2016BVDVIRPE1 ERNS	POS	POS	1
21	3	1	PT2016BVDVIRPE1 ERNS	POS	POS	1
22	3	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
23	3	3	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
24	3	4	PT2016BVDVIRPE1 ERNS	POS	POS	1
25	3	5	PT2016BVDVIRPE2 ERNS	POS	POS	1
26	3	6	PT2016BVDVIRPE3 ERNS	POS	POS	1
27	3	7	PT2016BVDVIRPE2 ERNS	POS	POS	1
28	3	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
29	3	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
30	3	10	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
31	4	1	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
32	4	2	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
33	4	3	PT2016BVDVIRPE2 ERNS	POS	POS	1
34	4	4	PT2016BVDVIRPE1 ERNS	POS	POS	1
35	4	5	PT2016BVDVIRPE2 ERNS	POS	POS	1
36	4	6	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
37	4	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
38	4	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
39	4	9	PT2016BVDVIRPE3 ERNS	POS	POS	1
40	4	10	PT2016BVDVIRPE1 ERNS	POS	POS	1

Table 8. RT-qPCR serum: 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.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	2	1	PT2016BVDVIRPE3 PCRSER	POS	POS	1
2	2	2	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
3	2	3	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
4	2	4	PT2016BVDVIRPE3 PCRSER	POS	POS	1
5	2	5	PT2016BVDVIRPE1 PCRSER	POS	POS	1
6	2	6	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
7	2	7	PT2016BVDVIRPE2 PCRSER	POS	POS	1
8	2	8	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
9	2	9	PT2016BVDVIRPE2 PCRSER	POS	POS	1
10	2	10	PT2016BVDVIRPE1 PCRSER	POS	POS	1
11	3	1	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
12	3	2	PT2016BVDVIRPE1 PCRSER	POS	POS	1
13	3	3	PT2016BVDVIRPE2 PCRSER	POS	POS	1
14	3	4	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
15	3	5	PT2016BVDVIRPE1 PCRSER	POS	POS	1
16	3	6	PT2016BVDVIRPE3 PCRSER	POS	POS	1
17	3	7	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
18	3	8	PT2016BVDVIRPE3 PCRSER	POS	POS	1
19	3	9	PT2016BVDVIRPE2 PCRSER	POS	POS	1
20	3	10	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
21	5	1	PT2016BVDVIRPE3 PCRSER	POS	POS	1
22	5	2	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
23	5	3	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
24	5	4	PT2016BVDVIRPE3 PCRSER	POS	POS	1
25	5	5	PT2016BVDVIRPE1 PCRSER	POS	POS	1
26	5	6	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
27	5	7	PT2016BVDVIRPE2 PCRSER	POS	POS	1
28	5	8	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
29	5	9	PT2016BVDVIRPE2 PCRSER	POS	POS	1
30	5	10	PT2016BVDVIRPE1 PCRSER	POS	POS	1
31	6	1	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1
32	6	2	PT2016BVDVIRPE1 PCRSER	POS	POS	1
33	6	3	PT2016BVDVIRPE2 PCRSER	POS	POS	1
34	6	4	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
35	6	5	PT2016BVDVIRPE1 PCRSER	POS	POS	1
36	6	6	PT2016BVDVIRPE3 PCRSER	POS	POS	1
37	6	7	PT2016BVDVIRNE1 PCRSER	NEG	NEG	1
38	6	8	PT2016BVDVIRPE3 PCRSER	POS	POS	1
39	6	9	PT2016BVDVIRPE2 PCRSER	POS	POS	1
40	6	10	PT2016BVDVIRNE2 PCRSER	NEG	NEG	1

