Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium
T + 32 2 642 51 11 • F + 32 2 642 50 01
www.sciensano.be

081-PT

PROFICIENCY TESTING 2019

Bovine Viral Diarrhea Virus (BVDV)

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

(ii) Detection of BVDV-specific antibodies in bovine serum by ELISA

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE START PT: 21 OCTOBER 2019

DATE REPORT: 24 JANUARY 2020

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of (i) BVDV-specific antigens in bovine ear notch samples by antigen ELISA and/or RT-qPCR and/or (ii) BVDV-specific antibodies in bovine serum by antibody ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

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

III.2. Reference samples

LAB1, LAB2, LAB3 and LAB4 received 40 aliquots, namely 10 aliquots of the matrix ear notch samples to perform BVDV antigen ELISA, 10 aliquots of the matrix ear notch samples to perform BVDV RT-qPCR and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB5 received 20 aliquots, namely 10 aliquots of the matrix ear notch samples to perform BVDV antigen ELISA and 10 aliquots of the matrix ear notch samples to perform BVDV RT-qPCR.
LAB6 received 30 aliquots, namely 10 aliquots of the matrix ear notch samples to perform BVDV antigen ELISA and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB7 received 30 aliquots, namely 10 aliquots of the matrix ear notch samples to perform BVDV RT-qPCR and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB8 and LAB9 received 10 aliquots, namely 10 aliquots of the matrix ear notch samples to perform BVDV RT-qPCR.
LAB11, LAB12 and LAB13 received 20 aliquots, namely 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
Each matrix was sent in a different block/bag with reference samples (position 1-10 or 1-20) .

III.2.1. Reference ear notch samples for antigen detection by BVDV antigen ELISA

Replicates of 10 reference ear notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=5; coded 'PT2019BVDAgNE1', 'PT2019BVDAgNE2', 'PT2019BVDAgNE3', 'PT2019BVDAgNE4' and 'PT2019BVDAgNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2019BVDAgPE1', 'PT2019BVDAgPE2', 'PT2019BVDAgPE3', 'PT2019BVDAgPE4', and 'PT2019BVDAgPE5'), were used.
In total, 60 aliquots of reference ear notch samples were distributed to 6 participating laboratories. All laboratories received 10 aliquots: 1 aliquot of each reference ear notch sample. (Table 2 and Table 5).

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 the BVDV antigen ELISA kit from IDEXX.

The reference ear notch samples PT2019BVDAgNE1, PT2019BVDAgNE2, PT2019BVDAgNE3, PT2019BVDAgNE4 and PT2019BVDAgNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2019BVDAgPE1, PT2019BVDAgPE2, PT2019BVDAgPE3, PT2019BVDAgPE4, and PT2019BVDAgPE5 were field samples obtained from 5 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 the BVDV antigen ELISA kit from IDEXX and the in-house developed BVDV RT-qPCR assays, except for the PT2019BVDAgPE5 which was only tested with the BVDV antigen ELISA kit from IDEXX.

After aliquoting the different ear notch samples, a homogeneity check was performed on 10 aliquots of each reference ear notch sample using 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 by BVDV antigen ELISA. 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 BVDV antigen ELISA kit from IDEXX.

III.2.2. Reference ear notch samples for antigen detection by BVDV RT-qPCR

Replicates of 10 reference ear notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=5; coded 'PT2019BVDVIRNE1', 'PT2019BVDVIRNE2', 'PT2019BVDVIRNE3', 'PT2019BVDVIRNE4' and 'PT2019BVDVIRNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2019BVDVIRPE1', 'PT2019BVDVIRPE2', 'PT2019BVDVIRPE3', 'PT2019BVDVIRPE4' and 'PT2019BVDVIRPE5'), were used.
In total, 80 aliquots of reference ear notch samples were distributed to 8 participating laboratories. All laboratories received 10 aliquots: 1 aliquot of each reference ear notch sample. (Table 3 and Table 6).

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 assay.

The reference ear notch samples PT2019BVDVIRNE1, PT2019BVDVIRNE2, PT2019BVDVIRNE3, PT2019BVDVIRNE4 and PT2019BVDVIRNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2019BVDVIRPE1, PT2019BVDVIRPE2, PT2019BVDVIRPE3, PT2019BVDVIRPE4 and PT2019BVDVIRPE5 were field samples obtained from 5 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 the BVDV antigen ELISA kit from IDEXX and the in-house developed BVDV RT-qPCR assay, except for the PT2019BVDVIRPE5 which was only tested with the in-house developed BVDV RT-qPCR assays.

After aliquoting the different ear notch samples, a homogeneity check was performed on 10 aliquots of each reference ear notch sample using an in-house developed BVDV RT-qPCR, 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 by BVDV RT-qPCR. 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.

