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172-PT

PROFICIENCY TESTING 2014

Bovine Viral Diarrhea Virus (BVDV)

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

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

THIS REPORT REPLACES AND CANCELS THE PREVIOUS REPORT PT2014BVDVIR:

Adaptation of Boxplots and legend for RT-PCR matrix Ear notch

DATE BEGIN PT: 03 NOVEMBER 2014

DATE REPORT: 16 MARCH 2015

I. Introduction

Details relevant to the proficiency test (PT) are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

II. Aim

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

III. Materials and methods

III.1. Conduct of diagnostic tests

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

III.2. Reference samples

LAB1 and LAB4 received 40 aliquots, namely 10 aliquots of the matrix serum and blood and 20 ear-notch samples. LAB2, LAB3, LAB6, LAB7 and LAB8 received 30 aliquots, namely 10 aliquots each matrix: serum, blood and ear-notch samples. LAB5 and LAB9 received 20 aliquots, namely 10 aliquots of the matrix blood and ear-notch. LAB10 received 10 aliquots of ear-notch samples. Each matrix was sent in a different block with reference samples (position 1-10).

III.2.1. Reference serum samples

Replicates of 5 reference serum samples of bovine origin, either free from detectable BVDV-specific antigens (n=2; coded 'PT2014BVDVIRNS1', 'PT2014BVDVIRNS2') or containing detectable BVDV-specific antigens (n=3; coded 'PT204BVDVIRPS1', 'PT2014BVDVIRPS2' and PT2014BVDVIRPS3), were used.
In total, 70 aliquots of reference serum samples were distributed to 7 participating laboratories. All participants received 10 aliquots: 2 alliquots of each sample were distributed. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 9 and Table 12).

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

The reference serum sample PT2014BVDVIRNS1 and PT2014BVDVIRNS2 were obtained from 2 different BVDV-free animals. The reference serum sample PT2014BVDVIRPS1 was a field sample obtained from an animal to be infected with a BVDV type 2 strain. The reference sample PT2014BVDVIRPS2 was obtained from a calve that was classified as immunotolerant persistently BVDV-infected (IPI) animal. The reference sample PT2014BVDVIRPS3 obtained from an animal to be infected with a BVDV type 1 strain

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

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 and 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. In addition, all reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX.

III.2.2. Reference blood samples

Replicates of 5 reference blood samples of bovine origin, either free from detectable BVDV-specific antigens (n=2; coded 'PT2014BVDVIRNB1' and 'PT2014BVDVIRNB2'') or containing detectable BVDV-specific antigens (n=3; coded 'PT2014BVDVIRPB1', 'PT2014BVDVIRPB2' and PT2014BVDVIRPB3), were used.
In total, 100 aliquots of reference blood samples were distributed to 10 participating laboratories. All participants received 10 aliquots: 2 alliquots of each sample were distributed. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 10 and Table 13).

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

The reference blood samples were obtained from the same animals of which the reference serum samples were obtained. For each reference blood sample, the same qualitative result was obtained with the in-house developed BVDV RT-qPCR assays and the BVDV antigen ELISA kit from IDEXX.

After aliquoting the different reference blood samples, a homogeneity check was performed on 10 alliquots of the reference blood sample using an in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX, hereby obtaining the same qualitative result for all aliquots of the same reference 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. 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 and the BVDV antigen ELISA kit from IDEXX.

III.2.1. Reference ear-notch samples

Replicates of 5 reference ear-notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=2; coded 'PT2014BVDVIRNE1' and 'PT2014BVDVIRNE2'') or containing detectable BVDV-specific antigens (n=3; coded 'PT2014BVDVIRPE1', 'PT2014BVDVIRPE2' and PT2014BVDVIRPE3), were used.
In total, 110 aliquots of reference ear-notch samples were distributed to 9 participating laboratories. All participants received 10 aliquots: 2 alliquots of each sample were distributed. The positions of the reference ear-notch samples in the sent blocks were randomized for each participant (Table 11 and Table 14).

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

The reference ear-notch samples were obtained from the same animals of which the reference serum samples were obtained. For each reference ear-notch sample, the same qualitative result was obtained with the in-house developed BVDV RT-qPCR assays and the BVDV antigen ELISA kit from IDEXX.

Using the in-house BVDV RT-qPCR and the antigen ELISA the same qualitative result was obtained for all the reference ear-notch samples. After aliquoting the different ear-notch samples, a homogeneity check was performed on 3 alliquots of each reference ear notch sample using an in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX, hereby obtaining the same qualitative result for all aliquots of the same reference ear notch sample. Consequently, all reference ear-notch samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BVDV-specific antigens in bovine ear notch samples. In addition, all reference ear notch samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX.

An overview of the different reference serum, blood and ear-notch samples and their assigned status for BVDV RT-qPCR and BVDV antigen ELISA is shown in Table 1.

Table 1. The different reference serum, blood and ear-notch samples and their assigned status (NEG: negative; POS: positive) for BVDV RT-qPCR and BVDV antigen ELISA.

SAMPLE	MATRIX	STATUS	
		RT-qPCR	Ag ELISA
PT2014BVDVIRNS1	SERUM	NEG	NEG
PT2014BVDVIRNS2	SERUM	NEG	NEG
PT2014BVDVIRPS1	SERUM	POS	POS
PT2014BVDVIRPS2	SERUM	POS	POS
PT2014BVDVIRPS3	SERUM	POS	POS
PT2014BVDVIRNB1	BLOOD	NEG	NEG
PT2014BVDVIRNB2	BLOOD	NEG	NEG
PT2014BVDVIRPB1	BLOOD	POS	POS
PT2014BVDVIRPB2	BLOOD	POS	POS
PT2014BVDVIRPB3	BLOOD	POS	POS
PT2014BVDVIRNE1	EAR NOTCH	NEG	NEG
PT2014BVDVIRNE2	EAR NOTCH	NEG	NEG
PT2014BVDVIRPE1	EAR NOTCH	POS	POS
PT2014BVDVIRPE2	EAR NOTCH	POS	POS
PT2014BVDVIRPE3	EAR NOTCH	POS	POS

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 this PT.

III.3.3. Threshold for qualification

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

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the operational unit CVD-ERA of CODA-CERVA.

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

LAB1 participated in the PT serum, blood and ear-notch for both RT-qPCR and Antigen ELISA. LAB2 participated in the PT serum, blood and ear-notch for the Antigen ELISA. LAB3 participated in the PT serum, blood and ear-notch for RT-qPCR and in the PT serum and blood for Antigen ELISA. LAB4 participated in the PT serum, blood and ear-notch for Antigen ELISA and in the PT earnotch for RT-qPCR. LAB5 participated in the PT ear-notch for Antigen ELISA. LAB6, LAB7 and LAB8 participated in the PT serum, blood and ear-notch for RT-qPCR. LAB9 participated in the PT blood and ear-notch for RT-qPCR. LAB10 participated in the PT blood for RT-qPCR.

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results were submitted to the operational unit CVD-ERA between 7th and 21th of November 2014.

