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172-PT

PROFICIENCY TESTING 2015

BLUE TONGUE VIRUS (BTV)

Detection of BTV-specific antibodies in serum/plasma by Enzyme Linked Immunosorbent Assay (ELISA)

and/or

Detection of BTV RNA in blood by real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

CODA-CERVA-UCCLE

DATE BEGIN PT: 8 JUNE 2015

DATE REPORT: 10 AUGUST 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 BTV-specific antibodies in serum/plasma of bovidae origin by ELISA and/or BTV RNA in blood of bovidae origin by RT-qPCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum/plasma samples must be tested by means of a BTV antibody ELISA test and/or predefined reference blood samples must be tested by means of RT-qPCR. The procedures for the ELISA tests and the RT-qPCR assays must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

LAB1 and LAB2 received 40 aliquots, namely 20 aliquots of the matrix serum/plasma and 20 aliquots of the matrix blood. LAB3 and LAB4 received 20 aliquots of the matrix serum/plasma. LAB5 received 20 aliquots of the matrix blood. Each matrix was sent in a different block with reference samples (position 1-20).

III.2.1. Reference serum samples

Replicates of 6 reference serum/plasma samples of bovidae origin, either free from detectable BTV-specific antibodies (n=2; coded 'PT2015BLTSERNS1' and 'PT2015BLTSERNS2') or containing detectable BTV-specific antibodies (n=4; coded 'PT2015BLTSERPS1', 'PT2015BLTSERPS2', 'PT2015BLTSERPS3' and 'PT2015BLTSERPS4'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference samples PT2015BLTSERNS1, PT2015BLTSERNS2, PT2015BLTSERPS1 and PT2015BLTSERPS3, and 4 aliquots of the reference samples PT2015BLTSERPS2 and PT2015BLTSERPS4. The positions of the reference serum/plasma samples in the sent blocks were randomized for each participant (Table 4a and 4b).

For each reference serum/plasma sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum/plasma samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification, hereby using the Bluetongue Virus (BTV) VP7 Antibody Test Kit from IDEXX and both the ID Screen® Bluetongue Competition and the ID Screen® Bluetongue Early Detection One-Step antibody ELISA test kits from ID VET.

The reference samples PT2015BLTSERNS1, PT2015BLTSERNS2, PT2015BLTSERPS1 and PT2015BLTSERPS2 were serum samples, whereas the reference samples PT2015BLTSERPS3 and PT2015BLTSERPS4 were plasma samples.
The reference serum samples PT2015BLTSERNS1 and PT2015BLTSERNS2 were obtained from BTV uninfected and non-vaccinated cattle (sentinel animals). The reference serum samples PT2015BLTSERPS1 and PT2015BLTSERPS2 were a 1/16 and a 1/32 dilution, respectively, of 2 different sera derived from BTV infected cattle. The reference plasma samples PT2015BLTSERPS3 and PT2015BLTSERPS4 were a 1/16 and a 1/4 dilution of 2 different plasma derived from BTV vaccinated sheep. Taken together, the reference serum samples PT2015BLTSERNS1 and PT2015BLTSERNS2 were considered as negative samples, and the reference serum/plasma samples PT2015BLTSERPS1, PT2015BLTSERPS2, PT2015BLTSERPS3 and PT2015BLTSERPS4 as positive samples in BTV antibody ELISA.

After aliquoting the different reference serum/plasma samples, a homogeneity check was performed on 10 aliquots of each reference serum/plasma sample using the the Bluetongue Virus (BTV) VP7 Antibody Test Kit from IDEXX and the

ID Screen® Bluetongue Competition ELISA test from ID VET. For all reference serum/plasma samples, the same qualitative result was obtained for all 10 aliquots of the same reference serum/plasma sample for both kits. Consequently, all reference serum/plasma samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BTV-specific antibodies in serum/plasma. In addition, 3 aliquots of each serum/plasma sample were tested once after the PT in order to confirm their stability and status (post-verification) using the Bluetongue Virus (BTV) VP7 Antibody Test Kit from IDEXX and the ID Screen® Bluetongue Competition ELISA test from ID VET.

