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081-PT

PROFICIENCY TESTING 2018

Bovine Viral Diarrhea Virus (BVDV)

Detection of BVDV-specific antigens in bovine blood and/or ear notch samples

By Enzyme Linked Immunosorbent Assay (ELISA) and/or Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE BEGIN PT: 8 OCTOBER 2018

DATE REPORT: 31 JANUARI 2019

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 BVDV-specific antigens in bovine blood and/or ear notch samples by antigen ELISA and/or RT-qPCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

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

III.2. Reference samples

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

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

Replicates of 6 reference blood samples of bovine origin, either free from detectable BVDV-specific antigens (n=3 coded 'PT2018BVDNB1', 'PT2018BVDNB2' and 'PT2018BVDNB3') or containing detectable BVDV-specific antigens (n=3 coded 'PT2018BVDPB1', 'PT2018BVDPB2', 'PT2018BVDPB3'), were used.
In total, 30 aliquots of reference blood samples were distributed to 3 participating laboratories. All laboratories received 10 aliquots: 2 aliquots of the reference blood samples PT2018BVDNB1, PT2018BVDNB2, PT2018BVDPB1, PT2018BVDPB2 and 1 aliquot of the reference blood sample PT2018BVDNB3, PT2018BVDPB3. The positions of the reference blood samples in the sent blocks were randomized for each participant. (Table 2 and Table 6).

For each reference blood sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference blood samples was based on (i) the historical background of the animals and (ii) the results obtained by the BVDV antigen test kit/blood plus ELISA from IDEXX (pre-verification).

The reference blood samples PT2018BVDNB1, PT2018BVDNB2 and PT2018BVDNB3 were obtained from 3 different BVDV-free animals from the field. The reference blood samples PT2018BVDPS1, PT2018BVDPS2 and PT2018BVDPS3 were obtained from 3 different calves that were classified as immunotolerant persistently (BVDV-1) infected (IPI) animals.

After aliquoting the different reference blood samples, a homogeneity check was performed on 10 aliquots of the reference blood samples using the BVDV antigen ELISA kit from IDEXX, hereby obtaining the same qualitative result for all aliquots

of the same reference blood sample. Consequently, all reference blood 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 blood by BVDV antigen ELISA. In addition, all reference blood samples were tested two 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 blood samples for antigen detection by BVDV RT-qPCR

Replicates of 4 reference blood samples of bovine origin, either free from detectable BVDV-specific antigens (n=2 coded 'PT2018BVDNB1' and 'PT2018BVDNB2') or containing detectable BVDV-specific antigens (n=2 coded 'PT2018BVDPB1' and 'PT2018BVDPB2'), were used.
In total, 70 aliquots of reference blood samples were distributed to 7 participating laboratories. All laboratories received 10 aliquots: 3 aliquots of the reference blood samples PT2018BVDNB1, PT2018BVDPB1 and 2 aliquots of the reference blood sample PT2018BVDNB2, PT2018BVDPB2. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 4 and Table 8).

For each reference blood sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference blood 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 blood samples PT2018BVDNB1 and PT2018BVDNB2 were obtained from 2 different BVDV-free animals from the field. The reference blood samples PT2018BVDPB1 and PT2018BVDPB2 were obtained from 2 different calves that were classified as immunotolerant persistently (BVDV-1) infected (IPI) animals.

After aliquoting the different reference blood samples, a homogeneity check was performed on 10 alliquots of the reference blood samples using an in-house developed BVDV RT-qPCR hereby obtaining the same qualitative result for all aliquots of the same reference blood sample. Consequently, all reference blood 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 by BVDV RT-qPCR in bovine blood. In addition, all reference blood samples were tested 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

Ten reference ear notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=5; coded 'PT2018BVDAgNE1', 'PT2018BVDAgNE2', 'PT2018BVDAgNE3', 'PT2018BVDAgNE4' and 'PT2018BVDAgNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2018BVDAgPE1', 'PT2018BVDAgPE2', 'PT2018BVDAgPE3', 'PT2018BVDAgPE4', and 'PT2018BVDAgPE5'), were used.
In total, 50 aliquots of reference ear notch samples were distributed to 5 participating laboratories. All laboratories received 10 aliquots: 1 aliquot of each reference ear notch sample. (Table 3 and Table 7).

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/blood plus ELISA from IDEXX.