Table 9. RT-qPCR ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference ear notch samples (SAMPLE), the positions (numbers) of the reference ear notch samples as provided for the laboratories (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
2	1	2	PT2016BVDVIRPE1 ERNS	POS	POS	1
3	1	3	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
4	1	4	PT2016BVDVIRPE2 ERNS	**POS**	**NEG**	**0**
5	1	5	PT2016BVDVIRPE3 ERNS	POS	POS	1
6	1	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
7	1	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
8	1	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
9	1	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
10	1	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
11	2	1	PT2016BVDVIRPE3 ERNS	POS	POS	1
12	2	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
13	2	3	PT2016BVDVIRPE1 ERNS	POS	POS	1
14	2	4	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
15	2	5	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
16	2	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
17	2	7	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
18	2	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
19	2	9	PT2016BVDVIRPE2 ERNS	POS	POS	1
20	2	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
21	3	1	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
22	3	2	PT2016BVDVIRPE1 ERNS	POS	POS	1
23	3	3	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
24	3	4	PT2016BVDVIRPE2 ERNS	POS	POS	1
25	3	5	PT2016BVDVIRPE3 ERNS	POS	POS	1
26	3	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
27	3	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
28	3	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
29	3	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
30	3	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
31	5	1	PT2016BVDVIRPE3 ERNS	POS	POS	1
32	5	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
33	5	3	PT2016BVDVIRPE1 ERNS	POS	POS	1
34	5	4	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
35	5	5	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
36	5	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
37	5	7	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
38	5	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
39	5	9	PT2016BVDVIRPE2 ERNS	POS	POS	1
40	5	10	PT2016BVDVIRPE2 ERNS	POS	POS	1

Table 9 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	6	1	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
42	6	2	PT2016BVDVIRPE1 ERNS	POS	POS	1
43	6	3	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
44	6	4	PT2016BVDVIRPE2 ERNS	POS	POS	1
45	6	5	PT2016BVDVIRPE3 ERNS	POS	POS	1
46	6	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
47	6	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
48	6	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
49	6	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
50	6	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
51	7	1	PT2016BVDVIRPE3 ERNS	POS	POS	1
52	7	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
53	7	3	PT2016BVDVIRPE1 ERNS	POS	POS	1
54	7	4	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
55	7	5	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
56	7	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
57	7	7	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
58	7	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
59	7	9	PT2016BVDVIRPE2 ERNS	POS	POS	1
60	7	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
61	8	1	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
62	8	2	PT2016BVDVIRPE1 ERNS	POS	POS	1
63	8	3	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
64	8	4	PT2016BVDVIRPE2 ERNS	POS	POS	1
65	8	5	PT2016BVDVIRPE3 ERNS	POS	POS	1
66	8	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
67	8	7	PT2016BVDVIRPE3 ERNS	POS	POS	1
68	8	8	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
69	8	9	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
70	8	10	PT2016BVDVIRPE2 ERNS	POS	POS	1
71	9	1	PT2016BVDVIRPE3 ERNS	POS	POS	1
72	9	2	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
73	9	3	PT2016BVDVIRPE1 ERNS	POS	POS	1
74	9	4	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
75	9	5	PT2016BVDVIRNE1 ERNS	NEG	NEG	1
76	9	6	PT2016BVDVIRPE1 ERNS	POS	POS	1
77	9	7	PT2016BVDVIRNE2 ERNS	NEG	NEG	1
78	9	8	PT2016BVDVIRPE3 ERNS	POS	POS	1
79	9	9	PT2016BVDVIRPE2 ERNS	POS	POS	1
80	9	10	PT2016BVDVIRPE2 ERNS	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing individual reference serum and/or ear notch samples of bovine origin for the detection of BVDV-specific antigens by antigen ELISA and/or RT-qPCR.

For the detection of BVDV-specific antigens by antigen ELISA in serum, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement).

For the detection of BVDV-specific antigens by antigen ELISA in ear notch, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement).

For the detection of BVDV-specific antigens by antigen ELISA, all laboratories used the same BVDV antigen test kit/serum plus ELISA kit from IDEXX, but 2 different batches were used: batch F551 (LAB1, LAB 3 and LAB4) and batch F781 (LAB2).