III.2.2. Reference blood samples

Replicates of 6 reference blood samples of bovidae origin, either free from detectable BTV RNA (n = 2; coded 'PT2015BLTVIRNB1' and 'PT2015BLTVIRNB2') or containing detectable BTV RNA (n = 4; coded 'PT2015BLTVIRPB1', 'PT2015BLTVIRPB3', 'PT2015BLTVIRPB4' and 'PT2015BLTVIRPB6') were used. In total, 60 aliquots were distributed to 3 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference blood samples PT2015BLTVIRNB1, PT2015BLTVIRNB2, PT2015BLTVIRPB1 and PT2015BLTVIRPB6, and 4 aliquots of the reference blood samples PT2015BLTVIRPB3 and PT2015BLTVIRPB4. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 5).

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 background of the samples and (ii) the results obtained during pre-verification, hereby using the LSI™ VetMAX™ BTV NS3 RT-qPCR kit from LSI (detecting all BTV serotypes) and 4 different in-house developed BTV RT-qPCR assays: one detecting all BTV serotypes, one detecting only BTV-1, one detecting only BTV-4 and one detecting only BTV-8.
The 2 reference blood samples free from detectable BTV RNA, namely PT2015BLTVIRNB1 and PT2015BLTVIRNB2, were derived from 2 different BTV negative animals, whereas the reference blood samples containing detectable BTV RNA were obtained by spiking blood derived from BTV negative animals with inactivated BTV-1 (PT2015BLTVIRPB1 and PT2015BLTVIRPB6) or BTV-4 (PT2015BLTVIRPB4) or BTV-8 (PT2015BLTVIRPB3). Hereby, the viral load of BTV-1 was 10 times higher in PT2015BLTVIRPB1 compared to PT2015BLTVIRPB6. For each reference blood sample, the same qualitative result was obtained with the commercial and the in-house BTV non serotype specific RT-qPCR assay. In addition, the reference blood samples PT2015BLTVIRPB1 and PT2015BLTVIRPB6 tested positive in the BTV-1 specific RT-qPCR but negative in BTV-4 and BTV-8 specific RT-qPCRs, reference blood sample PT2015BLTVIRPB4 is positive with the BTV-4 specific RT-qPCR but negative for BTV-1 and BTV-8 specific RT-qPCRs. PT2015BLTVIRPB3 tested positive with the BTV-8 specific RT-qPCR, but the sample was negative for the 2 other serotype specific RT-qPCRs. The reference blood samples PT2015BLTVIRNB1 and PT2015BLTVIRNB2 tested negative in the BTV-1, BTV-4 and BTV-8 specific RT-qPCR.
In conclusion, the reference blood samples PT20153BLTVIRNB1 and PT2015BLTVIRNB2 were considered as BTV negative samples, and the reference blood samples PT2015BLTVIRPB1, PT2015BLTVIRPB3, PT2015BLTVIRPB4 and PT2015BLTVIRPB6 as BTV positive samples in RT-qPCR.

After aliquoting the different reference blood samples, a homogeneity check was performed on 10 aliquots of each reference blood sample. The homogeneity check was performed using the in-house developed BTV non serotype specific RT-qPCR assay. For all reference blood samples, the same qualitative result was obtained for all 10 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 BTV RNA in blood of bovidae origin. In addition, 3 aliquots of each reference blood sample were tested after the PT using the in-house developed BTV non serotype specific RT-qPCR assay in order to confirm the stability and status of the reference blood samples (post-verification).

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* when the reported result does not match with the assigned status (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for each of the 20 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 20 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 CODA-CERVA-Uccle.