The reference ear notch samples PT2018BVDAgNE1, PT2018BVDAgNE2, PT2018BVDAgNE3, PT2018BVDAgNE4 and PT2018BVDAgNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2018BVDAgPE1, PT2018BVDAgPE2, PT2018BVDAgPE3, PT2018BVDAgPE4, and PT2018BVDAgPE5 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.

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 twice 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

Ten reference ear notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=5; coded 'PT2018BVDAgNE1', 'PT2018BVDAgNE2', 'PT2018BVDAgNE3', 'PT2018BVDAgNE4' and 'PT2018BVDAgNE5') or containing detectable BVDV-specific antigens (n=5; coded 'PT2018BVDAgPE1', 'PT2018BVDAgPE2', 'PT2018BVDAgPE3', 'PT2018BVDAgPE4', and 'PT2018BVDAgPE5'), 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 samples. (Table 5 and Table 9).

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

The reference ear notch samples PT2018BVDAgNE1, PT2018BVDAgNE2, PT2018BVDAgNE3, PT2018BVDAgNE4 and PT2018BVDAgNE5 were obtained from 5 different BVDV-free animals from the field. The reference ear notch samples PT2018BVDAgPE1, PT2018BVDAgPE2, PT2018BVDAgPE3, PT2018BVDAgPE4, and PT2018BVDAgPE5 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.

After aliquoting the different ear notch samples, a homogeneity check was performed previously (2017) 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. All reference ear notch samples were tested once before the PT to confirm their status (pré-verification). 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.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.

III.3.3. Threshold for qualification

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

IV. Results

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

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

The reference blood and ear notch samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 8th of October 2018. LAB1, LAB2, LAB3, LAB4 and LAB5 acknowledged receipt of the samples the same day, whereas LAB6, LAB7, LAB8 and LAB9 acknowledged receipt of the samples on 9th of October 2018.
LAB9 inform us that upon receipt of the blood samples the caps of tubes 5 and 7 were covered with blood but there was enough sample left to perform the analyses.
Analyses were performed between 9th and 29th of October 2018 (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 19th and 30th of October 2018 (Table 1). All participants, except LAB2 and LAB7 respected the deadline of 26st of October 2018 for submission of the results.

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	Submission of the results (Excel file)
LAB1	08/10/2018	12/10/2018 (Blood) 17/10/2018 (Ear Notch)	09/10/2018 (Blood) 17/10/2018 (Ear Notch)	22/10/2018 (ELISA) 26/10/2018 (RT-qPCR)
LAB2	08/10/2018	10/10/2018	09/10/2018 (Blood) 22/10/2018 (Ear Notch)	**29/10/2018**
LAB3	08/10/2018	10/10/2018	11/10/2018 (Ear Notch)	25/10/2018
LAB4	08/10/2018	16/10/2018 (Ear Notch)	09/10/2018 (Blood)	19/10/2018
LAB5	08/10/2018	NA	11/10/2018	23/10/2018
LAB6	09/10/2018	NA	18/10/2018	22/10/2018
LAB7	09/10/2018	NA	29/10/2018	**30/10/2018**
LAB8	09/10/2018	19/10/2018 (Ear Notch)	NA	19/10/2018
LAB9	09/10/2018	NA	15/10/2018 (Blood)	22/10/2018

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories except LAB2 have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

Qualitative data analysis showed that:

(i) For the detection of BVDV-specific antigens by **antigen ELISA** (Table 2 and 3): For the matrix blood, all laboratories (LAB1, LAB2 and LAB3) provided qualitative results that were in full agreement with the true status of the reference blood samples (100% of agreement). For the matrix ear notch, LAB2, LAB3 and LAB8 provided qualitative results that were in full agreement with the true status of the reference ear notch samples (100% of agreement). In contrast, LAB1 misclassified 8 aliquot (20% of agreement) of reference ear notch samples and LAB4 misclassified 6 aliquots (40% of agreement).

(ii) For the detection of BVDV-specific antigens by **RT-qPCR** (Table 4 and 5): For the matrix blood, all laboratories (LAB1, LAB2, LAB4, LAB5, LAB6, LAB7 and LAB9) provided qualitative results that were in full agreement with the true status of the reference blood samples (100% of agreement). For the matrix ear notch, all laboratories (LAB1, LAB2, LAB3, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the true status of the reference ear notch samples (100% of agreement).