For the detection of BVDV-specific antigens by RT-qPCR in serum, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement).

For the detection of BVDV-specific antigens by RT-qPCR in ear-notch, seven out of eight participating laboratories (LAB2, LAB3, LAB5, LAB6, LAB7, LAB8 and LAB9) provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement), whereas LAB1 misclassified 1 aliquot of the positive reference ear-notch sample PT2016BVDVIRPE2 ERNS (90% of agreement).

For the detection of BVDV-specific antigens by RT-qPCR, all participating laboratories, except LAB3, used a commercially available BVDV RT-qPCR: LAB1, LAB2, LAB5, LAB6 and LAB8 used the VetMax BVD4ALL Screening test kit from LSI (batch 007 (LAB2 and LAB5), batch 008 (LAB1, LAB6 and LAB8)), LAB7 used the Virotype® BVDV RT-PCR kit from Qiagen (batch 251120615) and LAB9 used the ID GeneTM IDBVD/BD Triplex from Idvet Genetics (batch 7). LAB3 used an in house RT-qPCR.

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, all participating laboratories achieved a satisfactory performance for the detection of BVDV-specific antigens by ELISA and RT-qPCR in serum and ear notch samples.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

DNAlysis Maastricht B.V. (Maastricht, The Nederlands)

ID. VET (Grabels, France)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Lavetan NV (Turnhout, Belgium)

Thermofisher Scientific -LSI (LSI) (Lissieu, 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 (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.

For the **antigen ELISA serum and ear notch reference samples,** box plots of the normalized OD values according the PT provider per reference sample and per participating laboratory are shown in Figure 1 and Figure 2, respectively.

Figure 1 (antigen ELISA serum reference samples)

