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172-PT

PROFICIENCY TESTING 2017

Bovine Viral Diarrhea Virus (BVDV)

(i) Detection of BVDV-specific antigens in bovine serum and/or ear notch samples 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

CODA-CERVA-UCCLE

DATE BEGIN PT: 23 OCTOBER 2017

DATE REPORT: 12 FEBRUARY 2018

THIS REPORT REPLACES AND CANCELS THE PREVIOUS REPORT PT2017BVDVIR+SER

Reason : change in the status of the serum sample PT2017BVDAbSERPS4 and possible results for the serum sample PT2017BVDAbSERPS7

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 (i) BVDV-specific antigens in bovine serum and/or 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 and LAB3 received 60 aliquots, namely 10 aliquots of the matrix serum and ear notch samples to perform BVDV antigen ELISA and BVDV RT-qPCR and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB6 received 50 aliquots, namely 10 aliquots of the matrix serum and 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.
LAB4 received 40 aliquots, namely 10 aliquots of the matrix serum and 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 antigen ELISA and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB12 received 30 aliquots, namely 10 aliquots of the matrix serum samples to perform BVDV RT-qPCR and 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB5, LAB8, LAB9, LAB10 and LAB11 received 20 aliquots, namely 10 aliquots of the matrix serum and ear notch samples to perform BVDV RT- qPCR.
LAB16, LAB17 and LAB18 received 20 aliquots of the matrix serum samples to perform BVDV antibody ELISA.
LAB13 received 10 aliquots of the matrix serum samples to perform BVDV RT- qPCR.
LAB14 and LAB15 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 or 1-20) .

III.2.1. Reference serum samples for antigen detection by BVDV antigen ELISA

Replicates of 5 reference serum samples of bovine origin, either free from detectable BVDV-specific antigens (n=2 coded 'PT2017BVDAgSERNS1' and 'PT2017BVDAgSERNS2') or containing detectable BVDV-specific antigens (n=3 coded 'PT2017BVDAgSERPS1', 'PT2017BVDAgSERPS2', 'PT2017BVDAgSERPS3'), were used.
In total, 50 aliquots of reference serum samples were distributed to 5 participating laboratories. All laboratories received 10 aliquots: 3 aliquots of the reference serum samples 'PT2017BVDAgSERNS1', 2 aliquots of the reference serum samples 'PT2017BVDAgSERNS2', 'PT2017BVDAgSERPS2' and 'PT2017BVDAgSERPS3' and 1 aliquots of the reference serum sample PT2017BVDAgSERPS1. The positions of the reference serum samples in the sent blocks were randomized for each participant. (Table 2 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 (ii) the results obtained by the BVDV antigen test kit/serum plus ELISA from IDEXX (pre-verification).

The reference serum samples PT2017BVDAgSERNS1 and PT2017BVDAgSERNS2 were obtained from 2 different BVDV-free animals from the field. The reference serum samples PT2017BVDAgSERPS1 and PT2017BVDAgSERPS2 were obtained from 2 different calves that were classified as immunotolerant persistently (BVDV-21) infected (IPI) animals. The reference serum sample PT2017BVDAgSERPS3 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.
After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of the reference serum samples using the BVDV antigen ELISA kit from IDEXX, 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 by BVDV antigen ELISA . 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 BVDV antigen ELISA kit from IDEXX.

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

Replicates of 5 reference serum samples of bovine origin, either free from detectable BVDV-specific antigens (n=2 coded 'PT2017BVDVIRSERNS1' and 'PT2017BVDVIRSERNS2') or containing detectable BVDV-specific antigens (n=3 coded 'PT2017BVDVIRSERPS1', 'PT2017BVDVIRSERPS2', 'PT2017BVDVIRSERPS3'), were used.
In total, 100 aliquots of reference serum samples were distributed to 10 participating laboratories. All laboratories received 10 aliquots: 2 aliquots of each reference serum samples. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 4 and Table 9).

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 (pre-verification).

The reference serum samples PT2017BVDVIRSERNS1 and PT2017BVDVIRSERNS2 were obtained from 2 different BVDV-free animals from the field. The reference serum samples PT2017BVDVIRSERPS1, PT2017BVDVIRSERPS2 and PT2017BVDVIRSERPS3 were obtained from 3 different calves that were classified as immunotolerant persistently (BVDV-1) infected (IPI) animals. The reference serum sample PT2017BVDVIRSERPS3 was a 1/1000 dilution in serum from a BVDV-free animal from the field.
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 hereby obtaining the same qualitative result for all aliquots of the same reference serum sample. Consequently, all reference serum samples, behalve PT2017BVDVIRSERPS1, were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BVDV-specific antigens by BVDV RT-qPCR in bovine serum. For serum sample PT2017BVDVIRSERPS1 positive, non-interpretable (doubtful) or negative results will be acceptable. 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.

III.2.3. 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 'PT2017BVDAgNE1', 'PT2017BVDAgNE2', 'PT2017BVDAgNE3', 'PT2017BVDAgNE4' and 'PT2017BVDAgNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2017BVDAgPE1', 'PT2017BVDAgPE2', 'PT2017BVDAgPE3', 'PT2017BVDAgPE4', and 'PT2017BVDAgPE5'), were used.
In total, 60 aliquots of reference ear notch samples were distributed to 6 participating laboratories. All laboratories received 10 aliquots: 1 aliquots of each reference ear notch samples. (Table 3 and Table 8).

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 test kit/serum plus ELISA from IDEXX.

The reference ear notch samples PT2017BVDAgNE1, PT2017BVDAgNE2, PT2017BVDAgNE3, PT2017BVDAgNE4 and PT2017BVDAgNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2017BVDAgPE1, PT2017BVDAgPE2, PT2017BVDAgPE3, PT2017BVDAgPE4, and PT2017BVDAgPE5 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 PT2017BVDAgPE5 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.2.4. 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 'PT2017BVDVIRNE1', 'PT2017BVDVIRNE2', 'PT2017BVDVIRNE3', 'PT2017BVDVIRNE4' and 'PT2017BVDVIRNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2017BVDVIRPE1', 'PT2017BVDVIRPE2', 'PT2017BVDVIRPE3', 'PT2017BVDVIRPE4' and 'PT2017BVDVIRPE5'), were used.
In total, 110 aliquots of reference ear notch samples were distributed to 11 participating laboratories. All laboratories received 10 aliquots: 1 aliquots of each reference ear notch samples. (Table 5 and Table 10).

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.

The reference ear notch samples PT2017BVDVIRNE1, PT2017BVDVIRNE2, PT2017BVDVIRNE3, PT2017BVDVIRNE4 and PT2017BVDVIRNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2017BVDVIRPE1, PT2017BVDVIRPE2, PT2017BVDVIRPE3, PT2017BVDVIRPE4 and PT2017BVDVIRPE5 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 PT2017BVDVIRPE5 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.2.5. Reference serum samples for antibody detection by BVDV antibody ELISA

Replicates of 10 reference serum samples of bovine origin, either free from detectable BVDV-specific antibodies (n=3; coded 'PT2017BVDAbSERNS1', 'PT2017BVDAbSERNS2' and 'PT2017BVDAbSERNS3') or containing detectable BVDV-specific antibodies (n=7; coded 'PT2017BVDAbSERPS1', 'PT2017BVDAbSERPS2', 'PT2017BVDAbSERPS3', 'PT2017BVDAbSERPS4', 'PT2017BVDAbSERPS5', 'PT2017BVDAbSERPS6' and 'PT2017BVDAbSERPS7'), were used.
In total, 200 aliquots were distributed to 10 laboratories. All laboratories received 20 aliquots: 5 aliquots of the reference serum samples 'PT2017BVDAbSERNS3' and 'PT2017BVDAbSERPS7', 3 aliquots of the reference serum sample 'PT2017BVDAbSERPS6' and 1 aliquots of the reference serum samples 'PT2017BVDAbSERNS1', 'PT2017BVDAbSERNS2', 'PT2017BVDAbSERPS1', 'PT2017BVDAbSERPS2', 'PT2017BVDAbSERPS3', 'PT2017BVDAbSERPS4' and 'PT2017BVDAbSERPS5' . The positions of the reference serum samples in the sent blocks were randomized for each participant. (Table 6 and Table 11).