Table 2. Antigen ELISA Blood: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference blood 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 blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0)	0 (0)	0 (0)
success	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 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**	**3**	**4**	**8**
failure	**8 (80)**	0 (0)	0 (0)	**6 (60)**	0 (0)
success	**2 (20)**	10 (100)	10 (100)	**4 (40)**	10 (100)

Table 4. RT-qPCR Blood: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference blood 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 blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1	**2**	**4**	**5**	**6**	**7**	**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 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 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	3	5	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)

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** for the matrix blood no variability between the participating laboratories could be observed since all participants correctly identified all reference blood samples. For the matrix ear notch no variability between LAB2, LAB3 and LAB8 could be observed since these participants correctly identified all reference ear notch samples. In contrast LAB1 misclassified the aliquot of the reference ear notch samples PT2018BVDAgNE1, PT2018BVDAgNE2, PT2018BVDAgNE3 and PT2018BVDAgNE5 (positive instead of negative) as well as PT2018BVDAgPE1, PT2018BVDAgPE2, PT2018BVDAgPE4 and PT2018BVDAgPE5 (negative instead of positive). LAB4 misclassified the aliquot of the reference ear notch samples PT2018BVDAgNE1, PT2018BVDAgNE2 and PT2018BVDAgNE3 (positive instead of negative) as well as PT2018BVDAgPE1, PT2018BVDAgPE2 and PT2018BVDAgPE4 (negative instead of positive).

(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 blood and ear notch samples.

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

Table 6. Antigen ELISA blood : The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference blood samples (SAMPLE), the external identification of the reference blood 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	PT2018BVDNB2	NEG	NEG	1
2	1	2	PT2018BVDPB1	POS	POS	1
3	1	3	PT2018BVDPB2	POS	POS	1
4	1	4	PT2018BVDNB1	NEG	NEG	1
5	1	5	PT2018BVDNB2	NEG	NEG	1
6	1	6	PT2018BVDNB3	NEG	NEG	1
7	1	7	PT2018BVDNB1	NEG	NEG	1
8	1	8	PT2018BVDPB1	POS	POS	1
9	1	9	PT2018BVDPB3	POS	POS	1
10	1	10	PT2018BVDPB2	POS	POS	1

(Table 6 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
11	2	1	PT2018BVDNB2	NEG	NEG	1
12	2	2	PT2018BVDNB1	NEG	NEG	1
13	2	3	PT2018BVDNB3	NEG	NEG	1
14	2	4	PT2018BVDPB1	POS	POS	1
15	2	5	PT2018BVDPB2	POS	POS	1
16	2	6	PT2018BVDNB1	NEG	NEG	1
17	2	7	PT2018BVDPB1	POS	POS	1
18	2	8	PT2018BVDPB3	POS	POS	1
19	2	9	PT2018BVDNB2	NEG	NEG	1
20	2	10	PT2018BVDPB2	POS	POS	1
21	3	1	PT2018BVDNB2	NEG	NEG	1
22	3	2	PT2018BVDPB1	POS	POS	1
23	3	3	PT2018BVDPB2	POS	POS	1
24	3	4	PT2018BVDNB1	NEG	NEG	1
25	3	5	PT2018BVDNB2	NEG	NEG	1
26	3	6	PT2018BVDNB3	NEG	NEG	1
27	3	7	PT2018BVDNB1	NEG	NEG	1
28	3	8	PT2018BVDPB1	POS	POS	1
29	3	9	PT2018BVDPB3	POS	POS	1
30	3	10	PT2018BVDPB2	POS	POS	1

Table 7. Antigen ELISA ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference ear notch samples (SAMPLE), the external identification of the reference 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	PT2018BVDAgNE3	**NEG**	**POS**	**0**
2	1	2	PT2018BVDAgPE1	**POS**	**NEG**	**0**
3	1	3	PT2018BVDAgNE1	**NEG**	**POS**	**0**
4	1	4	PT2018BVDAgPE3	POS	POS	1
5	1	5	PT2018BVDAgPE2	**POS**	**NEG**	**0**
6	1	6	PT2018BVDAgNE2	**NEG**	**POS**	**0**
7	1	7	PT2018BVDAgPE5	**POS**	**NEG**	**0**
8	1	8	PT2018BVDAgNE4	NEG	NEG	1
9	1	9	PT2018BVDAgNE5	**NEG**	**POS**	**0**
10	1	10	PT2018BVDAgPE4	**POS**	**NEG**	**0**