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

LAB1 and LAB2 participated in both the PT ELISA and the PT RT-qPCR and received 20 aliquots of reference serum/plasma samples and 20 aliquots of reference blood samples. LAB3 and LAB4 only participated in the PT ELISA and received 20 aliquots of reference serum/plasma samples. Lab 5 only participated in the PT RT-qPCR and received 20 aliquots of reference blood samples.
The reference serum/plasma and blood samples were sent frozen (dry ice) to the participating laboratories by national courier on 8th of June 2015 (140 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 9th and 15th of June 2015 (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 10th and 18th of June 2015 (Table 1). All participants hereby respected the deadline of 26th of June 2015 for submission of the results.

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

Participating laboratory	Reference samples received	Start of analysis ELISA	Start of analysis RT-qPCR	Submission of the results (Excel file)
LAB1	08/06/2015	09/06/2015	10/06/2015	16/06/2015
LAB2	08/06/2015	9-11/06/2015	11/06/2015	18/06/2015
LAB3	08/06/2015	10/06/2015	NA	10/06/2015
LAB4	08/06/2015	15/06/2015	NA	18/06/2015
LAB5	08/06/2015	NA	10/06/2015	18/06/2015

Legend: NA = not applicable;

IV.3. Compliance with the procedure

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

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that:

(i) For the detection of BTV-specific antibodies by **ELISA in serum/plasma** : all 4 participating laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum/plasma samples and hence reached 100% of agreement (Table 2a and Table 2b).

(ii) For the detection of BTV RNA by **RT-qPCR in blood** : 2 out of 3 participating laboratories (LAB2 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference blood samples (100% of agreement), whereas LAB1 misclassified 1 aliquots (95% of agreement) (Table 3).

A quantitative data analysis (box plots) is shown for educational purposes in Annex1.

Table 2a. ELISA (IDEXX) : Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum/plasma samples assigned by the BTV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum/plasma samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	2	**3**	**4**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)

Table 2b. ELISA (IDVET) : Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum/plasma samples assigned by the BTV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum/plasma 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	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. RT-qPCR : Agreement between results generated by the participating laboratories (LABNR) and the status of the reference blood samples assigned by the BTV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**5**
failure	1 (5.0)	0 (0.0)	0 (0.0)
success	19 (95.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

(i) For detection of BTV-specific antibodies by **ELISA in serum/plasma**, no variability between LAB1, LAB2, LAB3 and LAB4 could be observed since these participants correctly identified all reference serum samples.

(ii) For the detection of BTV RNA by **RT-qPCR in blood**, no variability between LAB2 and LAB5 could be observed since these laboratories correctly identified all reference samples. In contrast, LAB1 misclassified 1 out of 4 aliquots of the positive reference blood sample PT2015BLTVIRPB4 (1x NEG instead of POS).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum/plasma and blood samples are shown in Table 4a (ELISA IDEXX), Table 4b (ELISA IDVet) and 5 (RT-qPCR).

Table 4a. ELISA IDEXX: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum/plasma samples (SAMPLE), the positions of the reference serum/plasma samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2015BLTSERNS1	NEG	NEG	1
22	2	2	PT2015BLTSERPS1	POS	POS	1
23	2	3	PT2015BLTSERPS2	POS	POS	1
24	2	4	PT2015BLTSERPS4	POS	POS	1
25	2	5	PT2015BLTSERNS2	NEG	NEG	1
26	2	6	PT2015BLTSERPS2	POS	POS	1
27	2	7	PT2015BLTSERNS1	NEG	NEG	1
28	2	8	PT2015BLTSERPS4	POS	POS	1
29	2	9	PT2015BLTSERPS3	POS	POS	1
30	2	10	PT2015BLTSERNS2	NEG	NEG	1
31	2	11	PT2015BLTSERPS2	POS	POS	1
32	2	12	PT2015BLTSERPS1	POS	POS	1
33	2	13	PT2015BLTSERPS3	POS	POS	1
34	2	14	PT2015BLTSERNS2	NEG	NEG	1
35	2	15	PT2015BLTSERPS1	POS	POS	1
36	2	16	PT2015BLTSERPS4	POS	POS	1
37	2	17	PT2015BLTSERPS4	POS	POS	1
38	2	18	PT2015BLTSERNS1	NEG	NEG	1
39	2	19	PT2015BLTSERPS3	POS	POS	1
40	2	20	PT2015BLTSERPS2	POS	POS	1