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 PT2017BVDAbSERNS1, PT2017BVDAbSERNS2 and PT2017BVDAbSERNS3 were obtained from 3 animals from a BVDV-free unvaccinated herd. The reference serum samples PT2017BVDAbSERPS1, PT2017BVDAbSERPS2, PT2017BVDAbSERPS3, PT2017BVDAbSERPS4, PT2017BVDAbSERPS5 and PT2017BVDAbSERPS6 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 PT2017BVDAbSERPS4 that may not be detected by anti-p80 (or anti-NS3) competitive ELISA. The reference serum sample PT2017BVDAbSERPS7 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 PT2017BVDAbSERNS1, PT2017BVDAbSERNS2 and PT2017BVDAbSERNS3 were considered as negative sera and the reference serum samples PT2017BVDAbSERPS1, PT2017BVDAbSERPS2, PT2017BVDAbSERPS3, PT2017BVDAbSERPS5 and PT2017BVDAbSERPS6 as positive sera in BVDV antibody ELISA. The reference serum samples PT2017BVDAbSERPS7 was considered as weak positive serum in BVDV antibody ELISA. Therefore positive and non-interpretable (doubtful) ELISA results were accepted but negative results were rejected. The reference serum samples PT2017BVDAbSERPS4 was considered as doubtful serum in BVDV antibody ELISA. Therefore positive, non-interpretable (doubtful) or negative results were accepted.

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 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 CODA-CERVA-Uccle.

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 23th of October 2017. LAB2, LAB3, LAB4, LAB5, LAB6, LAB8, LAB14 and LAB15 acknowledged receipt of the samples on the same day, whereas LAB1, LAB7, LAB10, LAB11, LAB12, LAB13, LAB16 and LAB18 received the samples on 24th of October 2017 and LAB9 and LAB17 on 25th of October 2017.
Analyses were performed between 24th of October and 10th of November 2017 (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 6th and 10th of November 2017 (Table 1). All participants hereby respected the deadline of 10th of November 2017 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 Antigen ELISA	Start of analysis RT-qPCR	Start of analysis Antibody ELISA	Submission of the results (Excel file)
LAB1	24/10/2017	27/10/2017 (serum+ear-notch)	02/11/2017 (serum+ear-notch)	26/10/2017	07/11/2017
LAB2	23/10/2017	25/10/2017 (serum+ear-notch)	07/11/2017 (serum) 30/10/2017(ear-notch)	27/10/2017	09/11/2017
LAB3	23/10/2017	27/10/2017 (serum) 27+31/10/2017 (ear-notch)	26/10/2017 (serum+ear-notch)	26/10/2017	10/11/2017
LAB4	23/10/2017	24/10/2017 (serum+ear-notch)	NA	25/10/2017	10/11/2017
LAB5	23/10/2017	NA	24/10/2017 (serum+ear-notch)	NA	10/11/2017
LAB6	23/10/2017	30/10/2017 (serum+ear-notch)	NA (serum) 06/11/2017(ear-notch)	07/11/2017	8/11/2017
LAB7	24/10/2017	NA (serum) 10/11/2017 (ear-notch)	NA	08/11/2017	09+10/11/2017
LAB8	23/10/2017	NA	25/10/2017 (serum+ear-notch)	NA	07/11/2017
LAB9	25/10/2017	NA	07/11/2017 (serum+ear-notch)	NA	09/11/2017
LAB10	24/10/2017	NA	31/10/2017 (serum+ear-notch)	NA	08/11/2017
LAB11	24/10/2017	NA	02/11/2017 (serum+ear-notch)	NA	06/11/2017
LAB12	24/10/2017	NA	25/10/2017 (serum) NA (ear-notch)	26/10/2017	07/11/2017
LAB13	24/10/2017	NA	26/10/2017 (serum) NA (ear-notch)	NA	08/11/2017
LAB14	23/10/2017	NA	NA (serum) 30/10/2017(ear-notch)	NA	08/11/2017
LAB15	23/10/2017	NA	NA (serum) 08/11/2017(ear-notch)	NA	09/11/2017
LAB16	24/10/2017	NA	NA	02+03/11/2017	07/11/2017
LAB17	25/10/2017	NA	NA	26/10/2017	09/11/2017
LAB18	24/10/2017	NA	NA	06/11/2017	07/11/2017

Legend: NA = not applicable

IV.3. Compliance with the procedure

All laboratories have provided a duly dated and signed copy of the results except LAB1.

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, LAB4 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, all laboratories (LAB1, LAB2, LAB3, LAB4, LAB6 and LAB7) 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 (LAB1, LAB2, LAB3, LAB5, LAB8, LAB9, LAB10, LAB11, LAB12 and LAB13) 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, LAB5, LAB6, LAB8, LAB9, LAB10, LAB11, LAB14 and LAB15) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement).

(iii) For the detection of BVDV-specific antibodies by **antibody ELISA** (Table 6): 5 out of 10 laboratories (LAB2, LAB3, LAB4, LAB12 and LAB17) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). In contrast, LAB16 misclassified 1 aliquot (95% of agreement) of reference serum samples, LAB6 misclassified 2 aliquots (90% of agreement) and LAB1, LAB7 and LAB18 misclassified 5 aliquots (75% of agreement).

Table 2. Antigen 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	6
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

Table 3. 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 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	6	7
failure	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)

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									
	1	2	3	5	8	9	10	11	12	13
failure	0 (0)	0 (0)	0 (0)	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)	10 (100)	10 (100)	10 (100)

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**	**8**	**9**	**10**	**11**	**14**	**15**
failure	0 (0)	0 (0)	0 (0)	0 (0)	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)	10 (100)	10 (100)	10 (100)	10 (100)

Table 6. 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 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**	**6**	**7**	**12**	**16**	**17**	**18**
failure	**5 (25)**	0 (0)	0 (0)	0 (0)	**2 (10)**	**5 (25)**	0 (0)	**1 (5)**	0 (0)	**5 (25)**
success	**15 (75)**	20 (100)	20 (100)	20 (100)	**18 (90)**	**15 (75)**	20 (100)	**19 (95)**	20 (100)	**15 (75)**

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** no variability between the participating laboratories could be observed since all participants correctly identified all reference serum and ear notch samples.

(iii) For the detection of BVDV-specific antibodies by **antibody ELISA** no variability between LAB2, LAB3, LAB4, LAB12 and LAB17 could be observed since these participants correctly identified all reference serum samples. In contrast LAB16 misclassified 1 aliquot of the PT2017BVDAbSERPS7 reference serum sample (negative instead of positive or non interpretable). LAB1, LAB7 and LAB18 misclassified all aliquots of the PT2017BVDAbSERPS7 reference serum sample (negative instead of positive or non interpretable). LAB6 misclassified 2 aliquots of the PT2017BVDAbSERPS7 reference serum sample (two times negative instead of positive or non interpretable).

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 7 (serum) and Table 8 (ear notch) for antigen ELISA, in Table 9 (serum) and Table 10 (ear notch) for RT-qPCR and in Table 11 (serum) for antibody ELISA.

Table 7. 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	PT2017BVDAgSERPS3	POS	POS	1
2	1	2	PT2017BVDAgSERNS1	NEG	NEG	1
3	1	3	PT2017BVDAgSERPS1	POS	POS	1
4	1	4	PT2017BVDAgSERNS1	NEG	NEG	1
5	1	5	PT2017BVDAgSERPS2	POS	POS	1
6	1	6	PT2017BVDAgSERNS2	NEG	NEG	1
7	1	7	PT2017BVDAgSERNS1	NEG	NEG	1
8	1	8	PT2017BVDAgSERPS2	POS	POS	1
9	1	9	PT2017BVDAgSERNS2	NEG	NEG	1
10	1	10	PT2017BVDAgSERPS3	POS	POS	1
11	2	1	PT2017BVDAgSERNS2	NEG	NEG	1
12	2	2	PT2017BVDAgSERPS1	POS	POS	1
13	2	3	PT2017BVDAgSERNS1	NEG	NEG	1
14	2	4	PT2017BVDAgSERPS2	POS	POS	1
15	2	5	PT2017BVDAgSERNS1	NEG	NEG	1
16	2	6	PT2017BVDAgSERPS3	POS	POS	1
17	2	7	PT2017BVDAgSERPS3	POS	POS	1
18	2	8	PT2017BVDAgSERNS2	NEG	NEG	1
19	2	9	PT2017BVDAgSERNS1	NEG	NEG	1
20	2	10	PT2017BVDAgSERPS2	POS	POS	1
21	3	1	PT2017BVDAgSERPS3	POS	POS	1
22	3	2	PT2017BVDAgSERNS1	NEG	NEG	1
23	3	3	PT2017BVDAgSERPS1	POS	POS	1
24	3	4	PT2017BVDAgSERNS1	NEG	NEG	1
25	3	5	PT2017BVDAgSERPS2	POS	POS	1
26	3	6	PT2017BVDAgSERNS2	NEG	NEG	1
27	3	7	PT2017BVDAgSERNS1	NEG	NEG	1
28	3	8	PT2017BVDAgSERPS2	POS	POS	1
29	3	9	PT2017BVDAgSERNS2	NEG	NEG	1
30	3	10	PT2017BVDAgSERPS3	POS	POS	1
31	4	1	PT2017BVDAgSERNS2	NEG	NEG	1
32	4	2	PT2017BVDAgSERPS1	POS	POS	1
33	4	3	PT2017BVDAgSERNS1	NEG	NEG	1
34	4	4	PT2017BVDAgSERPS2	POS	POS	1
35	4	5	PT2017BVDAgSERNS1	NEG	NEG	1
36	4	6	PT2017BVDAgSERPS3	POS	POS	1
37	4	7	PT2017BVDAgSERPS3	POS	POS	1
38	4	8	PT2017BVDAgSERNS2	NEG	NEG	1
39	4	9	PT2017BVDAgSERNS1	NEG	NEG	1
40	4	10	PT2017BVDAgSERPS2	POS	POS	1
41	6	1	PT2017BVDAgSERPS3	POS	POS	1
42	6	2	PT2017BVDAgSERNS1	NEG	NEG	1
43	6	3	PT2017BVDAgSERPS1	POS	POS	1
44	6	4	PT2017BVDAgSERNS1	NEG	NEG	1
45	6	5	PT2017BVDAgSERPS2	POS	POS	1
46	6	6	PT2017BVDAgSERNS2	NEG	NEG	1
47	6	7	PT2017BVDAgSERNS1	NEG	NEG	1
48	6	8	PT2017BVDAgSERPS2	POS	POS	1
49	6	9	PT2017BVDAgSERNS2	NEG	NEG	1
50	6	10	PT2017BVDAgSERPS3	POS	POS	1