(Table 7 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
11	2	1	PT2018BVDAgPE2	POS	POS	1
12	2	2	PT2018BVDAgNE3	NEG	NEG	1
13	2	3	PT2018BVDAgNE1	NEG	NEG	1
14	2	4	PT2018BVDAgNE5	NEG	NEG	1
15	2	5	PT2018BVDAgPE1	POS	POS	1
16	2	6	PT2018BVDAgPE4	POS	POS	1
17	2	7	PT2018BVDAgNE2	NEG	NEG	1
18	2	8	PT2018BVDAgPE3	POS	POS	1
19	2	9	PT2018BVDAgNE4	NEG	NEG	1
20	2	10	PT2018BVDAgPE5	POS	POS	1
21	3	1	PT2018BVDAgNE3	NEG	NEG	1
22	3	2	PT2018BVDAgPE1	POS	POS	1
23	3	3	PT2018BVDAgNE1	NEG	NEG	1
24	3	4	PT2018BVDAgPE3	POS	POS	1
25	3	5	PT2018BVDAgPE2	POS	POS	1
26	3	6	PT2018BVDAgNE2	NEG	NEG	1
27	3	7	PT2018BVDAgPE5	POS	POS	1
28	3	8	PT2018BVDAgNE4	NEG	NEG	1
29	3	9	PT2018BVDAgNE5	NEG	NEG	1
30	3	10	PT2018BVDAgPE4	POS	POS	1
31	4	1	PT2018BVDAgPE2	**POS**	**NEG**	**0**
32	4	2	PT2018BVDAgNE3	**NEG**	**POS**	**0**
33	4	3	PT2018BVDAgNE1	**NEG**	**POS**	**0**
34	4	4	PT2018BVDAgNE5	NEG	NEG	1
35	4	5	PT2018BVDAgPE1	**POS**	**NEG**	**0**
36	4	6	PT2018BVDAgPE4	**POS**	**NEG**	**0**
37	4	7	PT2018BVDAgNE2	**NEG**	**POS**	**0**
38	4	8	PT2018BVDAgPE3	POS	POS	1
39	4	9	PT2018BVDAgNE4	NEG	NEG	1
40	4	10	PT2018BVDAgPE5	POS	POS	1
41	8	1	PT2018BVDAgNE3	NEG	NEG	1
42	8	2	PT2018BVDAgPE1	POS	POS	1
43	8	3	PT2018BVDAgNE1	NEG	NEG	1
44	8	4	PT2018BVDAgPE3	POS	POS	1
45	8	5	PT2018BVDAgPE2	POS	POS	1
46	8	6	PT2018BVDAgNE2	NEG	NEG	1
47	8	7	PT2018BVDAgPE5	POS	POS	1
48	8	8	PT2018BVDAgNE4	NEG	NEG	1
49	8	9	PT2018BVDAgNE5	NEG	NEG	1
50	8	10	PT2018BVDAgPE4	POS	POS	1

Table 8. RT-qPCR blood : The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference blood samples (SAMPLE), the external identification of the reference blood 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	PT2018BVDPB2	POS	POS	1
2	1	2	PT2018BVDNB1	NEG	NEG	1
3	1	3	PT2018BVDPB1	POS	POS	1
4	1	4	PT2018BVDNB1	NEG	NEG	1
5	1	5	PT2018BVDPB1	POS	POS	1
6	1	6	PT2018BVDNB2	NEG	NEG	1
7	1	7	PT2018BVDPB2	POS	POS	1
8	1	8	PT2018BVDNB1	NEG	NEG	1
9	1	9	PT2018BVDNB2	NEG	NEG	1
10	1	10	PT2018BVDPB1	POS	POS	1
11	2	1	PT2018BVDNB2	NEG	NEG	1
12	2	2	PT2018BVDPB1	POS	POS	1
13	2	3	PT2018BVDPB2	POS	POS	1
14	2	4	PT2018BVDNB2	NEG	NEG	1
15	2	5	PT2018BVDNB1	NEG	NEG	1
16	2	6	PT2018BVDPB1	POS	POS	1
17	2	7	PT2018BVDNB1	NEG	NEG	1
18	2	8	PT2018BVDPB2	POS	POS	1
19	2	9	PT2018BVDPB1	POS	POS	1
20	2	10	PT2018BVDNB1	NEG	NEG	1
21	4	1	PT2018BVDPB2	POS	POS	1
22	4	2	PT2018BVDNB1	NEG	NEG	1
23	4	3	PT2018BVDPB1	POS	POS	1
24	4	4	PT2018BVDNB1	NEG	NEG	1
25	4	5	PT2018BVDPB1	POS	POS	1
26	4	6	PT2018BVDNB2	NEG	NEG	1
27	4	7	PT2018BVDPB2	POS	POS	1
28	4	8	PT2018BVDNB1	NEG	NEG	1
29	4	9	PT2018BVDNB2	NEG	NEG	1
30	4	10	PT2018BVDPB1	POS	POS	1
31	5	1	PT2018BVDNB2	NEG	NEG	1
32	5	2	PT2018BVDPB1	POS	POS	1
33	5	3	PT2018BVDPB2	POS	POS	1
34	5	4	PT2018BVDNB2	NEG	NEG	1
35	5	5	PT2018BVDNB1	NEG	NEG	1
36	5	6	PT2018BVDPB1	POS	POS	1
37	5	7	PT2018BVDNB1	NEG	NEG	1
38	5	8	PT2018BVDPB2	POS	POS	1
39	5	9	PT2018BVDPB1	POS	POS	1
40	5	10	PT2018BVDNB1	NEG	NEG	1