Table 4a (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2015BLTSERPS1	POS	POS	1
42	3	2	PT2015BLTSERPS3	POS	POS	1
43	3	3	PT2015BLTSERNS2	NEG	NEG	1
44	3	4	PT2015BLTSERNS1	NEG	NEG	1
45	3	5	PT2015BLTSERPS2	POS	POS	1
46	3	6	PT2015BLTSERPS1	POS	POS	1
47	3	7	PT2015BLTSERNS2	NEG	NEG	1
48	3	8	PT2015BLTSERPS2	POS	POS	1
49	3	9	PT2015BLTSERPS4	POS	POS	1
50	3	10	PT2015BLTSERNS1	NEG	NEG	1
51	3	11	PT2015BLTSERPS3	POS	POS	1
52	3	12	PT2015BLTSERPS4	POS	POS	1
53	3	13	PT2015BLTSERPS4	POS	POS	1
54	3	14	PT2015BLTSERPS2	POS	POS	1
55	3	15	PT2015BLTSERPS4	POS	POS	1
56	3	16	PT2015BLTSERNS1	NEG	NEG	1
57	3	17	PT2015BLTSERPS2	POS	POS	1
58	3	18	PT2015BLTSERPS1	POS	POS	1
59	3	19	PT2015BLTSERPS3	POS	POS	1
60	3	20	PT2015BLTSERNS2	NEG	NEG	1

Table 4a (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2015BLTSERNS1	NEG	NEG	1
62	4	2	PT2015BLTSERPS1	POS	POS	1
63	4	3	PT2015BLTSERPS2	POS	POS	1
64	4	4	PT2015BLTSERPS4	POS	POS	1
65	4	5	PT2015BLTSERNS2	NEG	NEG	1
66	4	6	PT2015BLTSERPS2	POS	POS	1
67	4	7	PT2015BLTSERNS1	NEG	NEG	1
68	4	8	PT2015BLTSERPS4	POS	POS	1
69	4	9	PT2015BLTSERPS3	POS	POS	1
70	4	10	PT2015BLTSERNS2	NEG	NEG	1
71	4	11	PT2015BLTSERPS2	POS	POS	1
72	4	12	PT2015BLTSERPS1	POS	POS	1
73	4	13	PT2015BLTSERPS3	POS	POS	1
74	4	14	PT2015BLTSERNS2	NEG	NEG	1
75	4	15	PT2015BLTSERPS1	POS	POS	1
76	4	16	PT2015BLTSERPS4	POS	POS	1
77	4	17	PT2015BLTSERPS4	POS	POS	1
78	4	18	PT2015BLTSERNS1	NEG	NEG	1
79	4	19	PT2015BLTSERPS3	POS	POS	1
80	4	20	PT2015BLTSERPS2	POS	POS	1

Table 4b. ELISA IDVET: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum/plasma samples (SAMPLE), the positions of the reference serum/plasma samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BLTSERPS1	POS	POS	1
2	1	2	PT2015BLTSERPS3	POS	POS	1
3	1	3	PT2015BLTSERNS2	NEG	NEG	1
4	1	4	PT2015BLTSERNS1	NEG	NEG	1
5	1	5	PT2015BLTSERPS2	POS	POS	1
6	1	6	PT2015BLTSERPS1	POS	POS	1
7	1	7	PT2015BLTSERNS2	NEG	NEG	1
8	1	8	PT2015BLTSERPS2	POS	POS	1
9	1	9	PT2015BLTSERPS4	POS	POS	1
10	1	10	PT2015BLTSERNS1	NEG	NEG	1
11	1	11	PT2015BLTSERPS3	POS	POS	1
12	1	12	PT2015BLTSERPS4	POS	POS	1
13	1	13	PT2015BLTSERPS4	POS	POS	1
14	1	14	PT2015BLTSERPS2	POS	POS	1
15	1	15	PT2015BLTSERPS4	POS	POS	1
16	1	16	PT2015BLTSERNS1	NEG	NEG	1
17	1	17	PT2015BLTSERPS2	POS	POS	1
18	1	18	PT2015BLTSERPS1	POS	POS	1
19	1	19	PT2015BLTSERPS3	POS	POS	1
20	1	20	PT2015BLTSERNS2	NEG	NEG	1