Table 8. 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	PT2017BVDAgPE1	POS	POS	1
2	1	2	PT2017BVDAgNE3	NEG	NEG	1
3	1	3	PT2017BVDAgNE4	NEG	NEG	1
4	1	4	PT2017BVDAgPE2	POS	POS	1
5	1	5	PT2017BVDAgNE1	NEG	NEG	1
6	1	6	PT2017BVDAgPE3	POS	POS	1
7	1	7	PT2017BVDAgNE2	NEG	NEG	1
8	1	8	PT2017BVDAgPE4	POS	POS	1
9	1	9	PT2017BVDAgNE5	NEG	NEG	1
10	1	10	PT2017BVDAgPE5	POS	POS	1
11	2	1	PT2017BVDAgNE3	NEG	NEG	1
12	2	2	PT2017BVDAgNE4	NEG	NEG	1
13	2	3	PT2017BVDAgNE1	NEG	NEG	1
14	2	4	PT2017BVDAgNE5	NEG	NEG	1
15	2	5	PT2017BVDAgPE1	POS	POS	1
16	2	6	PT2017BVDAgNE2	NEG	NEG	1
17	2	7	PT2017BVDAgPE2	POS	POS	1
18	2	8	PT2017BVDAgPE4	POS	POS	1
19	2	9	PT2017BVDAgPE3	POS	POS	1
20	2	10	PT2017BVDAgPE5	POS	POS	1
21	3	1	PT2017BVDAgPE1	POS	POS	1
22	3	2	PT2017BVDAgNE3	NEG	NEG	1
23	3	3	PT2017BVDAgNE4	NEG	NEG	1
24	3	4	PT2017BVDAgPE2	POS	POS	1
25	3	5	PT2017BVDAgNE1	NEG	NEG	1
26	3	6	PT2017BVDAgPE3	POS	POS	1
27	3	7	PT2017BVDAgNE2	NEG	NEG	1
28	3	8	PT2017BVDAgPE4	POS	POS	1
29	3	9	PT2017BVDAgNE5	NEG	NEG	1
30	3	10	PT2017BVDAgPE5	POS	POS	1
31	4	1	PT2017BVDAgNE3	NEG	NEG	1
32	4	2	PT2017BVDAgNE4	NEG	NEG	1
33	4	3	PT2017BVDAgNE1	NEG	NEG	1
34	4	4	PT2017BVDAgNE5	NEG	NEG	1
35	4	5	PT2017BVDAgPE1	POS	POS	1
36	4	6	PT2017BVDAgNE2	NEG	NEG	1
37	4	7	PT2017BVDAgPE2	POS	POS	1
38	4	8	PT2017BVDAgPE4	POS	POS	1
39	4	9	PT2017BVDAgPE3	POS	POS	1
40	4	10	PT2017BVDAgPE5	POS	POS	1
41	6	1	PT2017BVDAgPE1	POS	POS	1
42	6	2	PT2017BVDAgNE3	NEG	NEG	1
43	6	3	PT2017BVDAgNE4	NEG	NEG	1
44	6	4	PT2017BVDAgPE2	POS	POS	1
45	6	5	PT2017BVDAgNE1	NEG	NEG	1
46	6	6	PT2017BVDAgPE3	POS	POS	1
47	6	7	PT2017BVDAgNE2	NEG	NEG	1
48	6	8	PT2017BVDAgPE4	POS	POS	1
49	6	9	PT2017BVDAgNE5	NEG	NEG	1
50	6	10	PT2017BVDAgPE5	POS	POS	1

Table 8 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
51	7	1	PT2017BVDAgNE3	NEG	NEG	1
52	7	2	PT2017BVDAgNE4	NEG	NEG	1
53	7	3	PT2017BVDAgNE1	NEG	NEG	1
54	7	4	PT2017BVDAgNE5	NEG	NEG	1
55	7	5	PT2017BVDAgPE1	POS	POS	1
56	7	6	PT2017BVDAgNE2	NEG	NEG	1
57	7	7	PT2017BVDAgPE2	POS	POS	1
58	7	8	PT2017BVDAgPE4	POS	POS	1
59	7	9	PT2017BVDAgPE3	POS	POS	1
60	7	10	PT2017BVDAgPE5	POS	POS	1

Table 9. 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; DBS: doubtful; NI: non interpretable (doubtful).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BVDVIRSERNS2	NEG	NEG	1
2	1	2	PT2017BVDVIRSERPS3	POS	POS	1
3	1	3	PT2017BVDVIRSERPS1	DBS	NEG	1
4	1	4	PT2017BVDVIRSERNS1	NEG	NEG	1
5	1	5	PT2017BVDVIRSERPS2	POS	POS	1
6	1	6	PT2017BVDVIRSERPS3	POS	POS	1
7	1	7	PT2017BVDVIRSERNS2	NEG	NEG	1
8	1	8	PT2017BVDVIRSERNS1	NEG	NEG	1
9	1	9	PT2017BVDVIRSERPS1	DBS	NEG	1
10	1	10	PT2017BVDVIRSERPS2	POS	POS	1
11	2	1	PT2017BVDVIRSERPS1	DBS	POS	1
12	2	2	PT2017BVDVIRSERPS3	POS	POS	1
13	2	3	PT2017BVDVIRSERNS2	NEG	NEG	1
14	2	4	PT2017BVDVIRSERPS1	DBS	POS	1
15	2	5	PT2017BVDVIRSERNS1	NEG	NEG	1
16	2	6	PT2017BVDVIRSERPS2	POS	POS	1
17	2	7	PT2017BVDVIRSERNS1	NEG	NEG	1
18	2	8	PT2017BVDVIRSERNS2	NEG	NEG	1
19	2	9	PT2017BVDVIRSERPS2	POS	POS	1
20	2	10	PT2017BVDVIRSERPS3	POS	POS	1
21	3	1	PT2017BVDVIRSERNS2	NEG	NEG	1
22	3	2	PT2017BVDVIRSERPS3	POS	POS	1
23	3	3	PT2017BVDVIRSERPS1	DBS	NI	1
24	3	4	PT2017BVDVIRSERNS1	NEG	NEG	1
25	3	5	PT2017BVDVIRSERPS2	POS	POS	1
26	3	6	PT2017BVDVIRSERPS3	POS	POS	1
27	3	7	PT2017BVDVIRSERNS2	NEG	NEG	1
28	3	8	PT2017BVDVIRSERNS1	NEG	NEG	1
29	3	9	PT2017BVDVIRSERPS1	DBS	NI	1
30	3	10	PT2017BVDVIRSERPS2	POS	POS	1