Table 2. Overview of the laboratories that participated with relation to starting date and submission of results towards the operational unit CVD-ERA of CODA-CERVA for the different assays as well as the different matrices

Laboratory	Reference samples received	Start of analysis RT-qPCR			Submission of the results
		serum	blood	earnotch	
LAB1	03/11/2014	13/11/2014	13/11/2014	13/11/2014	20/11/2014
LAB3	03/11/2014	14/11/2014	14/11/2014	13/11/2014	20/11/2014
LAB4	03/11/2014	NA	NA	20/11/201	21/11/2014
LAB6	04/11/2014	18/11/2014	18/11/2014	18/11/2014	21/11/2014
LAB7	03/11/2014	05+06/11/2014	05+06/11/2014	05+06/11/2014	07/11/2014
LAB8	03/11/2014	06/11/2014	06/11/2014	06/11/2014	14/11/2014
LAB9	04/11/2014	NA	19/11/2014	19/11/2014	20/11/2014
LAB10	04/11/2014	NA	05/11/2014	NA	06/11/2014

Laboratory	Reference samples received	Start of analysis Ag-ELISA			Submission of the results
		serum	blood	earnotch	
LAB1	03/11/2014	04/11/2014	04/11/2014	04/11/2014	20/11/2014
LAB2	03/11/2014	05/11/2014	05/11/2014	05/11/2014	12/11/2014
LAB3	03/11/2014	7/11/2014	7/11/2014	NA	20/11/2014
LAB4	03/11/2014	18/1/2014	18/11/2014	18/11/2014	20/11/2014
LAB5	05/11/2014	NA	NA	17/11/2014	21/11/2014

IV.3. Compliance with the procedure

LAB1, LAB2, LAB4, LAB5, LAB6, LAB7, LAB8 and LAB10 have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that:

(i) For the detection of BVDV-specific antigens by **RT-qPCR** (Tables 3, 4 and 5): For the matrix serum, 4 out of 5 laboratories (LAB3, LAB6, LAB7 and LAB8) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). For the matrix blood, 5 out of 7 laboratories (LAB3, LAB6, LAB7, LAB8 and LAB10) 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, 6 out of 7 laboratories (LAB1, LAB4, LAB6, LAB7, LAB8 and LAB9) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement).

(ii) For the detection of BVDV-specific antigens by **antigen ELISA** (Tables 6, 7 and 8). For the matrix serum, all participating laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). For the matrix blood all participating

laboratories (LAB1, LAB2 ,LAB3 and LAB4) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). For the matrix ear-notch all participating laboratories (LAB1, LAB2, LAB4 and LAB5) provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement).

A quantitative data analysis was done by making box plots of the data for each matrix separately. A real quantitative analysis (k and h plots) was not possible since only 2 repeats of each sample were sent. This quantitative analysis (boxplots) is shown for educational purposes in Annex 1.

Table 3. 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. All participating laboratories received 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**3**	**6**	**7**	**8**
failure	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	**8 (80.0)**	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

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 CODA-CERVA. All participating laboratories received 10 aliquots of reference blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1	**3**	**6**	**7**	**8**	**9**	**10**
failure	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)
success	9 (90.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	**8 (80.0)**	10 (100.0)

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

	LABNR						
	1	**3**	**4**	**6**	**7**	**8**	**9**
failure	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	**8 (80.0)**	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

Table 6. 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. All participating laboratories received 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

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

Table 7. 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 CODA-CERVA. All participating laboratories received 10 aliquots of reference blood samples. Results are presented as absolute values and percentages (in parentheses).

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

Table 8. 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. All participating laboratories received 10 aliquots of reference ear-notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	**2**	**4**	**5**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

(i) For the detection of BVDV-specific antigens by **RT-qPCR in serum**, no variability between LAB3, LAB6, LAB7 and LAB8 could be observed since these participants correctly identified all reference serum samples. In contrast LAB1 misclassified 1 aliquot of the negative reference serum samples PT2014BVDVIRNS1 and PT2014BVDVIRNS2. These serum samples were tested positive instead of negative.
For the detection of BVDV-specific antigens by **RT-qPCR in blood**, no variability between LAB3, LAB6, LAB7, LAB8 and LAB10 could be observed since these participants correctly identified all reference blood samples. In contrast LAB1 misclassified 1 aliquot of the negative reference blood sample PT2014BVDVIRNB1. This blood sample was tested positive instead of negative. LAB9 misclassified 2 aliquots of the positive reference blood sample PT2014BVDVIRPB3. These blood samples were tested negative instead of positive.
For the detection of BVDV-specific antigens by **RT-**qPCR in ear-notch, no variability between LAB1, LAB4, LAB6, LAB7, LAB8 and LAB9 could be observed since these participants correctly identified all reference ear-notch samples. In contrast LAB3 misclassified 1 aliquot of the negative reference ear-notch sample PT2014BVDVIRNE2 (positive instead of negative) and 1 aliquot of the positive reference ear-notch sample PT2014BVDVIRPE2. This sample was tested negative instead of positive.

(ii) For the detection of BVDV-specific antigens by **antigen ELISA**, no variability between the participating labs could be observed since these laboratories correctly identified all reference samples (all matrices).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum and blood samples are shown in Table 9 (serum), 10 (blood) and 11 (ear notch) for RT-qPCR and in Tables 12 (serum), 13 (blood) and 14 (ear notch) for antigen ELISA.

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 (STATUS). NEG: negative; POS: positive;.

	LABN	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRPS1	POS	POS	1
2	1	2	PT2014BVDVIRNS1	**NEG**	**POS**	**0**
3	1	3	PT2014BVDVIRPS2	POS	POS	1
4	1	4	PT2014BVDVIRNS2	NEG	NEG	1
5	1	5	PT2014BVDVIRPS3	POS	POS	1
6	1	6	PT2014BVDVIRPS1	POS	POS	1
7	1	7	PT2014BVDVIRNS1	NEG	NEG	1
8	1	8	PT2014BVDVIRPS3	POS	POS	1
9	1	9	PT2014BVDVIRNS2	**NEG**	**POS**	**0**
10	1	10	PT2014BVDVIRPS2	POS	POS	1
11	3	1	PT2014BVDVIRPS1	POS	POS	1
12	3	2	PT2014BVDVIRNS1	NEG	NEG	1
13	3	3	PT2014BVDVIRPS2	POS	POS	1
14	3	4	PT2014BVDVIRNS2	NEG	NEG	1
15	3	5	PT2014BVDVIRPS3	POS	POS	1
16	3	6	PT2014BVDVIRPS1	POS	POS	1
17	3	7	PT2014BVDVIRNS1	NEG	NEG	1
18	3	8	PT2014BVDVIRPS3	POS	POS	1
19	3	9	PT2014BVDVIRNS2	NEG	NEG	1
20	3	10	PT2014BVDVIRPS2	POS	POS	1
21	6	1	PT2014BVDVIRPS1	POS	POS	1
22	6	2	PT2014BVDVIRNS1	NEG	NEG	1
23	6	3	PT2014BVDVIRPS2	POS	POS	1
24	6	4	PT2014BVDVIRNS2	NEG	NEG	1
25	6	5	PT2014BVDVIRPS3	POS	POS	1
26	6	6	PT2014BVDVIRPS1	POS	POS	1
27	6	7	PT2014BVDVIRNS1	NEG	NEG	1
28	6	8	PT2014BVDVIRPS3	POS	POS	1
29	6	9	PT2014BVDVIRNS2	NEG	NEG	1
30	6	10	PT2014BVDVIRPS2	POS	POS	1
31	7	1	PT2014BVDVIRPS2	POS	POS	1
32	7	2	PT2014BVDVIRNS2	NEG	NEG	1
33	7	3	PT2014BVDVIRPS3	POS	POS	1
34	7	4	PT2014BVDVIRPS1	POS	POS	1
35	7	5	PT2014BVDVIRNS1	NEG	NEG	1
36	7	6	PT2014BVDVIRPS3	POS	POS	1
37	7	7	PT2014BVDVIRNS2	NEG	NEG	1
38	7	8	PT2014BVDVIRPS2	POS	POS	1
39	7	9	PT2014BVDVIRPS1	POS	POS	1
40	7	10	PT2014BVDVIRNS1	NEG	NEG	1

Table 9 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	8	1	PT2014BVDVIRPS1	POS	POS	1
42	8	2	PT2014BVDVIRNS1	NEG	NEG	1
43	8	3	PT2014BVDVIRPS2	POS	POS	1
44	8	4	PT2014BVDVIRNS2	NEG	NEG	1
45	8	5	PT2014BVDVIRPS3	POS	POS	1
46	8	6	PT2014BVDVIRPS1	POS	POS	1
47	8	7	PT2014BVDVIRNS1	NEG	NEG	1
48	8	8	PT2014BVDVIRPS3	POS	POS	1
49	8	9	PT2014BVDVIRNS2	NEG	NEG	1
50	8	10	PT2014BVDVIRPS2	POS	POS	1