Table 4b (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2015BLTSERNS1	NEG	NEG	1
22	2	2	PT2015BLTSERPS1	POS	POS	1
23	2	3	PT2015BLTSERPS2	POS	POS	1
24	2	4	PT2015BLTSERPS4	POS	POS	1
25	2	5	PT2015BLTSERNS2	NEG	NEG	1
26	2	6	PT2015BLTSERPS2	POS	POS	1
27	2	7	PT2015BLTSERNS1	NEG	NEG	1
28	2	8	PT2015BLTSERPS4	POS	POS	1
29	2	9	PT2015BLTSERPS3	POS	POS	1
30	2	10	PT2015BLTSERNS2	NEG	NEG	1
31	2	11	PT2015BLTSERPS2	POS	POS	1
32	2	12	PT2015BLTSERPS1	POS	POS	1
33	2	13	PT2015BLTSERPS3	POS	POS	1
34	2	14	PT2015BLTSERNS2	NEG	NEG	1
35	2	15	PT2015BLTSERPS1	POS	POS	1
36	2	16	PT2015BLTSERPS4	POS	POS	1
37	2	17	PT2015BLTSERPS4	POS	POS	1
38	2	18	PT2015BLTSERNS1	NEG	NEG	1
39	2	19	PT2015BLTSERPS3	POS	POS	1
40	2	20	PT2015BLTSERPS2	POS	POS	1

Table 4b (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2015BLTSERPS1	POS	POS	1
42	3	2	PT2015BLTSERPS3	POS	POS	1
43	3	3	PT2015BLTSERNS2	NEG	NEG	1
44	3	4	PT2015BLTSERNS1	NEG	NEG	1
45	3	5	PT2015BLTSERPS2	POS	POS	1
46	3	6	PT2015BLTSERPS1	POS	POS	1
47	3	7	PT2015BLTSERNS2	NEG	NEG	1
48	3	8	PT2015BLTSERPS2	POS	POS	1
49	3	9	PT2015BLTSERPS4	POS	POS	1
50	3	10	PT2015BLTSERNS1	NEG	NEG	1
51	3	11	PT2015BLTSERPS3	POS	POS	1
52	3	12	PT2015BLTSERPS4	POS	POS	1
53	3	13	PT2015BLTSERPS4	POS	POS	1
54	3	14	PT2015BLTSERPS2	POS	POS	1
55	3	15	PT2015BLTSERPS4	POS	POS	1
56	3	16	PT2015BLTSERNS1	NEG	NEG	1
57	3	17	PT2015BLTSERPS2	POS	POS	1
58	3	18	PT2015BLTSERPS1	POS	POS	1
59	3	19	PT2015BLTSERPS3	POS	POS	1
60	3	20	PT2015BLTSERNS2	NEG	NEG	1

Table 4b (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2015BLTSERNS1	NEG	NEG	1
62	4	2	PT2015BLTSERPS1	POS	POS	1
63	4	3	PT2015BLTSERPS2	POS	POS	1
64	4	4	PT2015BLTSERPS4	POS	POS	1
65	4	5	PT2015BLTSERNS2	NEG	NEG	1
66	4	6	PT2015BLTSERPS2	POS	POS	1
67	4	7	PT2015BLTSERNS1	NEG	NEG	1
68	4	8	PT2015BLTSERPS4	POS	POS	1
69	4	9	PT2015BLTSERPS3	POS	POS	1
70	4	10	PT2015BLTSERNS2	NEG	NEG	1
71	4	11	PT2015BLTSERPS2	POS	POS	1
72	4	12	PT2015BLTSERPS1	POS	POS	1
73	4	13	PT2015BLTSERPS3	POS	POS	1
74	4	14	PT2015BLTSERNS2	NEG	NEG	1
75	4	15	PT2015BLTSERPS1	POS	POS	1
76	4	16	PT2015BLTSERPS4	POS	POS	1
77	4	17	PT2015BLTSERPS4	POS	POS	1
78	4	18	PT2015BLTSERNS1	NEG	NEG	1
79	4	19	PT2015BLTSERPS3	POS	POS	1
80	4	20	PT2015BLTSERPS2	POS	POS	1