(Table 8 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	6	1	PT2018BVDPB2	POS	POS	1
42	6	2	PT2018BVDNB1	NEG	NEG	1
43	6	3	PT2018BVDPB1	POS	POS	1
44	6	4	PT2018BVDNB1	NEG	NEG	1
45	6	5	PT2018BVDPB1	POS	POS	1
46	6	6	PT2018BVDNB2	NEG	NEG	1
47	6	7	PT2018BVDPB2	POS	POS	1
48	6	8	PT2018BVDNB1	NEG	NEG	1
49	6	9	PT2018BVDNB2	NEG	NEG	1
50	6	10	PT2018BVDPB1	POS	POS	1
51	7	1	PT2018BVDNB2	NEG	NEG	1
52	7	2	PT2018BVDPB1	POS	POS	1
53	7	3	PT2018BVDPB2	POS	POS	1
54	7	4	PT2018BVDNB2	NEG	NEG	1
55	7	5	PT2018BVDNB1	NEG	NEG	1
56	7	6	PT2018BVDPB1	POS	POS	1
57	7	7	PT2018BVDNB1	NEG	NEG	1
58	7	8	PT2018BVDPB2	POS	POS	1
59	7	9	PT2018BVDPB1	POS	POS	1
60	7	10	PT2018BVDNB1	NEG	NEG	1
61	9	1	PT2018BVDPB2	POS	POS	1
62	9	2	PT2018BVDNB1	NEG	NEG	1
63	9	3	PT2018BVDPB1	POS	POS	1
64	9	4	PT2018BVDNB1	NEG	NEG	1
65	9	5	PT2018BVDPB1	POS	POS	1
66	9	6	PT2018BVDNB2	NEG	NEG	1
67	9	7	PT2018BVDPB2	POS	POS	1
68	9	8	PT2018BVDNB1	NEG	NEG	1
69	9	9	PT2018BVDNB2	NEG	NEG	1
70	9	10	PT2018BVDPB1	POS	POS	1

Table 9. RT-qPCR ear notch: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference ear notch samples (SAMPLE), the external identification of the reference 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	PT2018BVDAgNE3	NEG	NEG	1
2	1	2	PT2018BVDAgNE4	NEG	NEG	1
3	1	3	PT2018BVDAgPE1	POS	POS	1
4	1	4	PT2018BVDAgPE3	POS	POS	1
5	1	5	PT2018BVDAgNE1	NEG	NEG	1
6	1	6	PT2018BVDAgPE2	POS	POS	1
7	1	7	PT2018BVDAgNE5	NEG	NEG	1
8	1	8	PT2018BVDAgPE5	POS	POS	1
9	1	9	PT2018BVDAgNE2	NEG	NEG	1
10	1	10	PT2018BVDAgPE4	POS	POS	1
11	2	1	PT2018BVDAgNE5	NEG	NEG	1
12	2	2	PT2018BVDAgNE3	NEG	NEG	1
13	2	3	PT2018BVDAgPE4	POS	POS	1
14	2	4	PT2018BVDAgPE1	POS	POS	1
15	2	5	PT2018BVDAgNE1	NEG	NEG	1
16	2	6	PT2018BVDAgPE3	POS	POS	1
17	2	7	PT2018BVDAgPE5	POS	POS	1
18	2	8	PT2018BVDAgNE4	NEG	NEG	1
19	2	9	PT2018BVDAgNE2	NEG	NEG	1
20	2	10	PT2018BVDAgPE2	POS	POS	1
21	3	1	PT2018BVDAgNE3	NEG	NEG	1
22	3	2	PT2018BVDAgNE4	NEG	NEG	1
23	3	3	PT2018BVDAgPE1	POS	POS	1
24	3	4	PT2018BVDAgPE3	POS	POS	1
25	3	5	PT2018BVDAgNE1	NEG	NEG	1
26	3	6	PT2018BVDAgPE2	POS	POS	1
27	3	7	PT2018BVDAgNE5	NEG	NEG	1
28	3	8	PT2018BVDAgPE5	POS	POS	1
29	3	9	PT2018BVDAgNE2	NEG	NEG	1
30	3	10	PT2018BVDAgPE4	POS	POS	1
31	5	1	PT2018BVDAgNE5	NEG	NEG	1
32	5	2	PT2018BVDAgNE3	NEG	NEG	1
33	5	3	PT2018BVDAgPE4	POS	POS	1
34	5	4	PT2018BVDAgPE1	POS	POS	1
35	5	5	PT2018BVDAgNE1	NEG	NEG	1
36	5	6	PT2018BVDAgPE3	POS	POS	1
37	5	7	PT2018BVDAgPE5	POS	POS	1
38	5	8	PT2018BVDAgNE4	NEG	NEG	1
39	5	9	PT2018BVDAgNE2	NEG	NEG	1
40	5	10	PT2018BVDAgPE2	POS	POS	1