Table 10. RT-qPCR blood: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference blood samples (SAMPLE), the positions of the reference blood samples as placed in the block (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive;.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRPB1	POS	POS	1
2	1	2	PT2014BVDVIRNB1	NEG	NEG	1
3	1	3	PT2014BVDVIRNB2	NEG	NEG	1
4	1	4	PT2014BVDVIRPB2	POS	POS	1
5	1	5	PT2014BVDVIRPB1	POS	POS	1
6	1	6	PT2014BVDVIRPB3	POS	POS	1
7	1	7	PT2014BVDVIRNB1	**NEG**	**POS**	**0**
8	1	8	PT2014BVDVIRPB2	POS	POS	1
9	1	9	PT2014BVDVIRNB2	NEG	NEG	1
10	1	10	PT2014BVDVIRPB3	POS	POS	1
11	3	1	PT2014BVDVIRPB1	POS	POS	1
12	3	2	PT2014BVDVIRNB1	NEG	NEG	1
13	3	3	PT2014BVDVIRNB2	NEG	NEG	1
14	3	4	PT2014BVDVIRPB2	POS	POS	1
15	3	5	PT2014BVDVIRPB1	POS	POS	1
16	3	6	PT2014BVDVIRPB3	POS	POS	1
17	3	7	PT2014BVDVIRNB1	NEG	NEG	1
18	3	8	PT2014BVDVIRPB2	POS	POS	1
19	3	9	PT2014BVDVIRNB2	NEG	NEG	1
20	3	10	PT2014BVDVIRPB3	POS	POS	1

Table 10 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	6	1	PT2014BVDVIRNB2	NEG	NEG	1
22	6	2	PT2014BVDVIRPB2	POS	POS	1
23	6	3	PT2014BVDVIRPB1	POS	POS	1
24	6	4	PT2014BVDVIRPB3	POS	POS	1
25	6	5	PT2014BVDVIRNB1	NEG	NEG	1
26	6	6	PT2014BVDVIRPB2	POS	POS	1
27	6	7	PT2014BVDVIRNB2	NEG	NEG	1
28	6	8	PT2014BVDVIRPB3	POS	POS	1
29	6	9	PT2014BVDVIRPB1	POS	POS	1
30	6	10	PT2014BVDVIRNB1	NEG	NEG	1
31	7	1	PT2014BVDVIRPB1	POS	POS	1
32	7	2	PT2014BVDVIRNB1	NEG	NEG	1
33	7	3	PT2014BVDVIRNB2	NEG	NEG	1
34	7	4	PT2014BVDVIRPB2	POS	POS	1
35	7	5	PT2014BVDVIRPB1	POS	POS	1
36	7	6	PT2014BVDVIRPB3	POS	POS	1
37	7	7	PT2014BVDVIRNB1	NEG	NEG	1
38	7	8	PT2014BVDVIRPB2	POS	POS	1
39	7	9	PT2014BVDVIRNB2	NEG	NEG	1
40	7	10	PT2014BVDVIRPB3	POS	POS	1
41	8	1	PT2014BVDVIRNB2	NEG	NEG	1
42	8	2	PT2014BVDVIRPB2	POS	POS	1
43	8	3	PT2014BVDVIRPB1	POS	POS	1
44	8	4	PT2014BVDVIRPB3	POS	POS	1
45	8	5	PT2014BVDVIRNB1	NEG	NEG	1
46	8	6	PT2014BVDVIRPB2	POS	POS	1
47	8	7	PT2014BVDVIRNB2	NEG	NEG	1
48	8	8	PT2014BVDVIRPB3	POS	POS	1
49	8	9	PT2014BVDVIRPB1	POS	POS	1
50	8	10	PT2014BVDVIRNB1	NEG	NEG	1
51	9	1	PT2014BVDVIRPB1	POS	POS	1
52	9	2	PT2014BVDVIRNB1	NEG	NEG	1
53	9	3	PT2014BVDVIRNB2	NEG	NEG	1
54	9	4	PT2014BVDVIRPB2	POS	POS	1
55	9	5	PT2014BVDVIRPB1	POS	POS	1
56	9	6	PT2014BVDVIRPB3	**POS**	**NEG**	**0**
57	9	7	PT2014BVDVIRNB1	NEG	NEG	1
58	9	8	PT2014BVDVIRPB2	POS	POS	1
59	9	9	PT2014BVDVIRNB2	NEG	NEG	1
60	9	10	PT2014BVDVIRPB3	**POS**	**NEG**	**0**

Table 10 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	10	1	PT2014BVDVIRNB2	NEG	NEG	1
62	10	2	PT2014BVDVIRPB2	POS	POS	1
63	10	3	PT2014BVDVIRPB1	POS	POS	1
64	10	4	PT2014BVDVIRPB3	POS	POS	1
65	10	5	PT2014BVDVIRNB1	NEG	NEG	1
66	10	6	PT2014BVDVIRPB2	POS	POS	1
67	10	7	PT2014BVDVIRNB2	NEG	NEG	1
68	10	8	PT2014BVDVIRPB3	POS	POS	1
69	10	9	PT2014BVDVIRPB1	POS	POS	1
70	10	10	PT2014BVDVIRNB1	NEG	NEG	1

Table 11. 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 (STATUS). NEG: negative; POS: positive;

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRNE1	NEG	NEG	1
2	1	2	PT2014BVDVIRPE2	POS	POS	1
3	1	3	PT2014BVDVIRPE1	POS	POS	1
4	1	4	PT2014BVDVIRPE3	POS	POS	1
5	1	5	PT2014BVDVIRNE1	NEG	NEG	1
6	1	6	PT2014BVDVIRPE3	POS	POS	1
7	1	7	PT2014BVDVIRNE2	NEG	NEG	1
8	1	8	PT2014BVDVIRPE1	POS	POS	1
9	1	9	PT2014BVDVIRNE2	NEG	NEG	1
10	1	10	PT2014BVDVIRPE2	POS	POS	1
11	3	1	PT2014BVDVIRNE1	NEG	NEG	1
12	3	2	PT2014BVDVIRPE2	POS	POS	1
13	3	3	PT2014BVDVIRPE1	POS	POS	1
14	3	4	PT2014BVDVIRPE3	POS	POS	1
15	3	5	PT2014BVDVIRNE1	NEG	NEG	1
16	3	6	PT2014BVDVIRPE3	POS	POS	1
17	3	7	PT2014BVDVIRNE2	NEG	NEG	1
18	3	8	PT2014BVDVIRPE1	POS	POS	1
19	3	9	PT2014BVDVIRNE2	**NEG**	**POS**	**0**
20	3	10	PT2014BVDVIRPE2	**POS**	**NEG**	**0**