Table 5. RT-qPCR: 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 CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BLTVIRPB3	POS	POS	1
2	1	2	PT2015BLTVIRPB6	POS	POS	1
3	1	3	PT2015BLTVIRPB1	POS	POS	1
4	1	4	PT2015BLTVIRNB1	NEG	NEG	1
5	1	5	PT2015BLTVIRPB3	POS	POS	1
6	1	6	PT2015BLTVIRNB2	NEG	NEG	1
7	1	7	PT2015BLTVIRPB4	POS	POS	1
8	1	8	PT2015BLTVIRNB2	NEG	NEG	1
9	1	9	PT2015BLTVIRPB4	POS	POS	1
10	1	10	PT2015BLTVIRNB1	NEG	NEG	1
11	1	11	PT2015BLTVIRPB6	POS	POS	1
12	1	12	PT2015BLTVIRNB1	NEG	NEG	1
13	1	13	PT2015BLTVIRPB1	POS	POS	1
14	1	14	PT2015BLTVIRNB2	NEG	NEG	1
15	1	15	PT2015BLTVIRPB4	**POS**	**NEG**	**0**
16	1	16	PT2015BLTVIRPB3	POS	POS	1
17	1	17	PT2015BLTVIRPB6	POS	POS	1
18	1	18	PT2015BLTVIRPB4	POS	POS	1
19	1	19	PT2015BLTVIRPB3	POS	POS	1
20	1	20	PT2015BLTVIRPB1	POS	POS	1

Table 5 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2015BLTVIRPB4	POS	POS	1
22	2	2	PT2015BLTVIRPB1	POS	POS	1
23	2	3	PT2015BLTVIRPB6	POS	POS	1
24	2	4	PT2015BLTVIRPB3	POS	POS	1
25	2	5	PT2015BLTVIRNB1	NEG	NEG	1
26	2	6	PT2015BLTVIRPB4	POS	POS	1
27	2	7	PT2015BLTVIRNB2	NEG	NEG	1
28	2	8	PT2015BLTVIRPB3	POS	POS	1
29	2	9	PT2015BLTVIRNB2	NEG	NEG	1
30	2	10	PT2015BLTVIRPB1	POS	POS	1
31	2	11	PT2015BLTVIRNB1	NEG	NEG	1
32	2	12	PT2015BLTVIRPB3	POS	POS	1
33	2	13	PT2015BLTVIRNB2	NEG	NEG	1
34	2	14	PT2015BLTVIRNB1	NEG	NEG	1
35	2	15	PT2015BLTVIRPB4	POS	POS	1
36	2	16	PT2015BLTVIRPB6	POS	POS	1
37	2	17	PT2015BLTVIRPB3	POS	POS	1
38	2	18	PT2015BLTVIRPB1	POS	POS	1
39	2	19	PT2015BLTVIRPB6	POS	POS	1
40	2	20	PT2015BLTVIRPB4	POS	POS	1