(Table 9 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	6	1	PT2018BVDAgNE3	NEG	NEG	1
42	6	2	PT2018BVDAgNE4	NEG	NEG	1
43	6	3	PT2018BVDAgPE1	POS	POS	1
44	6	4	PT2018BVDAgPE3	POS	POS	1
45	6	5	PT2018BVDAgNE1	NEG	NEG	1
46	6	6	PT2018BVDAgPE2	POS	POS	1
47	6	7	PT2018BVDAgNE5	NEG	NEG	1
48	6	8	PT2018BVDAgPE5	POS	POS	1
49	6	9	PT2018BVDAgNE2	NEG	NEG	1
50	6	10	PT2018BVDAgPE4	POS	POS	1
51	7	1	PT2018BVDAgNE5	NEG	NEG	1
52	7	2	PT2018BVDAgNE3	NEG	NEG	1
53	7	3	PT2018BVDAgPE4	POS	POS	1
54	7	4	PT2018BVDAgPE1	POS	POS	1
55	7	5	PT2018BVDAgNE1	NEG	NEG	1
56	7	6	PT2018BVDAgPE3	POS	POS	1
57	7	7	PT2018BVDAgPE5	POS	POS	1
58	7	8	PT2018BVDAgNE4	NEG	NEG	1
59	7	9	PT2018BVDAgNE2	NEG	NEG	1
60	7	10	PT2018BVDAgPE2	POS	POS	1

V. Discussion

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

For the detection of BVDV-specific antigens by antigen ELISA in blood, 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, three out of five (LAB2, LAB3 and LAB8) participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference ear notch samples (100% of agreement) whereas LAB1 misclassified all the aliquot of the reference ear notch samples except PT2018BVDAgNE4 and PT2018BVDAgPE3 (20% of agreement) and LAB4 misclassified the aliquot of the reference ear notch samples PT2018BVDAgNE1, PT2018BVDAgNE2, PT2018BVDAgNE3, PT2018BVDAgPE1, PT2018BVDAgPE2 and PT2018BVDAgPE4 (40% of agreement). A possible explanation for LAB1 and LAB4 misclassification could be a shift in the samples order. Indeed if the LABPOSIT for LAB1 was 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 and for LAB4 was 3, 4, 5, 6, 7, 8, 9, 10, 1, 2 there would have been 100% of agreement. But it might be a coincidence.

For the detection of BVDV-specific antigens by antigen ELISA, all participating laboratories, except LAB8 used the ELISA kit BVDV Ag/Serum Plus Test from IDEXX, but 5 different batches were used: batch L121 (LAB1), batch L641 (LAB4), batch M141 (LAB3 for blood), batch M171 (LAB2) and batch M281 (LAB3 for ear notch). LAB7 used the SERELISA BVDV Erns Ag Capture ELISA kit from Zoetis (batch 234363).