Table 9 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	5	1	PT2017BVDVIRSERPS1	DBS	POS	1
32	5	2	PT2017BVDVIRSERPS3	POS	POS	1
33	5	3	PT2017BVDVIRSERNS2	NEG	NEG	1
34	5	4	PT2017BVDVIRSERPS1	DBS	POS	1
35	5	5	PT2017BVDVIRSERNS1	NEG	NEG	1
36	5	6	PT2017BVDVIRSERPS2	POS	POS	1
37	5	7	PT2017BVDVIRSERNS1	NEG	NEG	1
38	5	8	PT2017BVDVIRSERNS2	NEG	NEG	1
39	5	9	PT2017BVDVIRSERPS2	POS	POS	1
40	5	10	PT2017BVDVIRSERPS3	POS	POS	1
41	8	1	PT2017BVDVIRSERNS2	NEG	NEG	1
42	8	2	PT2017BVDVIRSERPS3	POS	POS	1
43	8	3	PT2017BVDVIRSERPS1	DBS	POS	1
44	8	4	PT2017BVDVIRSERNS1	NEG	NEG	1
45	8	5	PT2017BVDVIRSERPS2	POS	POS	1
46	8	6	PT2017BVDVIRSERPS3	POS	POS	1
47	8	7	PT2017BVDVIRSERNS2	NEG	NEG	1
48	8	8	PT2017BVDVIRSERNS1	NEG	NEG	1
49	8	9	PT2017BVDVIRSERPS1	DBS	POS	1
50	8	10	PT2017BVDVIRSERPS2	POS	POS	1
51	9	1	PT2017BVDVIRSERPS1	DBS	POS	1
52	9	2	PT2017BVDVIRSERPS3	POS	POS	1
53	9	3	PT2017BVDVIRSERNS2	NEG	NEG	1
54	9	4	PT2017BVDVIRSERPS1	DBS	POS	1
55	9	5	PT2017BVDVIRSERNS1	NEG	NEG	1
56	9	6	PT2017BVDVIRSERPS2	POS	POS	1
57	9	7	PT2017BVDVIRSERNS1	NEG	NEG	1
58	9	8	PT2017BVDVIRSERNS2	NEG	NEG	1
59	9	9	PT2017BVDVIRSERPS2	POS	POS	1
60	9	10	PT2017BVDVIRSERPS3	POS	POS	1
61	10	1	PT2017BVDVIRSERNS2	NEG	NEG	1
62	10	2	PT2017BVDVIRSERPS3	POS	POS	1
63	10	3	PT2017BVDVIRSERPS1	DBS	POS	1
64	10	4	PT2017BVDVIRSERNS1	NEG	NEG	1
65	10	5	PT2017BVDVIRSERPS2	POS	POS	1
66	10	6	PT2017BVDVIRSERPS3	POS	POS	1
67	10	7	PT2017BVDVIRSERNS2	NEG	NEG	1
68	10	8	PT2017BVDVIRSERNS1	NEG	NEG	1
69	10	9	PT2017BVDVIRSERPS1	DBS	POS	1
70	10	10	PT2017BVDVIRSERPS2	POS	POS	1
71	11	1	PT2017BVDVIRSERPS1	DBS	POS	1
72	11	2	PT2017BVDVIRSERPS3	POS	POS	1
73	11	3	PT2017BVDVIRSERNS2	NEG	NEG	1
74	11	4	PT2017BVDVIRSERPS1	DBS	POS	1
75	11	5	PT2017BVDVIRSERNS1	NEG	NEG	1
76	11	6	PT2017BVDVIRSERPS2	POS	POS	1
77	11	7	PT2017BVDVIRSERNS1	NEG	NEG	1
78	11	8	PT2017BVDVIRSERNS2	NEG	NEG	1
79	11	9	PT2017BVDVIRSERPS2	POS	POS	1
80	11	10	PT2017BVDVIRSERPS3	POS	POS	1

Table 9 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	12	1	PT2017BVDVIRSERNS2	NEG	NEG	1
82	12	2	PT2017BVDVIRSERPS3	POS	POS	1
83	12	3	PT2017BVDVIRSERPS1	DBS	POS	1
84	12	4	PT2017BVDVIRSERNS1	NEG	NEG	1
85	12	5	PT2017BVDVIRSERPS2	POS	POS	1
86	12	6	PT2017BVDVIRSERPS3	POS	POS	1
87	12	7	PT2017BVDVIRSERNS2	NEG	NEG	1
88	12	8	PT2017BVDVIRSERNS1	NEG	NEG	1
89	12	9	PT2017BVDVIRSERPS1	DBS	POS	1
90	12	10	PT2017BVDVIRSERPS2	POS	POS	1
91	13	1	PT2017BVDVIRSERPS1	DBS	POS	1
92	13	2	PT2017BVDVIRSERPS3	POS	POS	1
93	13	3	PT2017BVDVIRSERNS2	NEG	NEG	1
94	13	4	PT2017BVDVIRSERPS1	DBS	POS	1
95	13	5	PT2017BVDVIRSERNS1	NEG	NEG	1
96	13	6	PT2017BVDVIRSERPS2	POS	POS	1
97	13	7	PT2017BVDVIRSERNS1	NEG	NEG	1
98	13	8	PT2017BVDVIRSERNS2	NEG	NEG	1
99	13	9	PT2017BVDVIRSERPS2	POS	POS	1
100	13	10	PT2017BVDVIRSERPS3	POS	POS	1

Table 10. 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	PT2017BVDVIRNE5	NEG	NEG	1
2	1	2	PT2017BVDVIRPE1	POS	POS	1
3	1	3	PT2017BVDVIRNE2	NEG	NEG	1
4	1	4	PT2017BVDVIRPE5	POS	POS	1
5	1	5	PT2017BVDVIRNE4	NEG	NEG	1
6	1	6	PT2017BVDVIRNE1	NEG	NEG	1
7	1	7	PT2017BVDVIRPE2	POS	POS	1
8	1	8	PT2017BVDVIRNE3	NEG	NEG	1
9	1	9	PT2017BVDVIRPE3	POS	POS	1
10	1	10	PT2017BVDVIRPE4	POS	POS	1
11	2	1	PT2017BVDVIRNE3	NEG	NEG	1
12	2	2	PT2017BVDVIRPE4	POS	POS	1
13	2	3	PT2017BVDVIRNE1	NEG	NEG	1
14	2	4	PT2017BVDVIRPE1	POS	POS	1
15	2	5	PT2017BVDVIRPE2	POS	POS	1
16	2	6	PT2017BVDVIRNE4	NEG	NEG	1
17	2	7	PT2017BVDVIRPE3	POS	POS	1
18	2	8	PT2017BVDVIRNE2	NEG	NEG	1
19	2	9	PT2017BVDVIRNE5	NEG	NEG	1
20	2	10	PT2017BVDVIRPE5	POS	POS	1

Table 10 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2017BVDVIRNE5	NEG	NEG	1
22	3	2	PT2017BVDVIRPE1	POS	POS	1
23	3	3	PT2017BVDVIRNE2	NEG	NEG	1
24	3	4	PT2017BVDVIRPE5	POS	POS	1
25	3	5	PT2017BVDVIRNE4	NEG	NEG	1
26	3	6	PT2017BVDVIRNE1	NEG	NEG	1
27	3	7	PT2017BVDVIRPE2	POS	POS	1
28	3	8	PT2017BVDVIRNE3	NEG	NEG	1
29	3	9	PT2017BVDVIRPE3	POS	POS	1
30	3	10	PT2017BVDVIRPE4	POS	POS	1
31	5	1	PT2017BVDVIRNE3	NEG	NEG	1
32	5	2	PT2017BVDVIRPE4	POS	POS	1
33	5	3	PT2017BVDVIRNE1	NEG	NEG	1
34	5	4	PT2017BVDVIRPE1	POS	POS	1
35	5	5	PT2017BVDVIRPE2	POS	POS	1
36	5	6	PT2017BVDVIRNE4	NEG	NEG	1
37	5	7	PT2017BVDVIRPE3	POS	POS	1
38	5	8	PT2017BVDVIRNE2	NEG	NEG	1
39	5	9	PT2017BVDVIRNE5	NEG	NEG	1
40	5	10	PT2017BVDVIRPE5	POS	POS	1
41	6	1	PT2017BVDVIRNE5	NEG	NEG	1
42	6	2	PT2017BVDVIRPE1	POS	POS	1
43	6	3	PT2017BVDVIRNE2	NEG	NEG	1
44	6	4	PT2017BVDVIRPE5	POS	POS	1
45	6	5	PT2017BVDVIRNE4	NEG	NEG	1
46	6	6	PT2017BVDVIRNE1	NEG	NEG	1
47	6	7	PT2017BVDVIRPE2	POS	POS	1
48	6	8	PT2017BVDVIRNE3	NEG	NEG	1
49	6	9	PT2017BVDVIRPE3	POS	POS	1
50	6	10	PT2017BVDVIRPE4	POS	POS	1
51	8	1	PT2017BVDVIRNE3	NEG	NEG	1
52	8	2	PT2017BVDVIRPE4	POS	POS	1
53	8	3	PT2017BVDVIRNE1	NEG	NEG	1
54	8	4	PT2017BVDVIRPE1	POS	POS	1
55	8	5	PT2017BVDVIRPE2	POS	POS	1
56	8	6	PT2017BVDVIRNE4	NEG	NEG	1
57	8	7	PT2017BVDVIRPE3	POS	POS	1
58	8	8	PT2017BVDVIRNE2	NEG	NEG	1
59	8	9	PT2017BVDVIRNE5	NEG	NEG	1
60	8	10	PT2017BVDVIRPE5	POS	POS	1
61	9	1	PT2017BVDVIRNE5	NEG	NEG	1
62	9	2	PT2017BVDVIRPE1	POS	POS	1
63	9	3	PT2017BVDVIRNE2	NEG	NEG	1
64	9	4	PT2017BVDVIRPE5	POS	POS	1
65	9	5	PT2017BVDVIRNE4	NEG	NEG	1
66	9	6	PT2017BVDVIRNE1	NEG	NEG	1
67	9	7	PT2017BVDVIRPE2	POS	POS	1
68	9	8	PT2017BVDVIRNE3	NEG	NEG	1
69	9	9	PT2017BVDVIRPE3	POS	POS	1
70	9	10	PT2017BVDVIRPE4	POS	POS	1