III.2.3. Reference serum samples for antibody detection by BVDV antibody ELISA

Replicates of 20 reference serum samples of bovine origin, either free from detectable BVDV-specific antibodies (n=3; coded 'PT2019BVDAbSERNS1', 'PT2019BVDAbSERNS2' and 'PT2019BVDAbSERNS3') or containing detectable BVDV-specific antibodies (n=7; coded 'PT2019BVDAbSERPS1', 'PT2019BVDAbSERPS2', 'PT2019BVDAbSERPS3', 'PT2019BVDAbSERPS4', 'PT2019BVDAbSERPS5', 'PT2019BVDAbSERPS6' and 'PT2019BVDAbSERPS7'), were used.
In total, 180 aliquots were distributed to 9 laboratories. All laboratories received 20 aliquots: 4 aliquots of the reference serum sample 'PT2019BVDAbSERNS1', 2 aliquots of the reference serum samples 'PT2019BVDAbSERNS2', 'PT2019BVDAbSERNS3', 'PT2019BVDAbSERPS1', 'PT2019BVDAbSERPS2', 'PT2019BVDAbSERPS3', 'PT2019BVDAbSERPS6' and 'PT2019BVDAbSERPS7' and 1 aliquot of the reference serum samples 'PT2019BVDAbSERPS4' and 'PT2019BVDAbSERPS5'. The positions of the reference serum samples in the sent blocks were randomized for each participant. (Table 4 and Table 7).

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 Diagnostics.

The reference serum samples PT2019BVDAbSERNS1, PT2019BVDAbSERNS2 and PT2019BVDAbSERNS3 were obtained from 3 animals from a BVDV-free unvaccinated herd. The reference serum samples PT2019BVDAbSERPS1, PT2019BVDAbSERPS2, PT2019BVDAbSERPS3, PT2019BVDAbSERPS4, PT2019BVDAbSERPS5 and PT2019BVDAbSERPS6 were obtained from 6 seropositive animals from different herds and had a BVD-type 1 VNT titer of 1/480, 1/60, 1/640, 1/60, 1/60, 1/160 respectively. The true individual status (infected or vaccinated) of the animals is unknown but all the samples have generally been detected positive in previous PT except PT2019BVDAbSERPS4 that may not be detected by anti-p80 (or anti-NS3) competitive ELISA. The reference serum sample PT2019BVDAbSERPS7 was obtained after dilution 1/160 in a negative serum sample of a strong seropositive sample from an animal experimentally infected with a BVD type 2. The BVD-type 2 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 PT2019BVDAbSERNS1, PT2019BVDAbSERNS2 and PT2019BVDAbSERNS3 were considered as negative sera, the reference serum samples PT2019BVDAbSERPS1, PT2019BVDAbSERPS2, PT2019BVDAbSERPS3 and PT2019BVDAbSERPS6 as positive sera and the reference serum samples PT2019BVDAbSERPS7 as weak positive sera in BVDV antibody ELISA.
The reference serum sample PT2019BVDAbSERPS4 was positive on the Monoscreen Ab BVD ELISA kit from BioX Diagnostics and the ID Screen BVD p80 Antibody Competition from IDVET and negative on the Bovine Viral Diarrhoea Virus Antibody Test Kit from Idexx. The reference serum sample PT2019BVDAbSERPS5 was positive on the Bovine Viral Diarrhoea Virus Antibody Test Kit from Idexx and doubtful or negative on the Monoscreen Ab BVD ELISA kit from BioX Diagnostics and the ID Screen BVD p80 Antibody Competition from IDVET. Consequently all the results (Positive/Doublful/Negative) are acceptable (NI status) for the reference serum samples PT2019BVDAbSERPS4 and PT2019BVDAbSERPS5.

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 Diagnostics, 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 Diagnostics.

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 each of the 10 aliquots of reference samples used for BVDV-specific antigens and the 20 aliquots of reference samples used for BVDV-specific antibodies.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 aliquots or 20 aliquots of reference samples is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

The reference serum and ear notch samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 21st of October 2019. LAB1, LAB2, LAB3, LAB4, LAB8 and LAB13 acknowledged receipt of the samples on the same day, whereas LAB5, LAB7, LAB9 and LAB11 received the samples on 22nd of October 2019 and LAB6 and LAB12 on 23th of October 2019.
After receiving the samples LAB6 informed us that he was not able to carry out the analysis.
Analyses were performed between 22nd and 30th of October 2019 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 25th and 31st of October 2019 (Table 1).
LAB3 did not submit results on time.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Participating laboratory	Reference samples received	Start of analysis Antigen ELISA	Start of analysis RT-qPCR	Start of analysis Antibody ELISA	Submission of the results (Excel file)
LAB1	21/10/2019	25/10/2019	28/10/2019	23/10/2019	31/10/2019
LAB2	21/10/2019	22/10/2019	22/10/2019	28/10/2019	31/10/2019
LAB3	21/10/2019	NA	NA	NA	NA
LAB4	21/10/2019	22/10/2019	28/10/2019	23/10/2019	25/10/2019 30/10/2019 (PCR)
LAB5	22/10/2019	24/10/2019	25/10/2019	NA	30/10/2019
LAB6	23/10/2019	NA	NA	NA	NA
LAB7	22/10/2019	NA	23/10/2019	29/10/2019	25/10/2019 (PCR) 30/10/2019
LAB8	21/10/2019	NA	23/10/2019	NA	28/10/2019
LAB9	22/10/2019	NA	30/10/2019	NA	31/10/2019
LAB11	22/10/2019	NA	NA	28-29/10/2019	30/10/2019
LAB12	23/10/2019	NA	NA	24/10/2019	29/10/2019
LAB13	21/10/2019	NA	NA	29/10/2019	30/10/2019