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	4	1	PT2014BVDVIRPE1	POS	POS	1
22	4	2	PT2014BVDVIRPE3	POS	POS	1
23	4	3	PT2014BVDVIRNE1	NEG	NEG	1
24	4	4	PT2014BVDVIRPE3	POS	POS	1
25	4	5	PT2014BVDVIRNE2	NEG	NEG	1
26	4	6	PT2014BVDVIRPE1	POS	POS	1
27	4	7	PT2014BVDVIRNE2	NEG	NEG	1
28	4	8	PT2014BVDVIRPE2	POS	POS	1
29	4	9	PT2014BVDVIRNE1	NEG	NEG	1
30	4	10	PT2014BVDVIRPE2	POS	POS	1
31	6	1	PT2014BVDVIRPE1	POS	POS	1
32	6	2	PT2014BVDVIRPE3	POS	POS	1
33	6	3	PT2014BVDVIRNE1	NEG	NEG	1
34	6	4	PT2014BVDVIRPE3	POS	POS	1
35	6	5	PT2014BVDVIRNE2	NEG	NEG	1
36	6	6	PT2014BVDVIRPE1	POS	POS	1
37	6	7	PT2014BVDVIRNE2	NEG	NEG	1
38	6	8	PT2014BVDVIRPE2	POS	POS	1
39	6	9	PT2014BVDVIRNE1	NEG	NEG	1
40	6	10	PT2014BVDVIRPE2	POS	POS	1
41	7	1	PT2014BVDVIRNE1	NEG	NEG	1
42	7	2	PT2014BVDVIRPE2	POS	POS	1
43	7	3	PT2014BVDVIRPE1	POS	POS	1
44	7	4	PT2014BVDVIRPE3	POS	POS	1
45	7	5	PT2014BVDVIRNE1	NEG	NEG	1
46	7	6	PT2014BVDVIRPE3	POS	POS	1
47	7	7	PT2014BVDVIRNE2	NEG	NEG	1
48	7	8	PT2014BVDVIRPE1	POS	POS	1
49	7	9	PT2014BVDVIRNE2	NEG	NEG	1
50	7	10	PT2014BVDVIRPE2	POS	POS	1
51	8	1	PT2014BVDVIRPE1	POS	POS	1
52	8	2	PT2014BVDVIRPE3	POS	POS	1
53	8	3	PT2014BVDVIRNE1	NEG	NEG	1
54	8	4	PT2014BVDVIRPE3	POS	POS	1
55	8	5	PT2014BVDVIRNE2	NEG	NEG	1
56	8	6	PT2014BVDVIRPE1	POS	POS	1
57	8	7	PT2014BVDVIRNE2	NEG	NEG	1
58	8	8	PT2014BVDVIRPE2	POS	POS	1
59	8	9	PT2014BVDVIRNE1	NEG	NEG	1
60	8	10	PT2014BVDVIRPE2	POS	POS	1

Table 11 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	9	1	PT2014BVDVIRNE1	NEG	NEG	1
62	9	2	PT2014BVDVIRPE2	POS	POS	1
63	9	3	PT2014BVDVIRPE1	POS	POS	1
64	9	4	PT2014BVDVIRPE3	POS	POS	1
65	9	5	PT2014BVDVIRNE1	NEG	NEG	1
66	9	6	PT2014BVDVIRPE3	POS	POS	1
67	9	7	PT2014BVDVIRNE2	NEG	NEG	1
68	9	8	PT2014BVDVIRPE1	POS	POS	1
69	9	9	PT2014BVDVIRNE2	NEG	NEG	1
70	9	10	PT2014BVDVIRPE2	POS	POS	1

Table 12. 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 (STATUS). NEG: negative; POS: positive;.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRPS1	POS	POS	1
2	1	2	PT2014BVDVIRNS1	NEG	NEG	1
3	1	3	PT2014BVDVIRPS2	POS	POS	1
4	1	4	PT2014BVDVIRNS2	NEG	NEG	1
5	1	5	PT2014BVDVIRPS3	POS	POS	1
6	1	6	PT2014BVDVIRPS1	POS	POS	1
7	1	7	PT2014BVDVIRNS1	NEG	NEG	1
8	1	8	PT2014BVDVIRPS3	POS	POS	1
9	1	9	PT2014BVDVIRNS2	NEG	NEG	1
10	1	10	PT2014BVDVIRPS2	POS	POS	1
11	2	1	PT2014BVDVIRPS2	POS	POS	1
12	2	2	PT2014BVDVIRNS2	NEG	NEG	1
13	2	3	PT2014BVDVIRPS3	POS	POS	1
14	2	4	PT2014BVDVIRPS1	POS	POS	1
15	2	5	PT2014BVDVIRNS1	NEG	NEG	1
16	2	6	PT2014BVDVIRPS3	POS	POS	1
17	2	7	PT2014BVDVIRNS2	NEG	NEG	1
18	2	8	PT2014BVDVIRPS2	POS	POS	1
19	2	9	PT2014BVDVIRPS1	POS	POS	1
20	2	10	PT2014BVDVIRNS1	NEG	NEG	1

Table 12 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2014BVDVIRPS1	POS	POS	1
22	3	2	PT2014BVDVIRNS1	NEG	NEG	1
23	3	3	PT2014BVDVIRPS2	POS	POS	1
24	3	4	PT2014BVDVIRNS2	NEG	NEG	1
25	3	5	PT2014BVDVIRPS3	POS	POS	1
26	3	6	PT2014BVDVIRPS1	POS	POS	1
27	3	7	PT2014BVDVIRNS1	NEG	NEG	1
28	3	8	PT2014BVDVIRPS3	POS	POS	1
29	3	9	PT2014BVDVIRNS2	NEG	NEG	1
30	3	10	PT2014BVDVIRPS2	POS	POS	1
31	4	1	PT2014BVDVIRPS2	POS	POS	1
32	4	2	PT2014BVDVIRNS2	NEG	NEG	1
33	4	3	PT2014BVDVIRPS3	POS	POS	1
34	4	4	PT2014BVDVIRPS1	POS	POS	1
35	4	5	PT2014BVDVIRNS1	NEG	NEG	1
36	4	6	PT2014BVDVIRPS3	POS	POS	1
37	4	7	PT2014BVDVIRNS2	NEG	NEG	1
38	4	8	PT2014BVDVIRPS2	POS	POS	1
39	4	9	PT2014BVDVIRPS1	POS	POS	1
40	4	10	PT2014BVDVIRNS1	NEG	NEG	1

Table 13. Antigen ELISA blood: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference blood samples (SAMPLE), the positions of the reference blood samples as placed in the block (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive;.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRPB1	POS	POS	1
2	1	2	PT2014BVDVIRNB1	NEG	NEG	1
3	1	3	PT2014BVDVIRNB2	NEG	NEG	1
4	1	4	PT2014BVDVIRPB2	POS	POS	1
5	1	5	PT2014BVDVIRPB1	POS	POS	1
6	1	6	PT2014BVDVIRPB3	POS	POS	1
7	1	7	PT2014BVDVIRNB1	NEG	NEG	1
8	1	8	PT2014BVDVIRPB2	POS	POS	1
9	1	9	PT2014BVDVIRNB2	NEG	NEG	1
10	1	10	PT2014BVDVIRPB3	POS	POS	1
11	2	1	PT2014BVDVIRNB2	NEG	NEG	1
12	2	2	PT2014BVDVIRPB2	POS	POS	1
13	2	3	PT2014BVDVIRPB1	POS	POS	1
14	2	4	PT2014BVDVIRPB3	POS	POS	1
15	2	5	PT2014BVDVIRNB1	NEG	NEG	1
16	2	6	PT2014BVDVIRPB2	POS	POS	1
17	2	7	PT2014BVDVIRNB2	NEG	NEG	1
18	2	8	PT2014BVDVIRPB3	POS	POS	1
19	2	9	PT2014BVDVIRPB1	POS	POS	1
20	2	10	PT2014BVDVIRNB1	NEG	NEG	1
21	3	1	PT2014BVDVIRPB1	POS	POS	1
22	3	2	PT2014BVDVIRNB1	NEG	NEG	1
23	3	3	PT2014BVDVIRNB2	NEG	NEG	1
24	3	4	PT2014BVDVIRPB2	POS	POS	1
25	3	5	PT2014BVDVIRPB1	POS	POS	1
26	3	6	PT2014BVDVIRPB3	POS	POS	1
27	3	7	PT2014BVDVIRNB1	NEG	NEG	1
28	3	8	PT2014BVDVIRPB2	POS	POS	1
29	3	9	PT2014BVDVIRNB2	NEG	NEG	1
30	3	10	PT2014BVDVIRPB3	POS	POS	1
31	4	1	PT2014BVDVIRNB2	NEG	NEG	1
32	4	2	PT2014BVDVIRPB2	POS	POS	1
33	4	3	PT2014BVDVIRPB1	POS	POS	1
34	4	4	PT2014BVDVIRPB3	POS	POS	1
35	4	5	PT2014BVDVIRNB1	NEG	NEG	1
36	4	6	PT2014BVDVIRPB2	POS	POS	1
37	4	7	PT2014BVDVIRNB2	NEG	NEG	1
38	4	8	PT2014BVDVIRPB3	POS	POS	1
39	4	9	PT2014BVDVIRPB1	POS	POS	1
40	4	10	PT2014BVDVIRNB1	NEG	NEG	1