Table 10 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
71	10	1	PT2017BVDVIRNE3	NEG	NEG	1
72	10	2	PT2017BVDVIRPE4	POS	POS	1
73	10	3	PT2017BVDVIRNE1	NEG	NEG	1
74	10	4	PT2017BVDVIRPE1	POS	POS	1
75	10	5	PT2017BVDVIRPE2	POS	POS	1
76	10	6	PT2017BVDVIRNE4	NEG	NEG	1
77	10	7	PT2017BVDVIRPE3	POS	POS	1
78	10	8	PT2017BVDVIRNE2	NEG	NEG	1
79	10	9	PT2017BVDVIRNE5	NEG	NEG	1
80	10	10	PT2017BVDVIRPE5	POS	POS	1
81	11	1	PT2017BVDVIRNE5	NEG	NEG	1
82	11	2	PT2017BVDVIRPE1	POS	POS	1
83	11	3	PT2017BVDVIRNE2	NEG	NEG	1
84	11	4	PT2017BVDVIRPE5	POS	POS	1
85	11	5	PT2017BVDVIRNE4	NEG	NEG	1
86	11	6	PT2017BVDVIRNE1	NEG	NEG	1
87	11	7	PT2017BVDVIRPE2	POS	POS	1
88	11	8	PT2017BVDVIRNE3	NEG	NEG	1
89	11	9	PT2017BVDVIRPE3	POS	POS	1
90	11	10	PT2017BVDVIRPE4	POS	POS	1
91	14	1	PT2017BVDVIRNE3	NEG	NEG	1
92	14	2	PT2017BVDVIRPE4	POS	POS	1
93	14	3	PT2017BVDVIRNE1	NEG	NEG	1
94	14	4	PT2017BVDVIRPE1	POS	POS	1
95	14	5	PT2017BVDVIRPE2	POS	POS	1
96	14	6	PT2017BVDVIRNE4	NEG	NEG	1
97	14	7	PT2017BVDVIRPE3	POS	POS	1
98	14	8	PT2017BVDVIRNE2	NEG	NEG	1
99	14	9	PT2017BVDVIRNE5	NEG	NEG	1
100	14	10	PT2017BVDVIRPE5	POS	POS	1
101	15	1	PT2017BVDVIRNE5	NEG	NEG	1
102	15	2	PT2017BVDVIRPE1	POS	POS	1
103	15	3	PT2017BVDVIRNE2	NEG	NEG	1
104	15	4	PT2017BVDVIRPE5	POS	POS	1
105	15	5	PT2017BVDVIRNE4	NEG	NEG	1
106	15	6	PT2017BVDVIRNE1	NEG	NEG	1
107	15	7	PT2017BVDVIRPE2	POS	POS	1
108	15	8	PT2017BVDVIRNE3	NEG	NEG	1
109	15	9	PT2017BVDVIRPE3	POS	POS	1
110	15	10	PT2017BVDVIRPE4	POS	POS	1

Table 11. Antibody 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; NI : non interpretable (doubtful), DBS : doubtful.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017BVDAbSERPS5	POS	POS	1
2	1	2	PT2017BVDAbSERNS3	NEG	NEG	1
3	1	3	PT2017BVDAbSERPS4	DBS	POS	1
4	1	4	PT2017BVDAbSERPS6	POS	POS	1
5	1	5	PT2017BVDAbSERNS1	NEG	NEG	1
6	1	6	PT2017BVDAbSERNS3	NEG	NEG	1
7	1	7	PT2017BVDAbSERPS7	**POS**	**NEG**	0
8	1	8	PT2017BVDAbSERPS6	POS	POS	1
9	1	9	PT2017BVDAbSERNS2	NEG	NEG	1
10	1	10	PT2017BVDAbSERNS3	NEG	NEG	1
11	1	11	PT2017BVDAbSERPS3	POS	POS	1
12	1	12	PT2017BVDAbSERPS7	**POS**	**NEG**	0
13	1	13	PT2017BVDAbSERPS1	POS	POS	1
14	1	14	PT2017BVDAbSERNS3	NEG	NEG	1
15	1	15	PT2017BVDAbSERPS7	**POS**	**NEG**	0
16	1	16	PT2017BVDAbSERPS6	POS	POS	1
17	1	17	PT2017BVDAbSERPS7	**POS**	**NEG**	0
18	1	18	PT2017BVDAbSERPS2	POS	POS	1
19	1	19	PT2017BVDAbSERNS3	NEG	NEG	1
20	1	20	PT2017BVDAbSERPS7	**POS**	**NEG**	0
21	2	1	PT2017BVDAbSERNS3	NEG	NEG	1
22	2	2	PT2017BVDAbSERPS1	POS	POS	1
23	2	3	PT2017BVDAbSERPS7	POS	POS	1
24	2	4	PT2017BVDAbSERNS2	NEG	NEG	1
25	2	5	PT2017BVDAbSERPS4	DBS	POS	1
26	2	6	PT2017BVDAbSERPS2	POS	POS	1
27	2	7	PT2017BVDAbSERNS3	NEG	NEG	1
28	2	8	PT2017BVDAbSERPS3	POS	POS	1
29	2	9	PT2017BVDAbSERNS1	NEG	NEG	1
30	2	10	PT2017BVDAbSERPS7	POS	POS	1
31	2	11	PT2017BVDAbSERPS6	POS	POS	1
32	2	12	PT2017BVDAbSERPS5	POS	POS	1
33	2	13	PT2017BVDAbSERNS3	NEG	NEG	1
34	2	14	PT2017BVDAbSERPS7	POS	POS	1
35	2	15	PT2017BVDAbSERNS3	NEG	NEG	1
36	2	16	PT2017BVDAbSERPS7	POS	POS	1
37	2	17	PT2017BVDAbSERNS3	NEG	NEG	1
38	2	18	PT2017BVDAbSERPS7	POS	POS	1
39	2	19	PT2017BVDAbSERPS6	POS	POS	1
40	2	20	PT2017BVDAbSERPS6	POS	POS	1

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2017BVDAbSERPS5	POS	POS	1
42	3	2	PT2017BVDAbSERNS3	NEG	NEG	1
43	3	3	PT2017BVDAbSERPS4	DBS	POS	1
44	3	4	PT2017BVDAbSERPS6	POS	POS	1
45	3	5	PT2017BVDAbSERNS1	NEG	NEG	1
46	3	6	PT2017BVDAbSERNS3	NEG	NEG	1
47	3	7	PT2017BVDAbSERPS7	POS	POS	1
48	3	8	PT2017BVDAbSERPS6	POS	POS	1
49	3	9	PT2017BVDAbSERNS2	NEG	NEG	1
50	3	10	PT2017BVDAbSERNS3	NEG	NEG	1
51	3	11	PT2017BVDAbSERPS3	POS	POS	1
52	3	12	PT2017BVDAbSERPS7	POS	POS	1
53	3	13	PT2017BVDAbSERPS1	POS	POS	1
54	3	14	PT2017BVDAbSERNS3	NEG	NEG	1
55	3	15	PT2017BVDAbSERPS7	POS	POS	1
56	3	16	PT2017BVDAbSERPS6	POS	POS	1
57	3	17	PT2017BVDAbSERPS7	POS	POS	1
58	3	18	PT2017BVDAbSERPS2	POS	POS	1
59	3	19	PT2017BVDAbSERNS3	NEG	NEG	1
60	3	20	PT2017BVDAbSERPS7	POS	POS	1
61	4	1	PT2017BVDAbSERNS3	NEG	NEG	1
62	4	2	PT2017BVDAbSERPS1	POS	POS	1
63	4	3	PT2017BVDAbSERPS7	POS	POS	1
64	4	4	PT2017BVDAbSERNS2	NEG	NEG	1
65	4	5	PT2017BVDAbSERPS4	DBS	POS	1
66	4	6	PT2017BVDAbSERPS2	POS	POS	1
67	4	7	PT2017BVDAbSERNS3	NEG	NEG	1
68	4	8	PT2017BVDAbSERPS3	POS	POS	1
69	4	9	PT2017BVDAbSERNS1	NEG	NEG	1
70	4	10	PT2017BVDAbSERPS7	POS	POS	1
71	4	11	PT2017BVDAbSERPS6	POS	POS	1
72	4	12	PT2017BVDAbSERPS5	POS	POS	1
73	4	13	PT2017BVDAbSERNS3	NEG	NEG	1
74	4	14	PT2017BVDAbSERPS7	POS	POS	1
75	4	15	PT2017BVDAbSERNS3	NEG	NEG	1
76	4	16	PT2017BVDAbSERPS7	POS	POS	1
77	4	17	PT2017BVDAbSERNS3	NEG	NEG	1
78	4	18	PT2017BVDAbSERPS7	POS	POS	1
79	4	19	PT2017BVDAbSERPS6	POS	POS	1
80	4	20	PT2017BVDAbSERPS6	POS	POS	1