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories, except LAB5, 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 in ear notch**: all laboratories (LAB1, LAB2, LAB4 and LAB5) provided qualitative results that were in full agreement with the true status of the reference ear notch samples (100% of agreement) (Tabel 2).

(ii) For the detection of BVDV-specific antigens by **RT-qPCR in ear notch**: all laboratories (LAB1, LAB2, LAB4, LAB5, 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) (Tabel 3).

(iii) For the detection of BVDV-specific antibodies by **antibody ELISA in serum**: all laboratories (LAB1, LAB2, LAB4, LAB7, LAB11, LAB12 and LAB13) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement) (Tabel 4).

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

Table 2. Antigen 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 10 aliquots of reference ear notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	2	4	5
failure	0 (0)	0 (0)	0 (0)	0 (0)
success	10 (100)	10 (100)	10 (100)	10 (100)

Table 3. 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 10 aliquots of reference ear notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1	2	4	5	7	8	9
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

Table 4. Antibody 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1	2	4	7	11	12.1	12.2	13
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

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 ear notch samples.

(ii) For the detection of BVDV-specific antigens by **RT-qPCR** no variability between the participating laboratories could be observed since all participants correctly identified all reference ear notch samples.

(iii) For the detection of BVDV-specific antibodies by **antibody ELISA** no variability between the participating laboratories could be observed since all participants correctly identified all reference serum samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 5 for antigen ELISA, in Table 6 for RT-qPCR and in Table 7 for antibody ELISA.

Table 5 Antigen ELISA Ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the ear notch samples (SAMPLE), the external identification of the ear notch samples (LABPOSIT), and the status assigned by the BVDV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BVDAgPE5	POS	POS	1
2	1	2	PT2019BVDAgNE2	NEG	NEG	1
3	1	3	PT2019BVDAgPE1	POS	POS	1
4	1	4	PT2019BVDAgPE3	POS	POS	1
5	1	5	PT2019BVDAgNE4	NEG	NEG	1
6	1	6	PT2019BVDAgPE4	POS	POS	1
7	1	7	PT2019BVDAgNE3	NEG	NEG	1
8	1	8	PT2019BVDAgNE5	NEG	NEG	1
9	1	9	PT2019BVDAgPE2	POS	POS	1
10	1	10	PT2019BVDAgNE1	NEG	NEG	1
11	2	1	PT2019BVDAgPE5	POS	POS	1
12	2	2	PT2019BVDAgNE1	NEG	NEG	1
13	2	3	PT2019BVDAgPE2	POS	POS	1
14	2	4	PT2019BVDAgNE3	NEG	NEG	1
15	2	5	PT2019BVDAgNE5	NEG	NEG	1
16	2	6	PT2019BVDAgPE4	POS	POS	1
17	2	7	PT2019BVDAgNE2	NEG	NEG	1
18	2	8	PT2019BVDAgPE3	POS	POS	1
19	2	9	PT2019BVDAgNE4	NEG	NEG	1
20	2	10	PT2019BVDAgPE1	POS	POS	1
21	4	1	PT2019BVDAgPE5	POS	POS	1
22	4	2	PT2019BVDAgNE1	NEG	NEG	1
23	4	3	PT2019BVDAgPE2	POS	POS	1
24	4	4	PT2019BVDAgNE3	NEG	NEG	1
25	4	5	PT2019BVDAgNE5	NEG	NEG	1
26	4	6	PT2019BVDAgPE4	POS	POS	1
27	4	7	PT2019BVDAgNE2	NEG	NEG	1
28	4	8	PT2019BVDAgPE3	POS	POS	1
29	4	9	PT2019BVDAgNE4	NEG	NEG	1
30	4	10	PT2019BVDAgPE1	POS	POS	1
31	5	1	PT2019BVDAgPE5	POS	POS	1
32	5	2	PT2019BVDAgNE2	NEG	NEG	1
33	5	3	PT2019BVDAgPE1	POS	POS	1
34	5	4	PT2019BVDAgPE3	POS	POS	1
35	5	5	PT2019BVDAgNE4	NEG	NEG	1
36	5	6	PT2019BVDAgPE4	POS	POS	1
37	5	7	PT2019BVDAgNE3	NEG	NEG	1
38	5	8	PT2019BVDAgNE5	NEG	NEG	1
39	5	9	PT2019BVDAgPE2	POS	POS	1
40	5	10	PT2019BVDAgNE1	NEG	NEG	1