For the detection of BVDV-specific antigens by RT-qPCR in blood, 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 LAB2, used a commercially available BVDV RT-qPCR: LAB1 (for blood), LAB3, LAB4, LAB5 and LAB6 used the VetMax BVD4ALL Screening test kit from Thermofisher Scientific (LSI) [batch 014 (LAB4) and batch 015 (LAB1, LAB3, LAB5 and LAB6)], LAB1 (for ear notch) used the Virotype BVDV RT-PCR kit from Qiagen (batch 257121256), LAB7 the Bio-T kit BVDV/BVD Universal kit from Biosellal (batch BVDU-05C) and LAB9 used a kit from Life Technologies (batch B12S-130). LAB2 used an in house RT-qPCR.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by the BVDV reference laboratory of 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 ELISA in blood and RT-qPCR in blood and ear notch. For the detection of BVDV-specific antigens by ELISA in ear notch 3 out of 5 participating laboratories achieved a satisfactory performance. In contrast LAB1 and LAB4 did not achieved a satisfactory performance for the detection of BVDV-specific antigens by ELISA in reference ear notch samples

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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

Biosellal (Dardilly, France)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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)

LSI - Thermo Fisher Scientific (Lissieu, France)

Sciensano (Uccle, 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 box plot for the laboratories participating in the PT antigen ELISA blood is shown in Figures 1 and the box plot for the laboratories participating in the PT antigen RT-qPCR blood is shown in Figure 2.
No box plot was made for the PT antigen ELISA and antigen RT-qPCR ear notch because there was only one aliquot in the panel for these samples.

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.

The quantitative data analyse of the PT antigen ELISA blood was performed on the normalized data according to the instructions of the PT provider per reference blood sample and per participating laboratory. (Figure 1). The samples PT2018BVDNB3 and PT2018BVDPB3 were not included in the figure because there was only one aliquot in the panel for these samples.

The quantitative data analyse of the PT antigen RT-qPCR blood was performed on the normalized data according to the instructions of the PT provider per positive reference blood sample and per participating laboratory (Figure2).

Detection of BVDV-specific antigens by antigen ELISA for the matrix blood

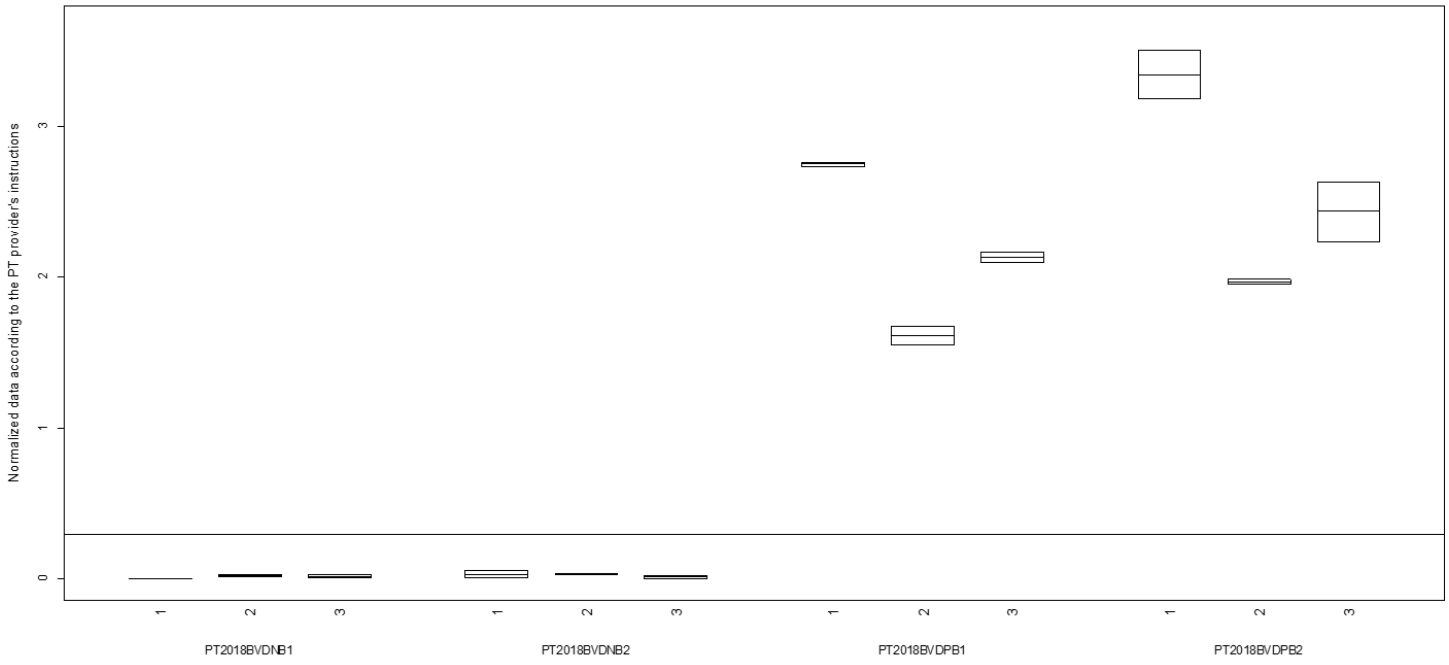

Figure 1. Box plots showing the normalized OD values according the PT provider per reference blood and per participating laboratory. All participating laboratories used the ELISA kit BVDV Ag/Serum Plus Test from IDEXX, but 3 different batches. Cut-off value (Idexx 0.3) is shown by a horizontal line.