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	6	1	PT2017BVDAbSERPS5	POS	POS	1
82	6	2	PT2017BVDAbSERNS3	NEG	NEG	1
83	6	3	PT2017BVDAbSERPS4	DBS	POS	1
84	6	4	PT2017BVDAbSERPS6	POS	POS	1
85	6	5	PT2017BVDAbSERNS1	NEG	NEG	1
86	6	6	PT2017BVDAbSERNS3	NEG	NEG	1
87	6	7	PT2017BVDAbSERPS7	POS	NI	1
88	6	8	PT2017BVDAbSERPS6	POS	POS	1
89	6	9	PT2017BVDAbSERNS2	NEG	NEG	1
90	6	10	PT2017BVDAbSERNS3	NEG	NEG	1
91	6	11	PT2017BVDAbSERPS3	POS	POS	1
92	6	12	PT2017BVDAbSERPS7	POS	NI	1
93	6	13	PT2017BVDAbSERPS1	POS	POS	1
94	6	14	PT2017BVDAbSERNS3	NEG	NEG	1
95	6	15	PT2017BVDAbSERPS7	**POS**	**NEG**	0
96	6	16	PT2017BVDAbSERPS6	POS	POS	1
97	6	17	PT2017BVDAbSERPS7	**POS**	**NEG**	0
98	6	18	PT2017BVDAbSERPS2	POS	POS	1
99	6	19	PT2017BVDAbSERNS3	NEG	NEG	1
100	6	20	PT2017BVDAbSERPS7	POS	NI	1
101	7	1	PT2017BVDAbSERNS3	NEG	NEG	1
102	7	2	PT2017BVDAbSERPS1	POS	POS	1
103	7	3	PT2017BVDAbSERPS7	**POS**	**NEG**	0
104	7	4	PT2017BVDAbSERNS2	NEG	NEG	1
105	7	5	PT2017BVDAbSERPS4	DBS	NI	1
106	7	6	PT2017BVDAbSERPS2	POS	POS	1
107	7	7	PT2017BVDAbSERNS3	NEG	NEG	1
108	7	8	PT2017BVDAbSERPS3	POS	POS	1
109	7	9	PT2017BVDAbSERNS1	NEG	NEG	1
110	7	10	PT2017BVDAbSERPS7	**POS**	**NEG**	0
111	7	11	PT2017BVDAbSERPS6	POS	POS	1
112	7	12	PT2017BVDAbSERPS5	POS	POS	1
113	7	13	PT2017BVDAbSERNS3	NEG	NEG	1
114	7	14	PT2017BVDAbSERPS7	**POS**	**NEG**	0
115	7	15	PT2017BVDAbSERNS3	NEG	NEG	1
116	7	16	PT2017BVDAbSERPS7	**POS**	**NEG**	0
117	7	17	PT2017BVDAbSERNS3	NEG	NEG	1
118	7	18	PT2017BVDAbSERPS7	**POS**	**NEG**	0
119	7	19	PT2017BVDAbSERPS6	POS	POS	1
120	7	20	PT2017BVDAbSERPS6	POS	POS	1

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	12	1	PT2017BVDAbSERPS5	POS	POS	1
122	12	2	PT2017BVDAbSERNS3	NEG	NEG	1
123	12	3	PT2017BVDAbSERPS4	DBS	POS	1
124	12	4	PT2017BVDAbSERPS6	POS	POS	1
125	12	5	PT2017BVDAbSERNS1	NEG	NEG	1
126	12	6	PT2017BVDAbSERNS3	NEG	NEG	1
127	12	7	PT2017BVDAbSERPS7	POS	POS	1
128	12	8	PT2017BVDAbSERPS6	POS	POS	1
129	12	9	PT2017BVDAbSERNS2	NEG	NEG	1
130	12	10	PT2017BVDAbSERNS3	NEG	NEG	1
131	12	11	PT2017BVDAbSERPS3	POS	POS	1
132	12	12	PT2017BVDAbSERPS7	POS	POS	1
133	12	13	PT2017BVDAbSERPS1	POS	POS	1
134	12	14	PT2017BVDAbSERNS3	NEG	NEG	1
135	12	15	PT2017BVDAbSERPS7	POS	POS	1
136	12	16	PT2017BVDAbSERPS6	POS	POS	1
137	12	17	PT2017BVDAbSERPS7	POS	POS	1
138	12	18	PT2017BVDAbSERPS2	POS	POS	1
139	12	19	PT2017BVDAbSERNS3	NEG	NEG	1
140	12	20	PT2017BVDAbSERPS7	POS	POS	1
141	16	1	PT2017BVDAbSERNS3	NEG	NEG	1
142	16	2	PT2017BVDAbSERPS1	POS	POS	1
143	16	3	PT2017BVDAbSERPS7	POS	POS	1
144	16	4	PT2017BVDAbSERNS2	NEG	NEG	1
145	16	5	PT2017BVDAbSERPS4	DBS	POS	1
146	16	6	PT2017BVDAbSERPS2	POS	POS	1
147	16	7	PT2017BVDAbSERNS3	NEG	NEG	1
148	16	8	PT2017BVDAbSERPS3	POS	POS	1
149	16	9	PT2017BVDAbSERNS1	NEG	NEG	1
150	16	10	PT2017BVDAbSERPS7	POS	POS	1
151	16	11	PT2017BVDAbSERPS6	POS	POS	1
152	16	12	PT2017BVDAbSERPS5	POS	POS	1
153	16	13	PT2017BVDAbSERNS3	NEG	NEG	1
154	16	14	PT2017BVDAbSERPS7	POS	POS	1
155	16	15	PT2017BVDAbSERNS3	NEG	NEG	1
156	16	16	PT2017BVDAbSERPS7	POS	POS	1
157	16	17	PT2017BVDAbSERNS3	NEG	NEG	1
158	16	18	PT2017BVDAbSERPS7	**POS**	**NEG**	0
159	16	19	PT2017BVDAbSERPS6	POS	POS	1
160	16	20	PT2017BVDAbSERPS6	POS	POS	1

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	17	1	PT2017BVDAbSERPS5	POS	POS	1
162	17	2	PT2017BVDAbSERNS3	NEG	NEG	1
163	17	3	PT2017BVDAbSERPS4	DBS	POS	1
164	17	4	PT2017BVDAbSERPS6	POS	POS	1
165	17	5	PT2017BVDAbSERNS1	NEG	NEG	1
166	17	6	PT2017BVDAbSERNS3	NEG	NEG	1
167	17	7	PT2017BVDAbSERPS7	POS	POS	1
168	17	8	PT2017BVDAbSERPS6	POS	POS	1
169	17	9	PT2017BVDAbSERNS2	NEG	NEG	1
170	17	10	PT2017BVDAbSERNS3	NEG	NEG	1
171	17	11	PT2017BVDAbSERPS3	POS	POS	1
172	17	12	PT2017BVDAbSERPS7	POS	POS	1
173	17	13	PT2017BVDAbSERPS1	POS	POS	1
174	17	14	PT2017BVDAbSERNS3	NEG	NEG	1
175	17	15	PT2017BVDAbSERPS7	POS	POS	1
176	17	16	PT2017BVDAbSERPS6	POS	POS	1
177	17	17	PT2017BVDAbSERPS7	POS	POS	1
178	17	18	PT2017BVDAbSERPS2	POS	POS	1
179	17	19	PT2017BVDAbSERNS3	NEG	NEG	1
180	17	20	PT2017BVDAbSERPS7	POS	POS	1
181	18	1	PT2017BVDAbSERNS3	NEG	NEG	1
182	18	2	PT2017BVDAbSERPS1	POS	POS	1
183	18	3	PT2017BVDAbSERPS7	**POS**	**NEG**	0
184	18	4	PT2017BVDAbSERNS2	NEG	NEG	1
185	18	5	PT2017BVDAbSERPS4	DBS	NEG	1
186	18	6	PT2017BVDAbSERPS2	POS	POS	1
187	18	7	PT2017BVDAbSERNS3	NEG	NEG	1
188	18	8	PT2017BVDAbSERPS3	POS	POS	1
189	18	9	PT2017BVDAbSERNS1	NEG	NEG	1
190	18	10	PT2017BVDAbSERPS7	**POS**	**NEG**	0
191	18	11	PT2017BVDAbSERPS6	POS	POS	1
192	18	12	PT2017BVDAbSERPS5	POS	POS	1
193	18	13	PT2017BVDAbSERNS3	NEG	NEG	1
194	18	14	PT2017BVDAbSERPS7	**POS**	**NEG**	0
195	18	15	PT2017BVDAbSERNS3	NEG	NEG	1
196	18	16	PT2017BVDAbSERPS7	**POS**	**NEG**	0
197	18	17	PT2017BVDAbSERNS3	NEG	NEG	1
198	18	18	PT2017BVDAbSERPS7	**POS**	**NEG**	0
199	18	19	PT2017BVDAbSERPS6	POS	POS	1
200	18	20	PT2017BVDAbSERPS6	POS	POS	1

V. Discussion

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

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 participating laboratories, except LAB7 used the ELISA kit BVDV Ag/Serum Plus Test from IDEXX, but 4 different batches were used: batch K031 (LAB6), batch K151 (LAB2 and

LAB4), batch K621 (LAB3) and batch K721 (LAB1). LAB7 used the SERELISA BVDV Erns Ag Capture ELISA kit from Zoetis (batch 234362).