Table 14. Antigen ELISA 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 (STATUS). NEG: negative; POS: positive;

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014BVDVIRNE1	NEG	NEG	1
2	1	2	PT2014BVDVIRPE2	POS	POS	1
3	1	3	PT2014BVDVIRPE1	POS	POS	1
4	1	4	PT2014BVDVIRPE3	POS	POS	1
5	1	5	PT2014BVDVIRNE1	NEG	NEG	1
6	1	6	PT2014BVDVIRPE3	POS	POS	1
7	1	7	PT2014BVDVIRNE2	NEG	NEG	1
8	1	8	PT2014BVDVIRPE1	POS	POS	1
9	1	9	PT2014BVDVIRNE2	NEG	NEG	1
10	1	10	PT2014BVDVIRPE2	POS	POS	1
11	2	1	PT2014BVDVIRPE1	POS	POS	1
12	2	2	PT2014BVDVIRPE3	POS	POS	1
13	2	3	PT2014BVDVIRNE1	NEG	NEG	1
14	2	4	PT2014BVDVIRPE3	POS	POS	1
15	2	5	PT2014BVDVIRNE2	NEG	NEG	1
16	2	6	PT2014BVDVIRPE1	POS	POS	1
17	2	7	PT2014BVDVIRNE2	NEG	NEG	1
18	2	8	PT2014BVDVIRPE2	POS	POS	1
19	2	9	PT2014BVDVIRNE1	NEG	NEG	1
20	2	10	PT2014BVDVIRPE2	POS	POS	1
21	4	1	PT2014BVDVIRPE1	POS	POS	1
22	4	2	PT2014BVDVIRPE3	POS	POS	1
23	4	3	PT2014BVDVIRNE1	NEG	NEG	1
24	4	4	PT2014BVDVIRPE3	POS	POS	1
25	4	5	PT2014BVDVIRNE2	NEG	NEG	1
26	4	6	PT2014BVDVIRPE1	POS	POS	1
27	4	7	PT2014BVDVIRNE2	NEG	NEG	1
28	4	8	PT2014BVDVIRPE2	POS	POS	1
29	4	9	PT2014BVDVIRNE1	NEG	NEG	1
30	4	10	PT2014BVDVIRPE2	POS	POS	1
31	5	1	PT2014BVDVIRNE1	NEG	NEG	1
32	5	2	PT2014BVDVIRPE2	POS	POS	1
33	5	3	PT2014BVDVIRPE1	POS	POS	1
34	5	4	PT2014BVDVIRPE3	POS	POS	1
35	5	5	PT2014BVDVIRNE1	NEG	NEG	1
36	5	6	PT2014BVDVIRPE3	POS	POS	1
37	5	7	PT2014BVDVIRNE2	NEG	NEG	1
38	5	8	PT2014BVDVIRPE1	POS	POS	1
39	5	9	PT2014BVDVIRNE2	NEG	NEG	1
40	5	10	PT2014BVDVIRPE2	POS	POS	1

V. Discussion

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

For the detection of BVDV-specific antigens by RT-qPCR in serum, four out of five participating laboratories (LAB3, LAB6, LAB7 and LAB8) provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement), whereas LAB1 misclassified 1 aliquot of the negative reference serum samples PT2014BVDVIRNS1 and PT2014BVDVIRNS2 (80% of agreement). For the detection of BVDV-specific antigens by RT-qPCR in blood, five out of seven participating laboratories (LAB3, LAB6, LAB7, LAB8 and LAB10) provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement), whereas LAB1 misclassified 1 aliquot of the negative reference blood sample PT2014BVDVIRNB1 (90% of agreement) and LAB9 misclassified 2 aliquots of the positive reference blood sample PT2014BVDVIRPB3 (80% of agreement). For the detection of BVDV-specific antigens by RT-qPCR in ear-notch, six out of seven participating laboratories (LAB1, LAB4, LAB6, LAB7, LAB8 and LAB9) provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement), whereas LAB3 misclassified 1 aliquot of the negative reference ear-notch sample PT2014BVDVIRNE2 and 1 aliquot of the positive reference ear-notch sample PT2014BVDVIRPE2 (80% of agreement). All participating laboratories, except LAB7, used a commercially available BVDV RT-qPCR: LAB1, LAB8 used the VetMax BVDV screening test from LSI (batch B12S-106), LAB3 used the TaqVet BVDV Screening Kit from LSI (batch B12S-104) and LAB 4, LAB6 and LAB9 used the VetMax BVD4ALL from LSI (batch 004 (LAB4 and LAB6) and batch 003 (LAB9)), LAB10 used the BVD real time form Adiagène (btach10K4TR139).

For the detection of BVDV-specific antigens by antigen ELISA, all participating provided qualitative results that were in full agreement with the true status of the reference samples for all matrices (100% agreement). LAB1, LAB2 , LAB3 and LAB4 used the BVDV antigen test kit/serum plus ELISA from IDEXX, but 2 different batches were used: batch D371 (LAB1,LAB2 and LAB4), batch D001 (LAB3). LAB5 used the ID Screen BVDVAg Capture form IDVET (Lot 001).

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 (see III.3.3.). Consequently, all participating laboratories achieved a satisfactory performance for the detection of BVDV-specific antigens by ELISA in serum, blood and ear-notch samples.

LAB3, LAB6, LAB7 and LAB8 achieved a satisfactory performance for the detection of BVDV-specific antigens in reference serum samples by RT-qPCR. LAB1 did not obtain the required 90% of agreement for RT-qPCR in serum samples.
LAB1, LAB3, LAB6, LAB7, LAB8 and LAB10 achieved a satisfactory performance for the detection of BVDV-, specific antigens in reference blood samples by RT-qPCR. LAB9 did not obtain the required 90% of agreement for RT-qPCR in blood samples.
LAB1, LAB4, LAB6, LAB7, LAB8 and LAB9 achieved a satisfactory performance for the detection of BVDV-, specific antigens in reference ear-notch samples by RT-qPCR. LAB3 did not obtain the required 90% of agreement for RT-qPCR in ear-notch samples.

Head CVD-ERA
Yves Van der Stede

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) (Ciney, Belgium)

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

IDVET (Grabels, france)

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

Laboratoire Service International (LSI) (Lissieu, France)

Lavetan NV (Turnhout, Belgium)

PRIONICS AG (Schlieren, Switzerland)

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

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R (box plots) and SAS 9.2 (summary statistics).

During this PT only 2 alliquots of each sample (serum, blood and ear notch) were provided. Therefore the analysis in this PT was limited to perform the boxplots in R and this for the RT-qPCR and antigen ELISA separately for each matrix. When comparing the quantitative results obtained by RT-qPCR, it should be noted that the Ct or Cp values are not normalized with the internal controls. In addition, modifiable factors such as extraction protocol, PCR machine and calculation of Ct or Cp values are not taken into account.
For the antigen ELISA, the normalized OD values, calculated according to the instructions of the PT provider, were used.

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.

I. Box plots: RT-qPCR and antigen ELISA for serum, blood and ear notch samples

For the **RT-qPCR serum, blood and ear notch reference samples** box plots of the Ct or Cp values per reference sample and per participating laboratory were made using the statistical software R and are shown in Figure 1, Figure 2 and Figure 3, respectively.