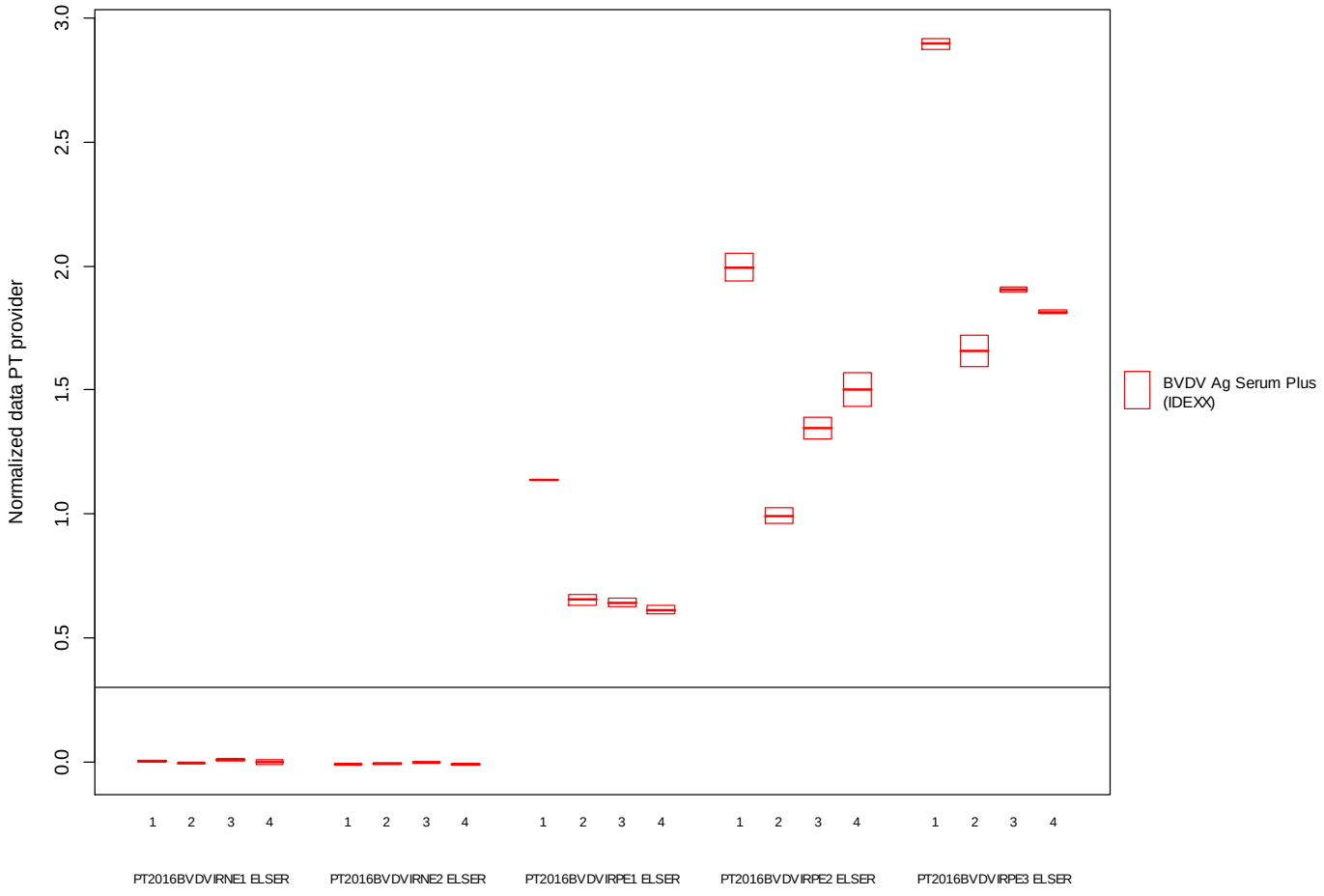

Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory. All laboratories used the same BVDV antigen test kit/serum plus ELISA kit from IDEXX, but 2 different batches: batch F551 (LAB1, LAB 3 and LAB4) and batch F781 (LAB2). Cut-off value (0.3) is shown by a horizontal line.

Figure 2 (antigen ELISA ear notch reference samples)