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, 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 LAB3, used a commercially available BVDV RT-qPCR: LAB2 (for serum), LAB5, LAB6, LAB8, LAB10 and LAB13 used the VetMax BVD4ALL Screening test kit from Thermofisher Scientific (LSI) [batch 013 (LAB2, LAB5, LAB6 and LAB8), batch 011 (LAB10) and batch B12S-127 (LAB13)], LAB2 (for ear notch) and LAB14 used the Virotype BVDV RT-PCR kit from Qiagen [batch 254121248 (LAB2) and batch 257120207 (LAB14)], LAB1 used the RealPCR BVDV RNA Mix and RealPCR RNA Master Mix from IDEXX (batch 42G178 and 35L132), LAB9 used the IDBVD kit from IDVET Genetics (batch 24), LAB11 used the Bio-T kit BVDV/BVD Universal kit from Biosellal (batch BVDVU-06B), LAB12 used the LSIVetMAX BVDV Screening kit from Life Technologies (batch B12S-126) and LAB15 used the ADIAVET BVDV REAL TIME from BioX Diagnostics/Adiagene (batch 10K4TR169). LAB3 used an in house RT-qPCR.

For the detection of BVDV-specific antibodies in reference serum samples, five out of ten (LAB2, LAB3, LAB4, LAB12 and LAB17) participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) whereas LAB16 misclassified 1 aliquot of the PT2017BVDAbSERPS7 reference serum sample (95% of agreement), LAB6 misclassified 2 aliquots of the PT2017BVDAbSERPS7 reference serum sample (90% of agreement) and LAB1, LAB7 and LAB18 misclassified all the aliquots of the PT2017BVDAbSERPS7 reference serum sample (75% of agreement). The PT2017BVDAbSERPS7 reference sample was obtained after dilution of a strong positive sample from an animal experimentally infected with BVD type 2. 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-1 and BVD-2 in order to be used in an official monitoring programme.

For the detection of BVDV-specific antibodies by antibody ELISA: LAB2, LAB3, LAB4 and LAB12 used the Monoscreen Ab ELISA BVDV kit from BioX Diagnostics/Adiagene (batch 16L19), LAB1 and LAB6 used the BVDV Total Ab Test ELISA kit from IDEXX [batch J621 (LAB1) and batch J511 (LAB6)], LAB18 used the BVDV p80 Ab ELISA kit from IDEXX (batch 6156), LAB7 used the SERELISA BVD Ab Mono Blocking ELISA kit from Zoetis (batch 17ZEAK005), LAB16 used the CIVTEST BOVIS BVD/BD P80 ELISA kit from HIPRA (batch CVD.4P32) and LAB17 used the ID Screen BVD p80 Antibody Competition ELISA kit from IDVET (batch C07).

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. LAB2, LAB3, LAB4, LAB6, LAB12, LAB16 and LAB17 achieved a satisfactory performance for the detection of BVDV-specific antibodies by ELISA in reference serum samples. In contrast LAB1, LAB7 and LAB18 did not achieved a satisfactory performance for the detection of BVDV-specific antibodies by ELISA in reference 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)

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

Biosellal (Dardilly, France)

Bio-X Diagnostics (Rochefort, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

HIPRA Scientific SLU (Amer (Griona), Spain)

IDEXX technonogies AG (Liebefeld-Bern, Switzerland)

ID. VET (Grabels, France)

IDVet Genetics (Grabels, France)

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

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

Laboratoire Vétérinaire Départemental (LVD) de l'Aude (Carcassonne, France)

Lavetan NV (Turnhout, Belgium)

The Maastricht Forensic Institute B.V. / BeterVee (Maastricht, The Nederlands)

Thermofisher Scientific - LSI (Lissieu, France)

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

Zoetis France (Lyon, France)

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,** a box plot of the normalized OD values according the PT provider per reference serum sample and per participating laboratory using the ELISA kit BVDV Ag/Serum Plus Test from IDEXX is shown in Figure 1. It was not possible to make a box plot for the reference ear notch samples since there was only one aliquot per sample.

Figure 1 (antigen ELISA serum reference samples)

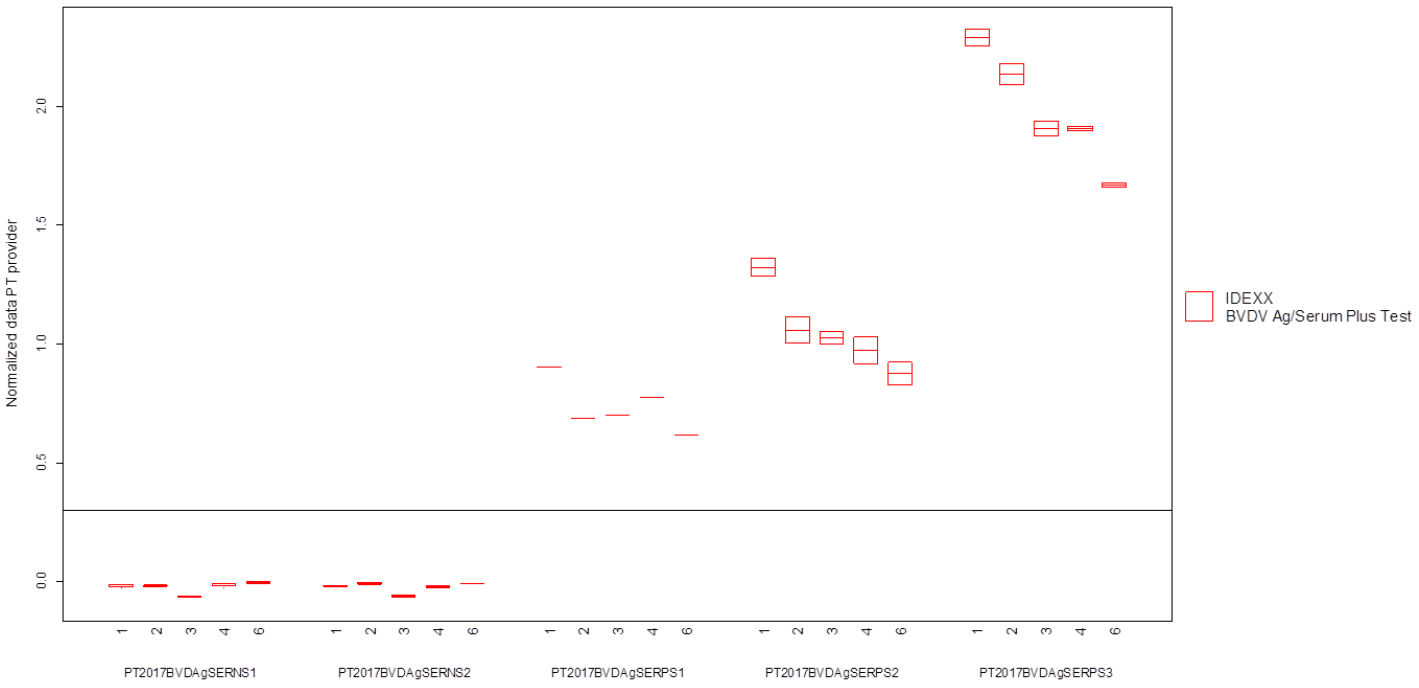

Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory using the ELISA kit BVDV Ag/Serum Plus Test from IDEXX. Different batches were used : batch K031 (LAB6), batch K151 (LAB2 and LAB4), batch K621 (LAB3) and batch K721 (LAB1). Cut-off value (0.3) is shown by a horizontal line.

For the **RT-qPCR serum and ear notch reference samples,** a box plot of the Ct or Cp values for the positive reference serum samples PT2017BVDVIRSERPS2 and PT2017BVDVIRSERPS3 and per participating laboratory is shown in Figure 2. It was not possible to make a box plot for the reference ear notch samples since there was only one aliquot for these samples.