Table 5 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2015BLTVIRPB3	POS	POS	1
42	5	2	PT2015BLTVIRPB6	POS	POS	1
43	5	3	PT2015BLTVIRPB1	POS	POS	1
44	5	4	PT2015BLTVIRNB1	NEG	NEG	1
45	5	5	PT2015BLTVIRPB3	POS	POS	1
46	5	6	PT2015BLTVIRNB2	NEG	NEG	1
47	5	7	PT2015BLTVIRPB4	POS	POS	1
48	5	8	PT2015BLTVIRNB2	NEG	NEG	1
49	5	9	PT2015BLTVIRPB4	POS	POS	1
50	5	10	PT2015BLTVIRNB1	NEG	NEG	1
51	5	11	PT2015BLTVIRPB6	POS	POS	1
52	5	12	PT2015BLTVIRNB1	NEG	NEG	1
53	5	13	PT2015BLTVIRPB1	POS	POS	1
54	5	14	PT2015BLTVIRNB2	NEG	NEG	1
55	5	15	PT2015BLTVIRPB4	POS	POS	1
56	5	16	PT2015BLTVIRPB3	POS	POS	1
57	5	17	PT2015BLTVIRPB6	POS	POS	1
58	5	18	PT2015BLTVIRPB4	POS	POS	1
59	5	19	PT2015BLTVIRPB3	POS	POS	1
60	5	20	PT2015BLTVIRPB1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum/plasma samples of bovidae origin for the detection of BTV-specific antibodies by ELISA and/or analyzing reference blood samples of bovidae origin for the detection of BTV RNA by RT-qPCR.

For the detection of BTV-specific antibodies in reference serum/plasma samples, all participating laboratories (LAB1, LAB2, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum/plasma samples (100% of agreement) (Table 2a, Table 2b, Table 4a and Table4b).

BTV antibody ELISA kits from 2 different producers as well as different batches from the same ELISA kit were used: IDEXX (2 batches: 4069 and 5046N) and ID VET (2 batches: 517 and 656). LAB2, LAB3 and LAB4 used BTV antibody ELISA kits from the 2 producers (same bactch for ID VET and different batches for IDEXX), LAB1 used only a BTV antibody ELISA kit from one producer (ID VET).

For the detection of BTV RNA reference blood samples, 2 out of 3 participating laboratories (LAB2 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference blood samples (100% of agreement). LAB1 misclassified 1 out of 4 aliqouts of the positive reference blood sample PT2015BLTVIRPB4 (95% of agreement) (Table 3 and Table 5).

Three different RNA extraction kits were used: the MagVet Universal Isolation Kit from Life Technologies by LAB1 (batch MV384-056), the Nucleospin RNA Virus from Macherey-Nagel by LAB2 (batch 1409/007) and the QIAamp Viral RNA mini kit from Qiagen by LAB5 (batch 148026860). Also different RT-qPCR assays were used by the participating laboratories: LAB2 used an in-house developed BTV non serotype specific BTV RT-qPCR, while LAB1 and LAB5 used the ADIAVET® BTV REALTIME kit from Adiagene (2 batches: 35K2TR193 and 35K2TR191). All used RT-qPCR assays were able to detect RNA from all BTV serotypes.

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 BTV reference laboratory of CODA-CERVA-Uccle (see III.3.3.). Consequently, all participating laboratories achieved a satisfactory performance for the detection of BTV-specific antibodies by ELISA and the detection of BTV RNA by RT-qPCR.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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)

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

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).

The quantitative data analysis in this PT was limited to perform boxplots.

All quantitative data analyses for ELISA were performed on the normalized data, namely the percentages negativity calculated according to the instructions for this PT: OD_{Sample} / mean $OD_{Negative\ Kit\ Controls}$ x 100.

For RT-qPCR, it should be noted that the Ct or Cp values were not normalized with the internal controls. In addition, modifiable factors such as extraction protocol, PCR machine and calculation of Ct or Cp values were also not taken into account.

In the framework of the PT, the virus was heat-inactivated in order to provide the participating laboratories with non-infectious material. Noteworthy, heat inactivation can result in damage of the genomic material of the virus. The degree of genomic damage can be influenced by a number of factors such as segment inherent sequence stability and stabilizing interactions (e.g. with proteins). Consequently, heat-induced genomic damage may differently influence RT-qPCR assays targeting different genomic segments.

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.

Box plots

For the ELISA box plots of the percentages negativity per reference serum/plasma sample and per participating laboratory were made using the statistical software R and are shown in Figure 1a for IDEXX and Figure 1b for IDVet.