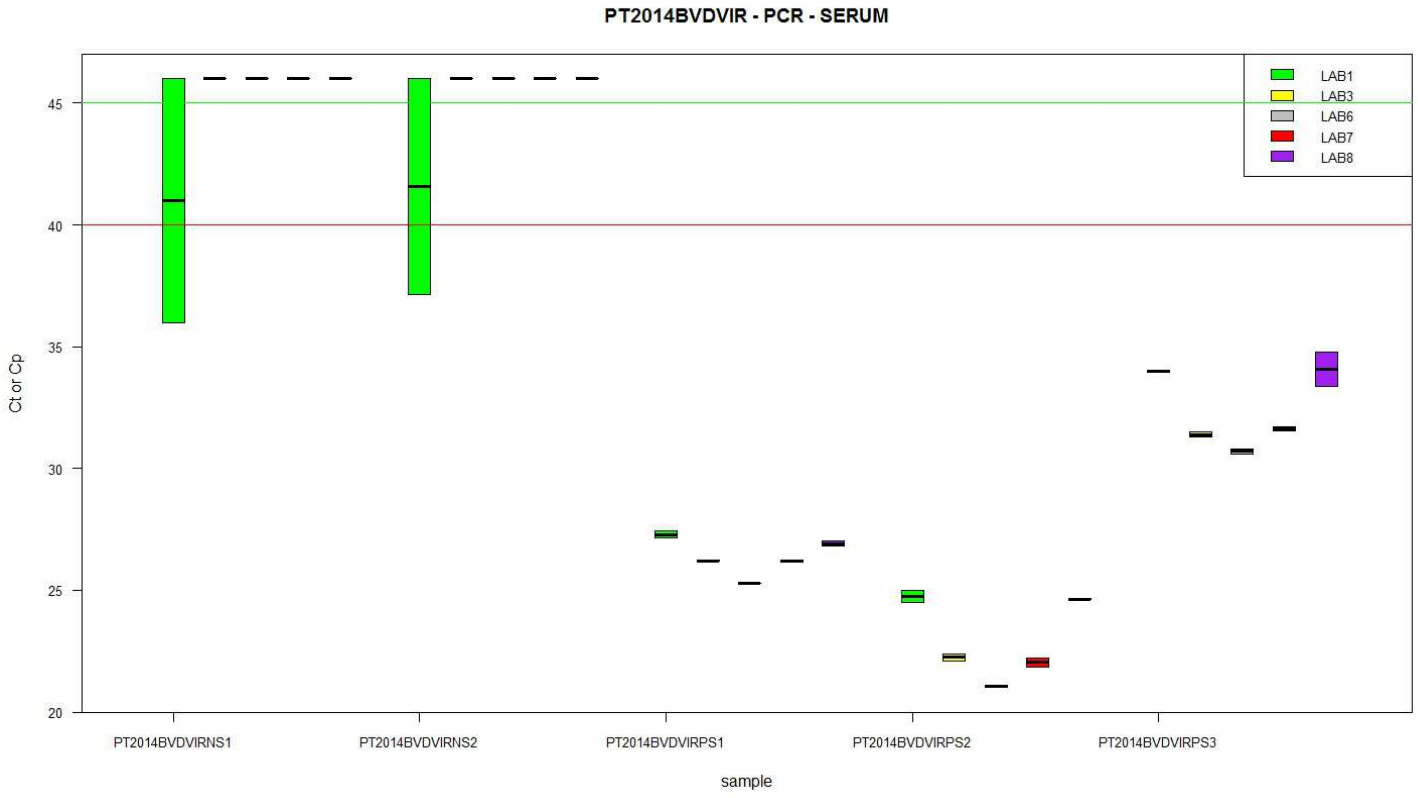

Figure 1. Box plots showing the Ct or Cp values per reference serum and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line). Cut-off values for the different RT-qPCR assays are shown in red (40) and green (45). A default Ct or Cp value of 46 was assigned to negative results, according to the corresponding RT-qPCR.

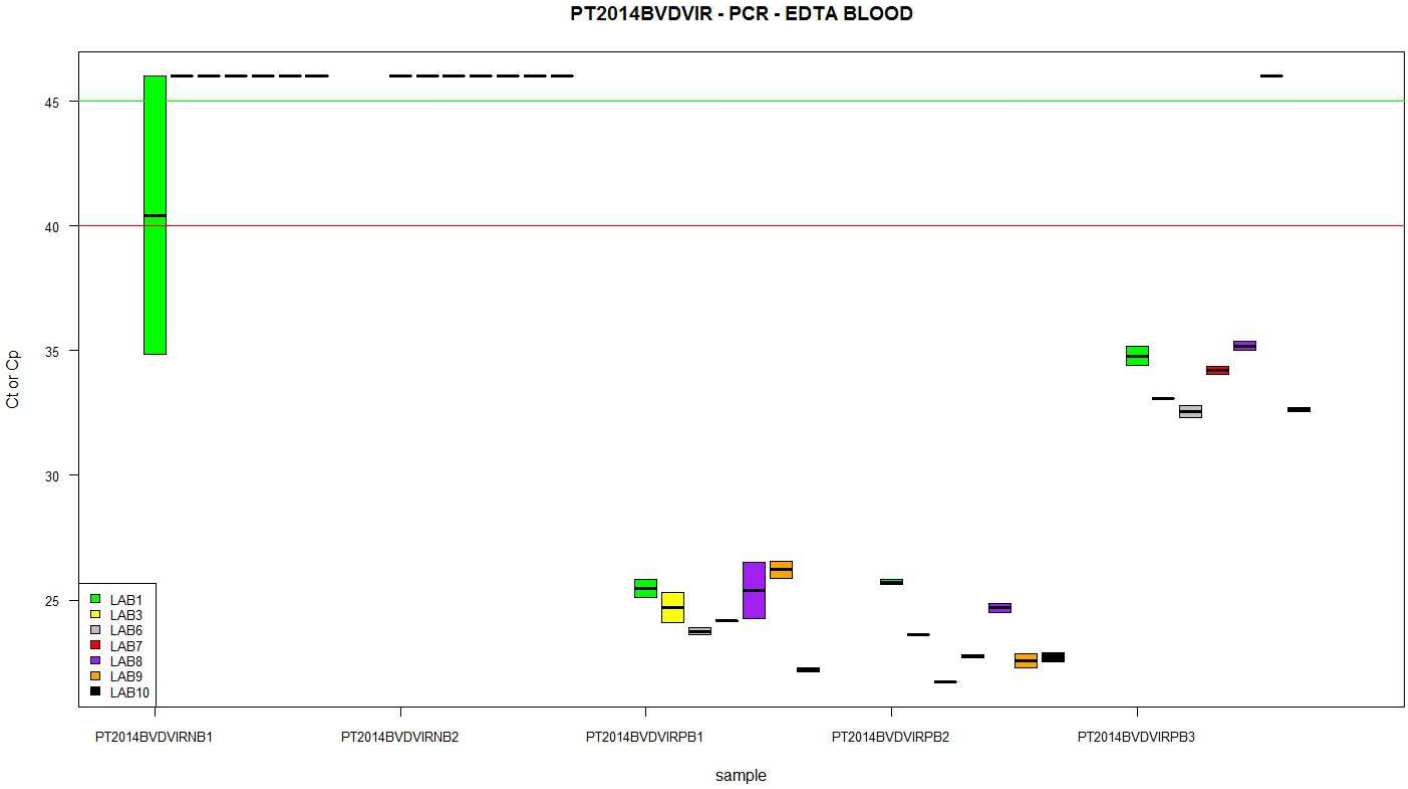

Figure 2. Box plots showing the Ct or Cp values per reference blood sample and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line). Cut-off values for the different RT-qPCR assays are shown in red (40) and green (45). A default Ct or Cp value of 46 was assigned to negative results, according to the corresponding RT-qPCR.