Figure 2 (RT-qPCR serum reference samples)

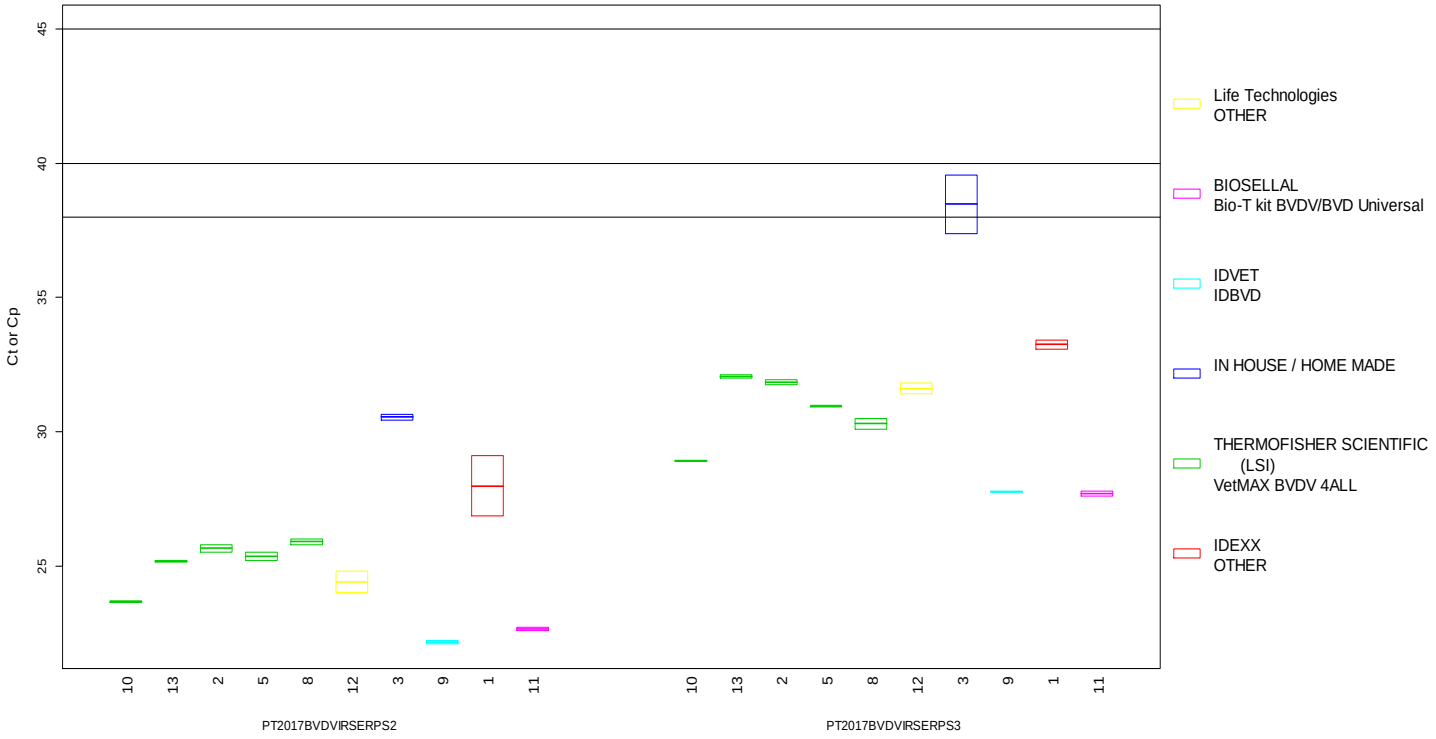

Figure 2. Box plots showing the Ct or Cp values for the positive reference serum samples PT2017BVDVIRSERPS2 and PT2017BVDVIRSERPS3 per participating laboratory. LAB2, LAB5, LAB8, LAB10 and LAB13 used the VetMax BVD4ALL Screening test kit from Thermofisher Scientific (LSI) (green box plots), LAB12 used the LSIVetMAX BVDV Screening kit from Life Technologies (yellow box plots), LAB3 used an in house RT-qPCR (blue box plots), LAB9 used the IDBVD kit from IDVET Genetics (light blue box plots), LAB1 used the RealPCR BVDV RNA Mix and RealPCR RNA Master Mix from IDEXX (red box plots) and LAB11 used the Bio-T kit BVDV/BVD Universal kit from Biosellal (pink box plots). Cut-off values for the different kits (38-40-45) are shown by horizontal lines.

For the **antibody ELISA serum reference samples,** a box plot of the normalized OD values according the PT provider for the reference serum samples PT2017BVDAbSERNS3, PT2017BVDAbSERPS6 and PT2017BVDAbSERPS7 per participating laboratory is shown in Figure 3. It was not possible to make a box plot for the other reference serum samples since there was only one aliquot for these samples.

Figure 3 (antibody ELISA serum reference samples)

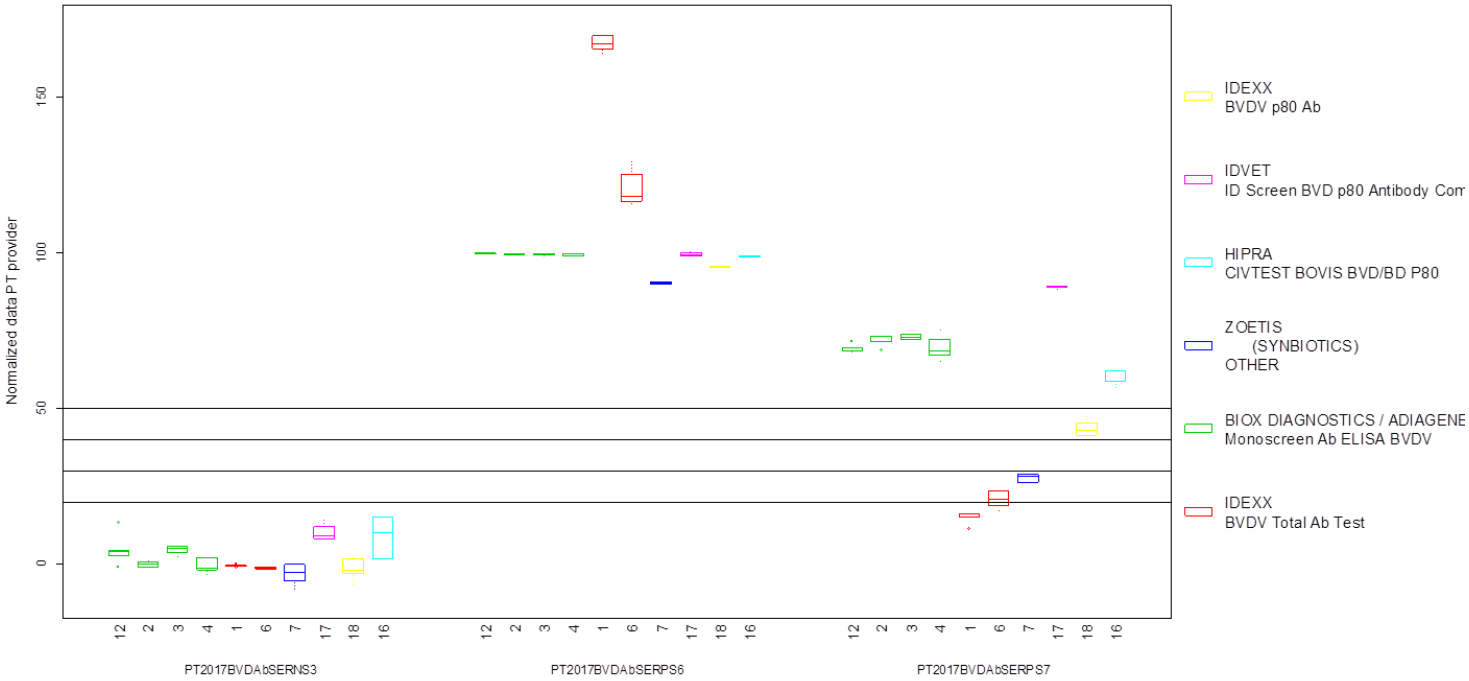

Figure 3. Box plots showing the normalized OD values according the PT provider for the reference serum samples PT2017BVDAbSERNS3, PT2017BVDAbSERPS6 and PT2017BVDAbSERPS7 per participating laboratory. LAB2, LAB3, LAB4 and LAB12 used the Monoscreen Ab ELISA BVDV kit from BioX Diagnostics/Adiagene (green box plots), LAB1 and LAB6 used the BVDV Total Ab Test ELISA kit from IDEXX (red box plots), LAB7 used the SERELISA BVD Ab Mono Blocking ELISA kit from Zoetis (blue box plot), LAB17 used the ID Screen BVD p80 Antibody Competition ELISA kit from IDVET (pink box plots), LAB18 used the BVDV p80 Ab ELISA kit from IDEXX (yellow box plots) and LAB16 used the CIVTEST BOVIS BVD/BD P80 ELISA kit from HIPRA (light blue box plots). Cut-off values (20-30-40-50) for the different kits except for the BVDV Total Ab Test ELISA kit from IDEXX are shown by horizontal lines.