Table 6 RT-qPCR Ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the ear notch samples (SAMPLE), the external identification of the ear notch samples (LABPOSIT), and the status assigned by the BVDV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BVDVIRNE1	NEG	NEG	1
2	1	2	PT2019BVDVIRPE3	POS	POS	1
3	1	3	PT2019BVDVIRNE4	NEG	NEG	1
4	1	4	PT2019BVDVIRPE1	POS	POS	1
5	1	5	PT2019BVDVIRPE5	POS	POS	1
6	1	6	PT2019BVDVIRNE2	NEG	NEG	1
7	1	7	PT2019BVDVIRPE4	POS	POS	1
8	1	8	PT2019BVDVIRNE3	NEG	NEG	1
9	1	9	PT2019BVDVIRPE2	POS	POS	1
10	1	10	PT2019BVDVIRNE5	NEG	NEG	1
11	2	1	PT2019BVDVIRPE3	POS	POS	1
12	2	2	PT2019BVDVIRPE5	POS	POS	1
13	2	3	PT2019BVDVIRNE2	NEG	NEG	1
14	2	4	PT2019BVDVIRPE2	POS	POS	1
15	2	5	PT2019BVDVIRNE4	NEG	NEG	1
16	2	6	PT2019BVDVIRNE5	NEG	NEG	1
17	2	7	PT2019BVDVIRPE4	POS	POS	1
18	2	8	PT2019BVDVIRNE3	NEG	NEG	1
19	2	9	PT2019BVDVIRNE1	NEG	NEG	1
20	2	10	PT2019BVDVIRPE1	POS	POS	1
21	4	1	PT2019BVDVIRPE3	POS	POS	1
22	4	2	PT2019BVDVIRPE5	POS	POS	1
23	4	3	PT2019BVDVIRNE2	NEG	NEG	1
24	4	4	PT2019BVDVIRPE2	POS	POS	1
25	4	5	PT2019BVDVIRNE4	NEG	NEG	1
26	4	6	PT2019BVDVIRNE5	NEG	NEG	1
27	4	7	PT2019BVDVIRPE4	POS	POS	1
28	4	8	PT2019BVDVIRNE3	NEG	NEG	1
29	4	9	PT2019BVDVIRNE1	NEG	NEG	1
30	4	10	PT2019BVDVIRPE1	POS	POS	1
31	5	1	PT2019BVDVIRNE1	NEG	NEG	1
32	5	2	PT2019BVDVIRPE3	POS	POS	1
33	5	3	PT2019BVDVIRNE4	NEG	NEG	1
34	5	4	PT2019BVDVIRPE1	POS	POS	1
35	5	5	PT2019BVDVIRPE5	POS	POS	1
36	5	6	PT2019BVDVIRNE2	NEG	NEG	1
37	5	7	PT2019BVDVIRPE4	POS	POS	1
38	5	8	PT2019BVDVIRNE3	NEG	NEG	1
39	5	9	PT2019BVDVIRPE2	POS	POS	1
40	5	10	PT2019BVDVIRNE5	NEG	NEG	1
41	7	1	PT2019BVDVIRPE3	POS	POS	1
42	7	2	PT2019BVDVIRPE5	POS	POS	1
43	7	3	PT2019BVDVIRNE2	NEG	NEG	1
44	7	4	PT2019BVDVIRPE2	POS	POS	1
45	7	5	PT2019BVDVIRNE4	NEG	NEG	1
46	7	6	PT2019BVDVIRNE5	NEG	NEG	1
47	7	7	PT2019BVDVIRPE4	POS	POS	1
48	7	8	PT2019BVDVIRNE3	NEG	NEG	1
49	7	9	PT2019BVDVIRNE1	NEG	NEG	1
50	7	10	PT2019BVDVIRPE1	POS	POS	1
51	8	1	PT2019BVDVIRNE1	NEG	NEG	1
52	8	2	PT2019BVDVIRPE3	POS	POS	1
53	8	3	PT2019BVDVIRNE4	NEG	NEG	1
54	8	4	PT2019BVDVIRPE1	POS	POS	1
55	8	5	PT2019BVDVIRPE5	POS	POS	1
56	8	6	PT2019BVDVIRNE2	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
57	8	7	PT2019BVDVIRPE4	POS	POS	1
58	8	8	PT2019BVDVIRNE3	NEG	NEG	1
59	8	9	PT2019BVDVIRPE2	POS	POS	1
60	8	10	PT2019BVDVIRNE5	NEG	NEG	1
61	9	1	PT2019BVDVIRPE3	POS	POS	1
62	9	2	PT2019BVDVIRPE5	POS	POS	1
63	9	3	PT2019BVDVIRNE2	NEG	NEG	1
64	9	4	PT2019BVDVIRPE2	POS	POS	1
65	9	5	PT2019BVDVIRNE4	NEG	NEG	1
66	9	6	PT2019BVDVIRNE5	NEG	NEG	1
67	9	7	PT2019BVDVIRPE4	POS	POS	1
68	9	8	PT2019BVDVIRNE3	NEG	NEG	1
69	9	9	PT2019BVDVIRNE1	NEG	NEG	1
70	9	10	PT2019BVDVIRPE1	POS	POS	1