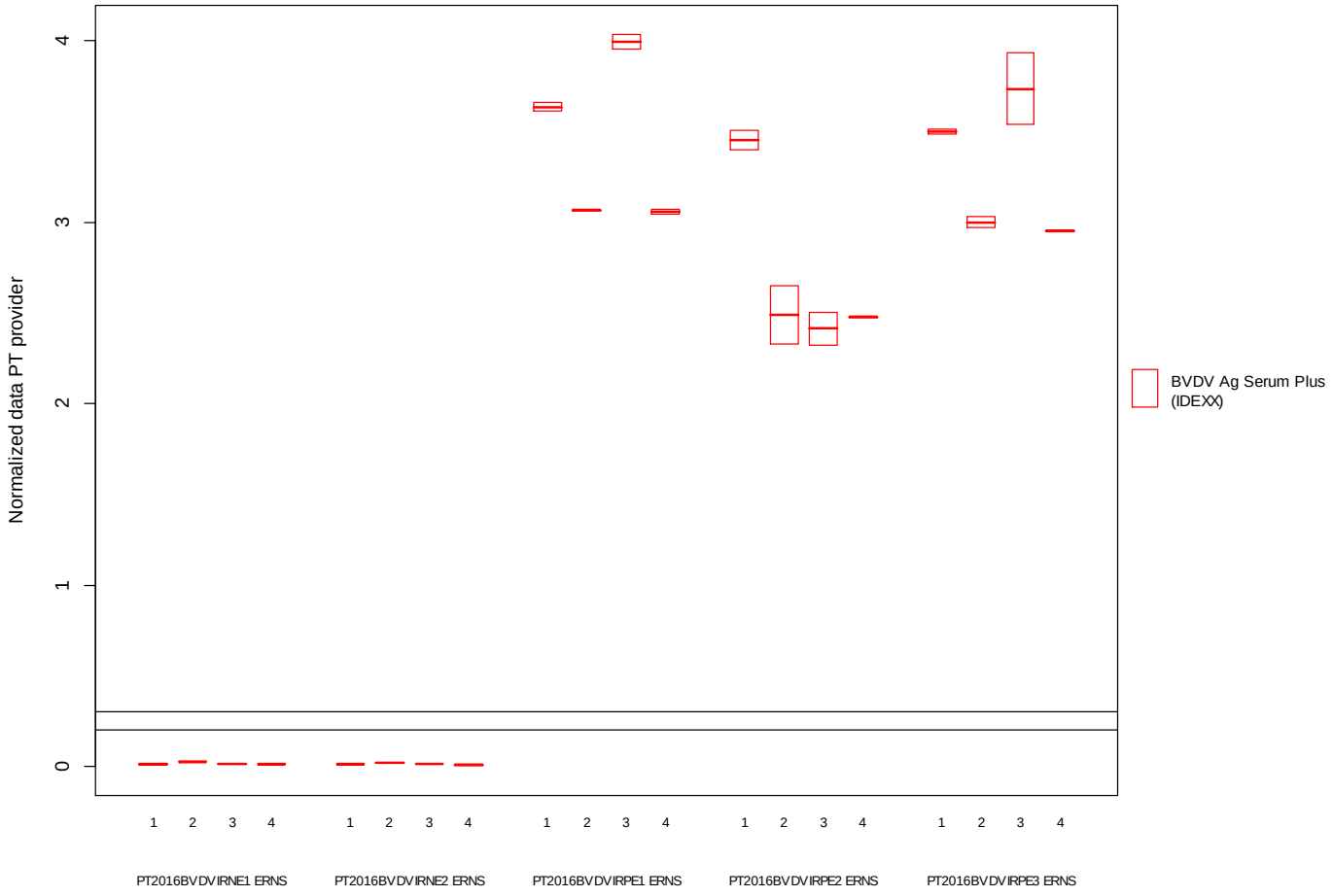

Figure 2. Box plots showing the normalized OD values according the PT provider per reference ear notch and per participating laboratory. All laboratories used the same BVDV antigen test kit/serum plus ELISA kit from IDEXX, but 2 different batches: batch F551 (LAB1, LAB 3 and LAB4) and batch F781 (LAB2). Cut-off values (0.2-0.3) are shown by horizontal lines.

For the **RT-qPCR serum and ear notch reference samples,** box plots of the Ct or Cp values per positive reference sample and per participating laboratory are shown in Figure 3 and Figure 4, respectively.

Figure 3 (RT-qPCR serum reference samples)