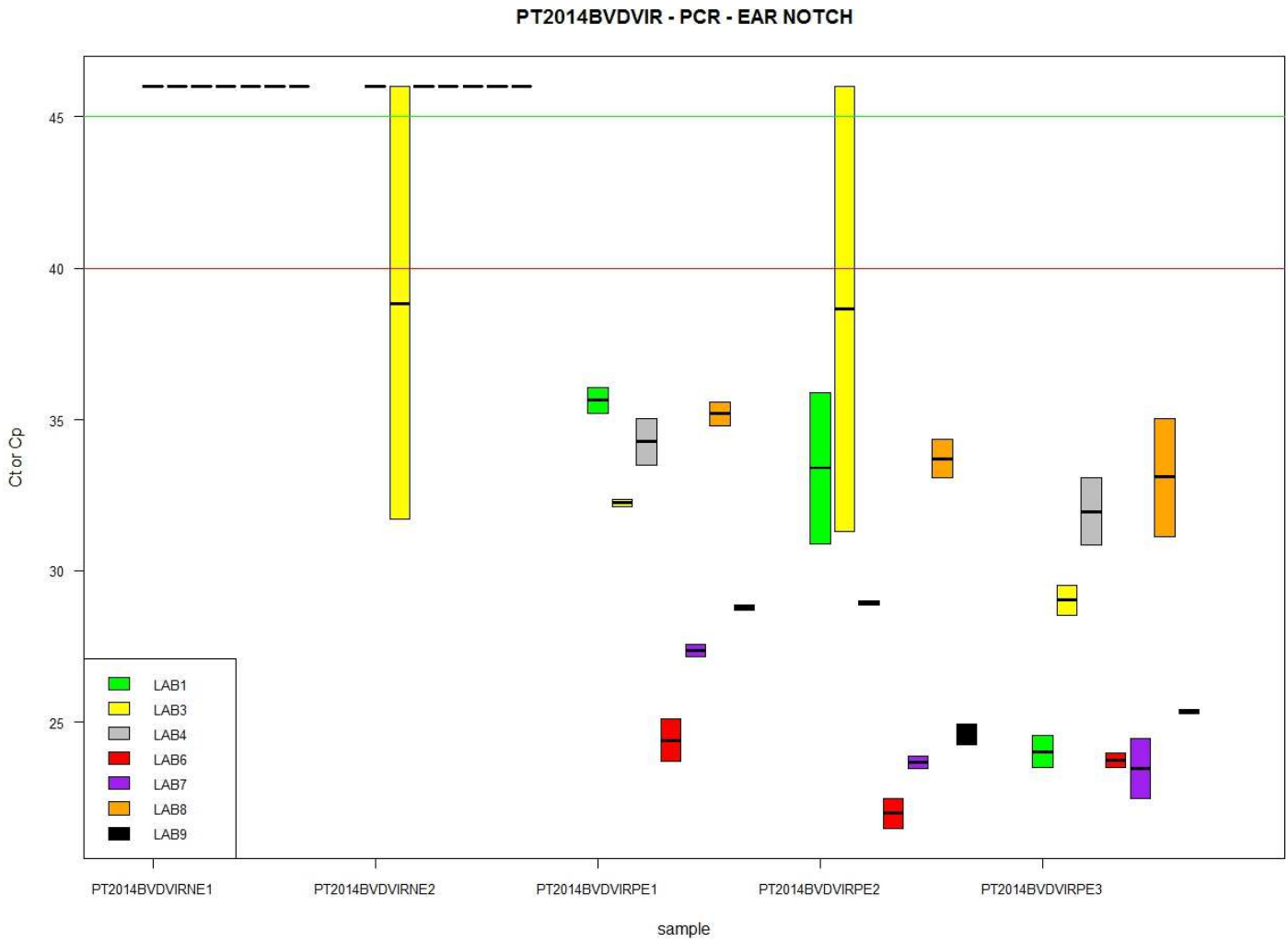

Figure 3. Box plots showing the Ct or Cp values per reference ear notch sample and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line). Cut-off values for the different RT-qPCR assays are shown in red (40) and green (45). A default Ct or Cp value of 46 was assigned to negative results, according to the corresponding RT-qPCR. For

For the **antigen ELISA** , **<u>serum, blood and ear notch reference samples</u>** box plots of the corrected/normalized OD values per reference sample and per participating laboratory were made using the statistical software R and are shown in Figure 4, Figure 5 and Figure 6, respectively.

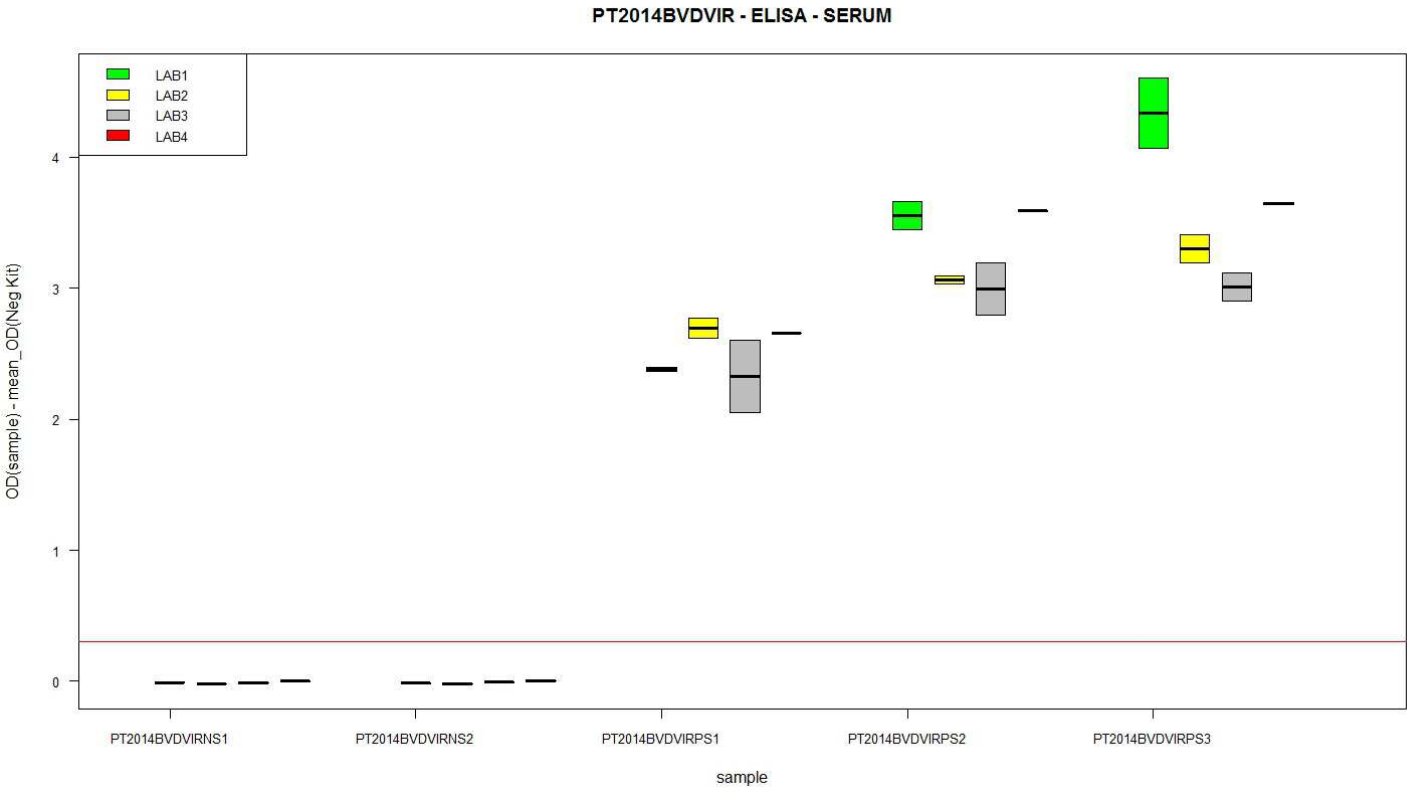

Figure 4. Box plots showing the normalized OD values per reference serum sample and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line).. The cut-off value of 0.3 is shown in red.