Table 7 Antibody ELISA Serum: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the BVDV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; NI (non-interpretable / doubtful); POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BVDAbSERNS1	NEG	NEG	1
2	1	2	PT2019BVDAbSERNS2	NEG	NEG	1
3	1	3	PT2019BVDAbSERNS2	NEG	NEG	1
4	1	4	PT2019BVDAbSERPS2	POS	POS	1
5	1	5	PT2019BVDAbSERNS1	NEG	NEG	1
6	1	6	PT2019BVDAbSERPS3	POS	POS	1
7	1	7	PT2019BVDAbSERNS3	NEG	NEG	1
8	1	8	PT2019BVDAbSERPS6	POS	POS	1
9	1	9	PT2019BVDAbSERPS7	POS	POS	1
10	1	10	PT2019BVDAbSERPS2	POS	POS	1
11	1	11	PT2019BVDAbSERNS3	NEG	NEG	1
12	1	12	PT2019BVDAbSERPS4	NI	POS	1
13	1	13	PT2019BVDAbSERNS1	NEG	NEG	1
14	1	14	PT2019BVDAbSERPS1	POS	POS	1
15	1	15	PT2019BVDAbSERPS5	NI	NEG	1
16	1	16	PT2019BVDAbSERNS1	NEG	NEG	1
17	1	17	PT2019BVDAbSERPS7	POS	POS	1
18	1	18	PT2019BVDAbSERPS6	POS	POS	1
19	1	19	PT2019BVDAbSERPS3	POS	POS	1
20	1	20	PT2019BVDAbSERPS1	POS	POS	1
21	2	1	PT2019BVDAbSERPS1	POS	POS	1
22	2	2	PT2019BVDAbSERNS2	NEG	NEG	1
23	2	3	PT2019BVDAbSERPS2	POS	POS	1
24	2	4	PT2019BVDAbSERPS6	POS	POS	1
25	2	5	PT2019BVDAbSERPS5	NI	NEG	1
26	2	6	PT2019BVDAbSERPS3	POS	POS	1
27	2	7	PT2019BVDAbSERNS1	NEG	NEG	1
28	2	8	PT2019BVDAbSERNS3	NEG	NEG	1
29	2	9	PT2019BVDAbSERPS7	POS	POS	1
30	2	10	PT2019BVDAbSERPS2	POS	POS	1
31	2	11	PT2019BVDAbSERPS7	POS	POS	1
32	2	12	PT2019BVDAbSERNS1	NEG	NEG	1
33	2	13	PT2019BVDAbSERPS3	POS	POS	1
34	2	14	PT2019BVDAbSERNS3	NEG	NEG	1
35	2	15	PT2019BVDAbSERPS6	POS	POS	1
36	2	16	PT2019BVDAbSERNS2	NEG	NEG	1
37	2	17	PT2019BVDAbSERPS4	NI	POS	1
38	2	18	PT2019BVDAbSERPS1	POS	POS	1
39	2	19	PT2019BVDAbSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
40	2	20	PT2019BVDAbSERNS1	NEG	NEG	1
41	4	1	PT2019BVDAbSERPS1	POS	POS	1
42	4	2	PT2019BVDAbSERNS2	NEG	NEG	1
43	4	3	PT2019BVDAbSERPS2	POS	POS	1
44	4	4	PT2019BVDAbSERPS6	POS	POS	1
45	4	5	PT2019BVDAbSERPS5	NI	NEG	1
46	4	6	PT2019BVDAbSERPS3	POS	POS	1
47	4	7	PT2019BVDAbSERNS1	NEG	NEG	1
48	4	8	PT2019BVDAbSERNS3	NEG	NEG	1
49	4	9	PT2019BVDAbSERPS7	POS	POS	1
50	4	10	PT2019BVDAbSERPS2	POS	POS	1
51	4	11	PT2019BVDAbSERPS7	POS	POS	1
52	4	12	PT2019BVDAbSERNS1	NEG	NEG	1
53	4	13	PT2019BVDAbSERPS3	POS	POS	1
54	4	14	PT2019BVDAbSERNS3	NEG	NEG	1
55	4	15	PT2019BVDAbSERPS6	POS	POS	1
56	4	16	PT2019BVDAbSERNS2	NEG	NEG	1
57	4	17	PT2019BVDAbSERPS4	NI	POS	1
58	4	18	PT2019BVDAbSERPS1	POS	POS	1
59	4	19	PT2019BVDAbSERNS1	NEG	NEG	1
60	4	20	PT2019BVDAbSERNS1	NEG	NEG	1
61	7	1	PT2019BVDAbSERPS1	POS	POS	1
62	7	2	PT2019BVDAbSERNS2	NEG	NEG	1
63	7	3	PT2019BVDAbSERPS2	POS	POS	1
64	7	4	PT2019BVDAbSERPS6	POS	POS	1
65	7	5	PT2019BVDAbSERPS5	NI	NI	1
66	7	6	PT2019BVDAbSERPS3	POS	POS	1
67	7	7	PT2019BVDAbSERNS1	NEG	NEG	1
68	7	8	PT2019BVDAbSERNS3	NEG	NEG	1
69	7	9	PT2019BVDAbSERPS7	POS	POS	1
70	7	10	PT2019BVDAbSERPS2	POS	POS	1
71	7	11	PT2019BVDAbSERPS7	POS	POS	1
72	7	12	PT2019BVDAbSERNS1	NEG	NEG	1
73	7	13	PT2019BVDAbSERPS3	POS	POS	1
74	7	14	PT2019BVDAbSERNS3	NEG	NEG	1
75	7	15	PT2019BVDAbSERPS6	POS	POS	1
76	7	16	PT2019BVDAbSERNS2	NEG	NEG	1
77	7	17	PT2019BVDAbSERPS4	NI	POS	1
78	7	18	PT2019BVDAbSERPS1	POS	POS	1
79	7	19	PT2019BVDAbSERNS1	NEG	NEG	1
80	7	20	PT2019BVDAbSERNS1	NEG	NEG	1
81	11	1	PT2019BVDAbSERNS1	NEG	NEG	1