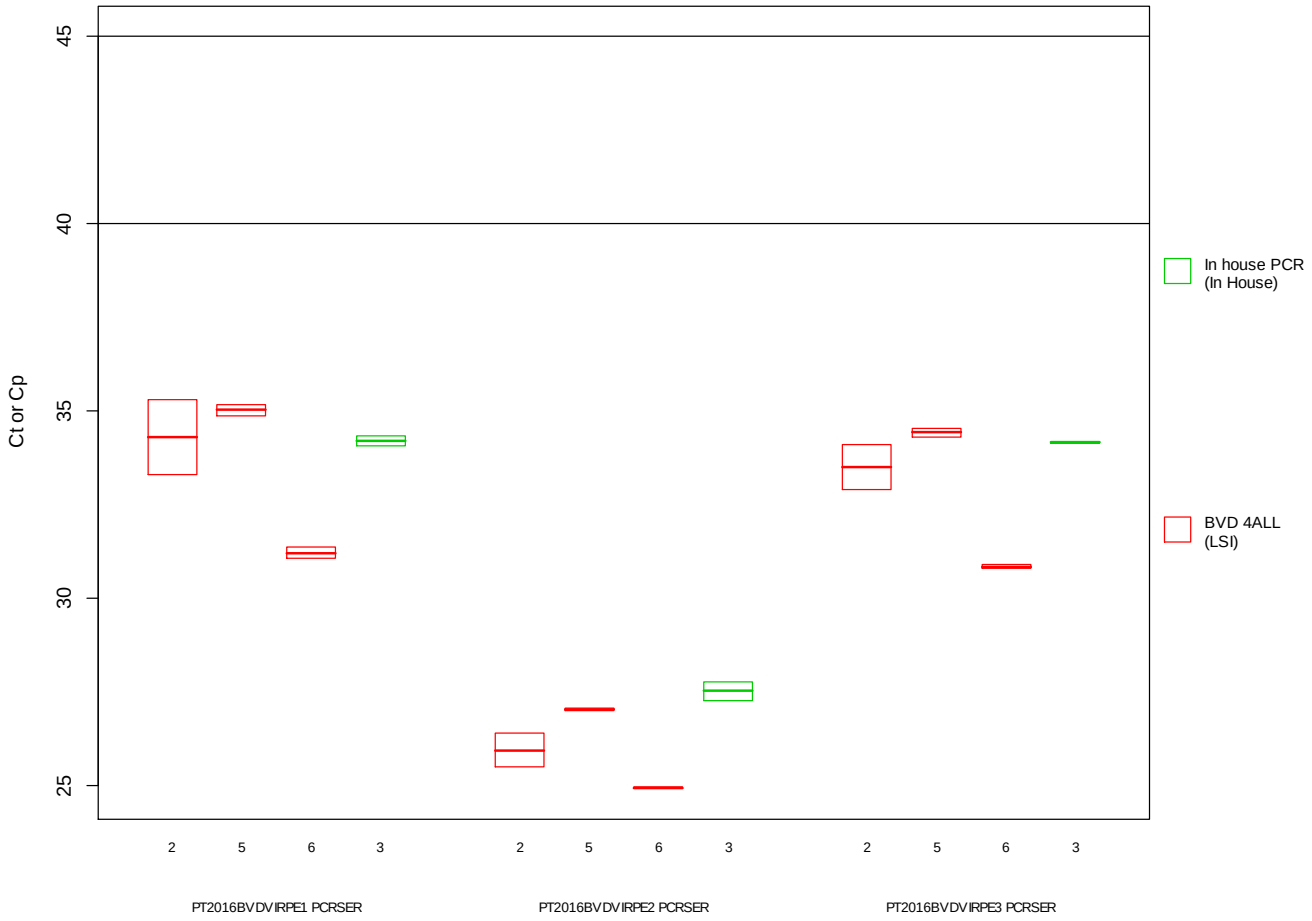

Figure 3. Box plots showing the Ct or Cp values per positive reference serum sample and per participating laboratory.
LAB2, LAB5 and LAB6 used the VetMax BVD4ALL Screening test kit from LSI (red box plots) but 2 different batches: batch 007 (LAB2 and LAB5), batch 008 (LAB6). LAB3 used an in house RT-qPCR (green box plots). Cut-off values (40-45) are shown by horizontal lines.

Figure 4 (RT-qPCR ear notch reference samples)