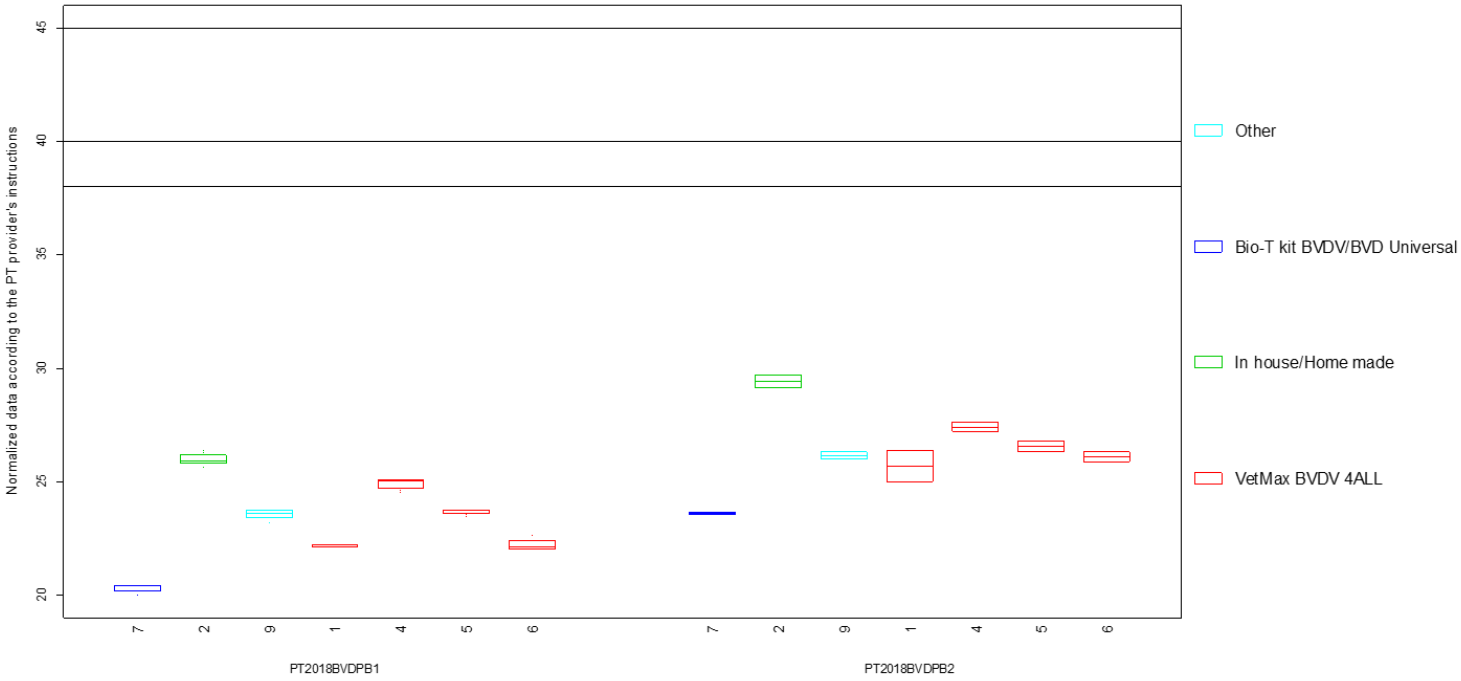

Figure 2. Box plots showing the normalized OD values according the PT provider per reference blood and per participating laboratory. All participating laboratories, except LAB2, used a commercially available BVDV RT-qPCR: Thermofisher Scientific LSI (red plots), Biosellal (blue plots) and Life Technologies (light blue plots). LAB2 used an in house RT-qPCR (green plots). Cut-off value (Thermofisher Scientific 38-45 or 45, Biosellal 40, Life Technologies 45) are shown by a horizontal line.