82	11	2	PT2019BVDAbSERNS2	NEG	NEG	1
83	11	3	PT2019BVDAbSERNS2	NEG	NEG	1
84	11	4	PT2019BVDAbSERPS2	POS	POS	1
85	11	5	PT2019BVDAbSERNS1	NEG	NEG	1
86	11	6	PT2019BVDAbSERPS3	POS	POS	1
87	11	7	PT2019BVDAbSERNS3	NEG	NEG	1
88	11	8	PT2019BVDAbSERPS6	POS	POS	1
89	11	9	PT2019BVDAbSERPS7	POS	POS	1
90	11	10	PT2019BVDAbSERPS2	POS	POS	1
91	11	11	PT2019BVDAbSERNS3	NEG	NEG	1
92	11	12	PT2019BVDAbSERPS4	NI	POS	1
93	11	13	PT2019BVDAbSERNS1	NEG	NEG	1
94	11	14	PT2019BVDAbSERPS1	POS	POS	1
95	11	15	PT2019BVDAbSERPS5	NI	NEG	1
96	11	16	PT2019BVDAbSERNS1	NEG	NEG	1
97	11	17	PT2019BVDAbSERPS7	POS	POS	1
98	11	18	PT2019BVDAbSERPS6	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
99	11	19	PT2019BVDAbSERPS3	POS	POS	1
100	11	20	PT2019BVDAbSERPS1	POS	POS	1
101	12.1	1	PT2019BVDAbSERPS1	POS	POS	1
102	12.1	2	PT2019BVDAbSERNS2	NEG	NEG	1
103	12.1	3	PT2019BVDAbSERPS2	POS	POS	1
104	12.1	4	PT2019BVDAbSERPS6	POS	POS	1
105	12.1	5	PT2019BVDAbSERPS5	NI	NEG	1
106	12.1	6	PT2019BVDAbSERPS3	POS	POS	1
107	12.1	7	PT2019BVDAbSERNS1	NEG	NEG	1
108	12.1	8	PT2019BVDAbSERNS3	NEG	NEG	1
109	12.1	9	PT2019BVDAbSERPS7	POS	POS	1
110	12.1	10	PT2019BVDAbSERPS2	POS	POS	1
111	12.1	11	PT2019BVDAbSERPS7	POS	POS	1
112	12.1	12	PT2019BVDAbSERNS1	NEG	NEG	1
113	12.1	13	PT2019BVDAbSERPS3	POS	POS	1
114	12.1	14	PT2019BVDAbSERNS3	NEG	NEG	1
115	12.1	15	PT2019BVDAbSERPS6	POS	POS	1
116	12.1	16	PT2019BVDAbSERNS2	NEG	NEG	1
117	12.1	17	PT2019BVDAbSERPS4	NI	NEG	1
118	12.1	18	PT2019BVDAbSERPS1	POS	POS	1
119	12.1	19	PT2019BVDAbSERNS1	NEG	NEG	1
120	12.1	20	PT2019BVDAbSERNS1	NEG	NEG	1
121	12.2	1	PT2019BVDAbSERPS1	POS	POS	1
122	12.2	2	PT2019BVDAbSERNS2	NEG	NEG	1
123	12.2	3	PT2019BVDAbSERPS2	POS	POS	1
124	12.2	4	PT2019BVDAbSERPS6	POS	POS	1
125	12.2	5	PT2019BVDAbSERPS5	NI	POS	1
126	12.2	6	PT2019BVDAbSERPS3	POS	POS	1
127	12.2	7	PT2019BVDAbSERNS1	NEG	NEG	1
128	12.2	8	PT2019BVDAbSERNS3	NEG	NEG	1
129	12.2	9	PT2019BVDAbSERPS7	POS	POS	1
130	12.2	10	PT2019BVDAbSERPS2	POS	POS	1
131	12.2	11	PT2019BVDAbSERPS7	POS	POS	1
132	12.2	12	PT2019BVDAbSERNS1	NEG	NEG	1
133	12.2	13	PT2019BVDAbSERPS3	POS	POS	1
134	12.2	14	PT2019BVDAbSERNS3	NEG	NEG	1
135	12.2	15	PT2019BVDAbSERPS6	POS	POS	1
136	12.2	16	PT2019BVDAbSERNS2	NEG	NEG	1
137	12.2	17	PT2019BVDAbSERPS4	NI	POS	1
138	12.2	18	PT2019BVDAbSERPS1	POS	POS	1
139	12.2	19	PT2019BVDAbSERNS1	NEG	NEG	1
140	12.2	20	PT2019BVDAbSERNS1	NEG	NEG	1
141	13	1	PT2019BVDAbSERNS1	NEG	NEG	1
142	13	2	PT2019BVDAbSERNS2	NEG	NEG	1
143	13	3	PT2019BVDAbSERNS2	NEG	NEG	1
144	13	4	PT2019BVDAbSERPS2	POS	POS	1
145	13	5	PT2019BVDAbSERNS1	NEG	NEG	1
146	13	6	PT2019BVDAbSERPS3	POS	POS	1
147	13	7	PT2019BVDAbSERNS3	NEG	NEG	1
148	13	8	PT2019BVDAbSERPS6	POS	POS	1
149	13	9	PT2019BVDAbSERPS7	POS	POS	1
150	13	10	PT2019BVDAbSERPS2	POS	POS	1
151	13	11	PT2019BVDAbSERNS3	NEG	NEG	1
152	13	12	PT2019BVDAbSERPS4	NI	POS	1
153	13	13	PT2019BVDAbSERNS1	NEG	NEG	1
154	13	14	PT2019BVDAbSERPS1	POS	POS	1
155	13	15	PT2019BVDAbSERPS5	NI	NEG	1
156	13	16	PT2019BVDAbSERNS1	NEG	NEG	1
157	13	17	PT2019BVDAbSERPS7	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
158	13	18	PT2019BVDAbSERPS6	POS	POS	1
159	13	19	PT2019BVDAbSERPS3	POS	POS	1
160	13	20	PT2019BVDAbSERPS1	POS	POS	1