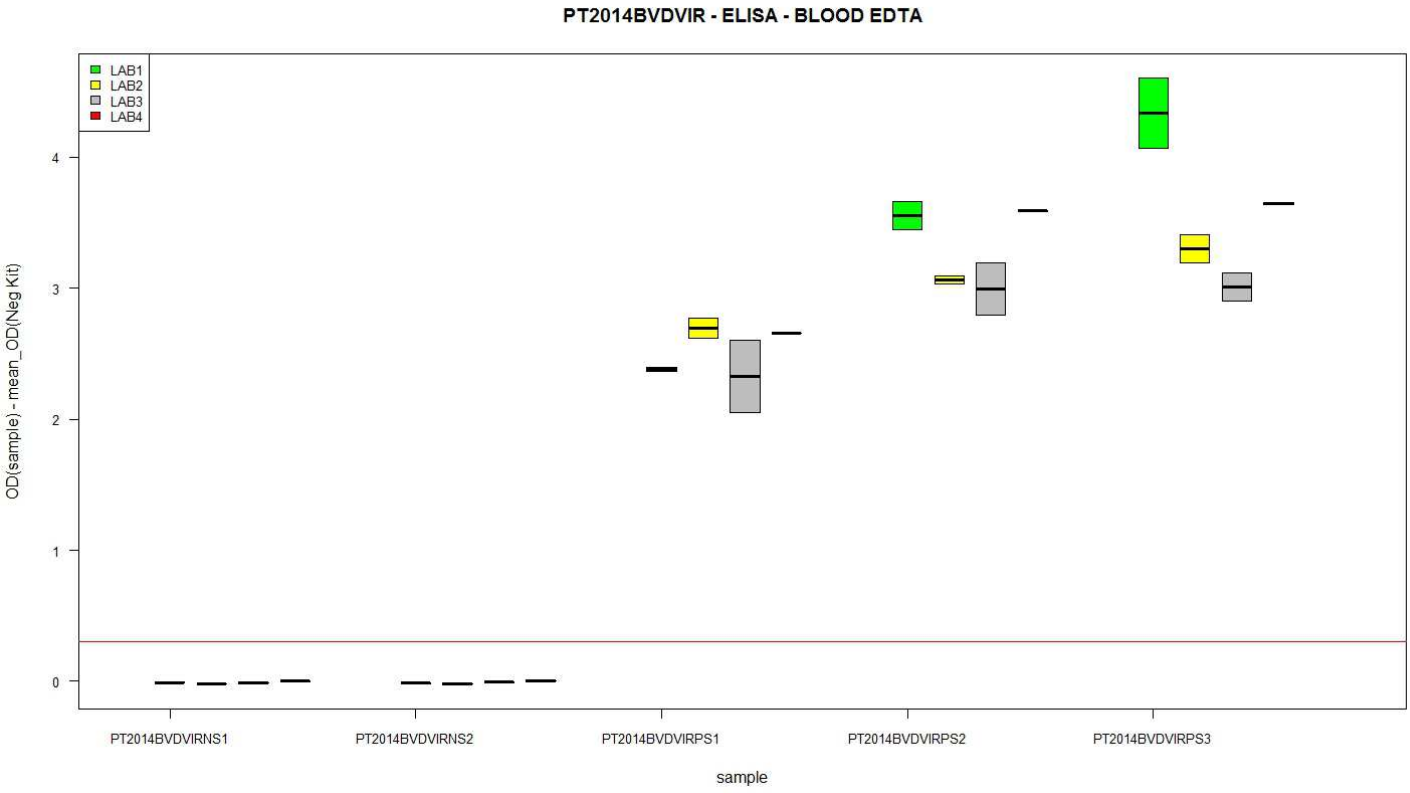

Figure 5. Box plots showing the normalized OD values per reference blood sample and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line).. The cut-off value of 0.3 is shown in red.

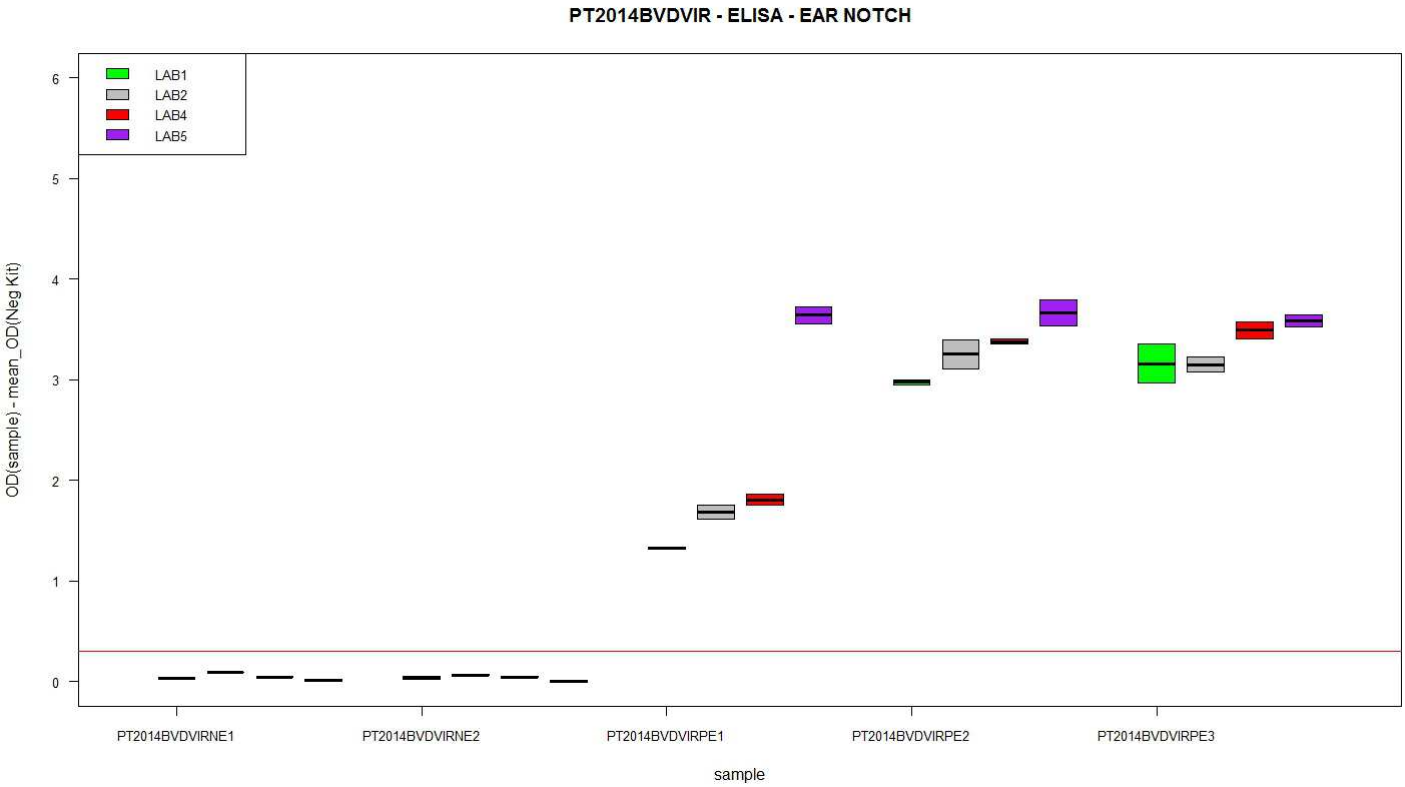

Figure 6. Box plots showing the normalized OD values per reference ear notch sample and per participating laboratory. Box plots represent the minimum value and the maximum value (only 2 alliquots per sample) and the median value (line). The cut-off value of 0.3 is shown in red.