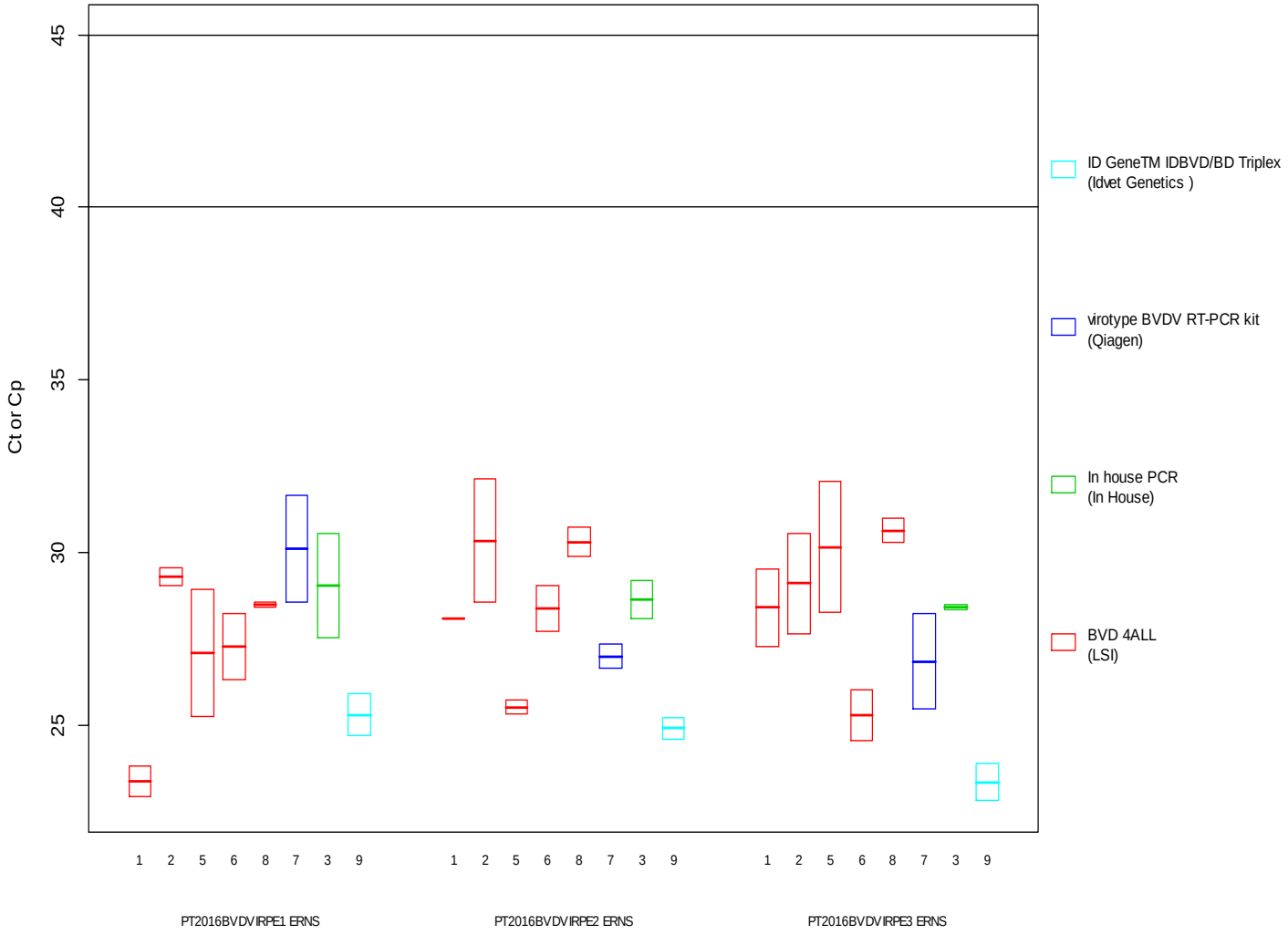

Figure 4. Box plots showing the Ct or Cp values per positive reference ear notch sample and per participating laboratory.
LAB1, LAB2, LAB5, LAB6 and LAB8 used the VetMax BVD4ALL Screening test kit from LSI (red box plots) [batch 007 (LAB2 and LAB5), batch 008 (LAB1, LAB6 and LAB8)]. LAB7 used the Virotype® BVDV RT-PCR kit from Qiagen batch 251120615 (dark blue box plots). LAB3 used an in house RT-qPCR (green box plots). LAB9 used the ID GeneTM IDBVD/BD Triplex from Idvet Genetics batch 7 (light blue box plots). Cut-off values (40-45) are shown by horizontal lines.
Remark : for LAB1 only the correctly classified aliquot for PT2016BVDVIRPE2 ERNS was taken into account for the boxplot.