V. Discussion

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

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 participating laboratories used the ELISA kit BVDV Ag/Serum Plus Test from IDEXX, but 4 different batches were used: batch Q251 (LAB1), batch P321 (LAB2), batch P211 (LAB4) and batch Q211 (LAB5).

For the detection of BVDV-specific antigens by RT-qPCR 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 RT-qPCR, all participating laboratories, except LAB5, used a commercially available BVDV RT-qPCR: LAB1, LAB2 and LAB8 used the VetMax BVD4ALL Screening test kit from Thermofisher Scientific (LSI) (batch 017), LAB4 used the Virotype BVDV PCR kit from Indical Bioscience (batch F201900031), LAB7 used the VetMAX BVDV Screening kit from Thermofisher (batch B12S-135) and LAB9 used the Bio-T kit BVDV/BVD Universal kit from Biosellal (batch BVDVU-05D). LAB5 used an in house RT-qPCR (batch 42F389/35D341).

For the detection of BVDV-specific antibodies in reference serum samples, 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 antibodies by antibody ELISA: LAB1 and LAB7 used the ID Screen BVD p80 Antibody Competition ELISA kit from IDVET (batch C89 and G02), LAB2, LAB4 and LAB13 used the Monoscreen Ab ELISA BVDV kit from BioX Diagnostics/Adiagene (batch 149B19 and 19B19), LAB11 used the CIVTEST BOVIS BVD/BD P80 ELISA kit from HIPRA (batch CVD.03L6) and LAB12 used two ELISA kits from Thermo Fisher Scientific the PrioCHECK™ Rum. BVD p80 Ab Serum & Milk Kit (batch BB181101L) and the PrioCHECK™ Bovine BVDV Ab Plate Kit (batch D190201L).

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 the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.).
Consequently, all participating laboratories achieved a satisfactory performance for the detection of BVDV-specific antigens by antigen ELISA and/or RT-qPCR in ear notch samples and for the detection of BVDV-specific antibodies by antibody ELISA in serum samples.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

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

Biosellal (Dardilly, France)

BIO-X Diagnostics S.A. (Rochefort, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

HIPRA Scientific SLU (Girona, Spain)

IDEXX technologies GmbH (Bäch, Switzerland)

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

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

Lavetan NV (Turnhout, Belgium)

Thermo Fisher Scientific Prionics AG (Schlieren, Switzerland)

Sciensano (Ukkel, Belgium)

Zoetis France (Lyon, France)

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 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 antibody ELISA, box plots of the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory are shown in Figure 1. The samples PT2019BVDAbSERPS4 and PT2019BVDAbSERPS5 are not shown because there was only one aliquot of these.

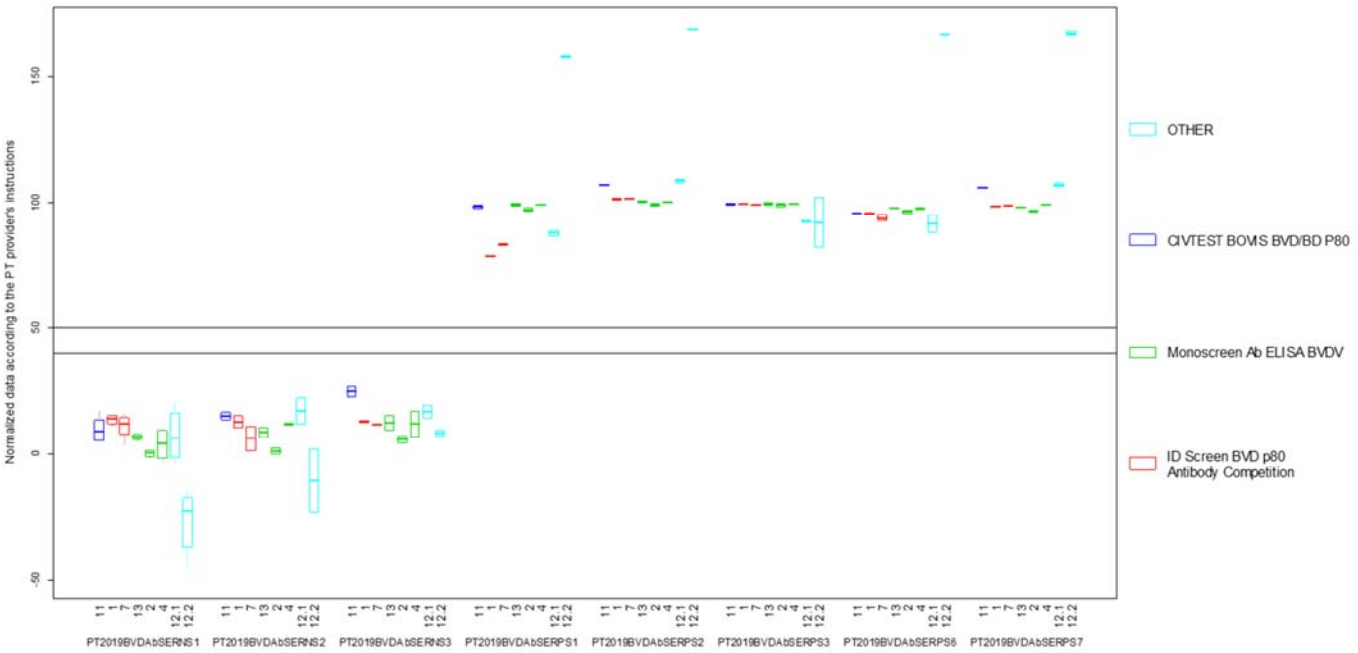

Figure 1. Box plots showing the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory.
LAB1 and LAB7 used the ID Screen BVD p80 Antibody Competition ELISA kit from IDVET, LAB2, LAB4 and LAB13 used the Monoscreen Ab ELISA BVDV kit from BioX Diagnostics/Adiagene, LAB11 used the CIVTEST BOVIS BVD/BD P80 ELISA kit from HIPRA and LAB12 used two ELISA kits from Thermo Fisher Scientific the PrioCHECK™ Rum. BVD p80 Ab Serum & Milk Kit and the PrioCHECK™ Bovine BVDV Ab Plate Kit.
Cut-off values applied by the participating laboratories are shown by horizontal lines (IDVet 40-50, BioX Diagnostics/Adiagene 50, HIPRA 50 and Thermo Fisher Scientific 50).