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

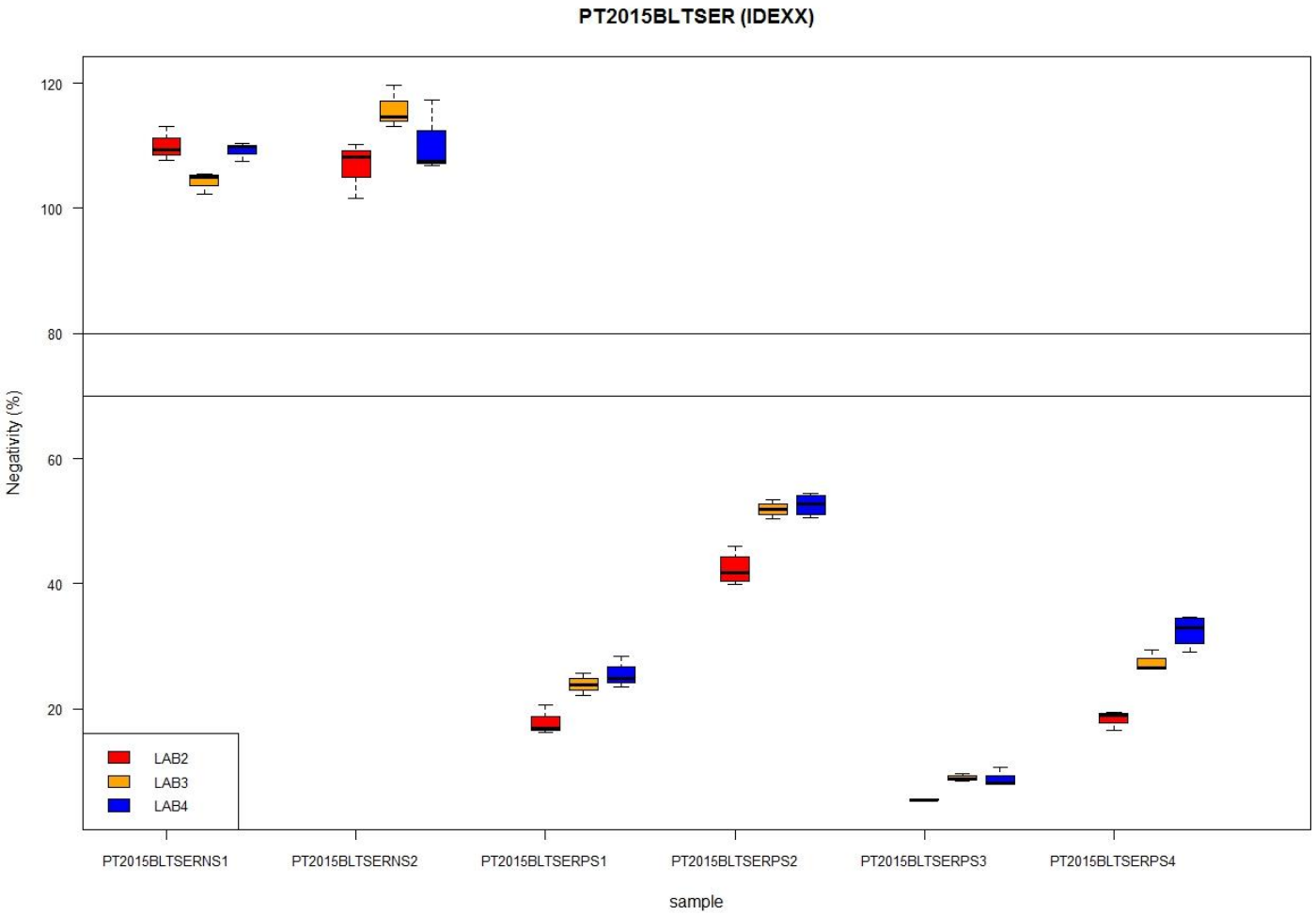

Figure 1a (ELISA IDEXX). Box plots showing the percentage negativity per reference serum/plasma sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in black (70-80%).

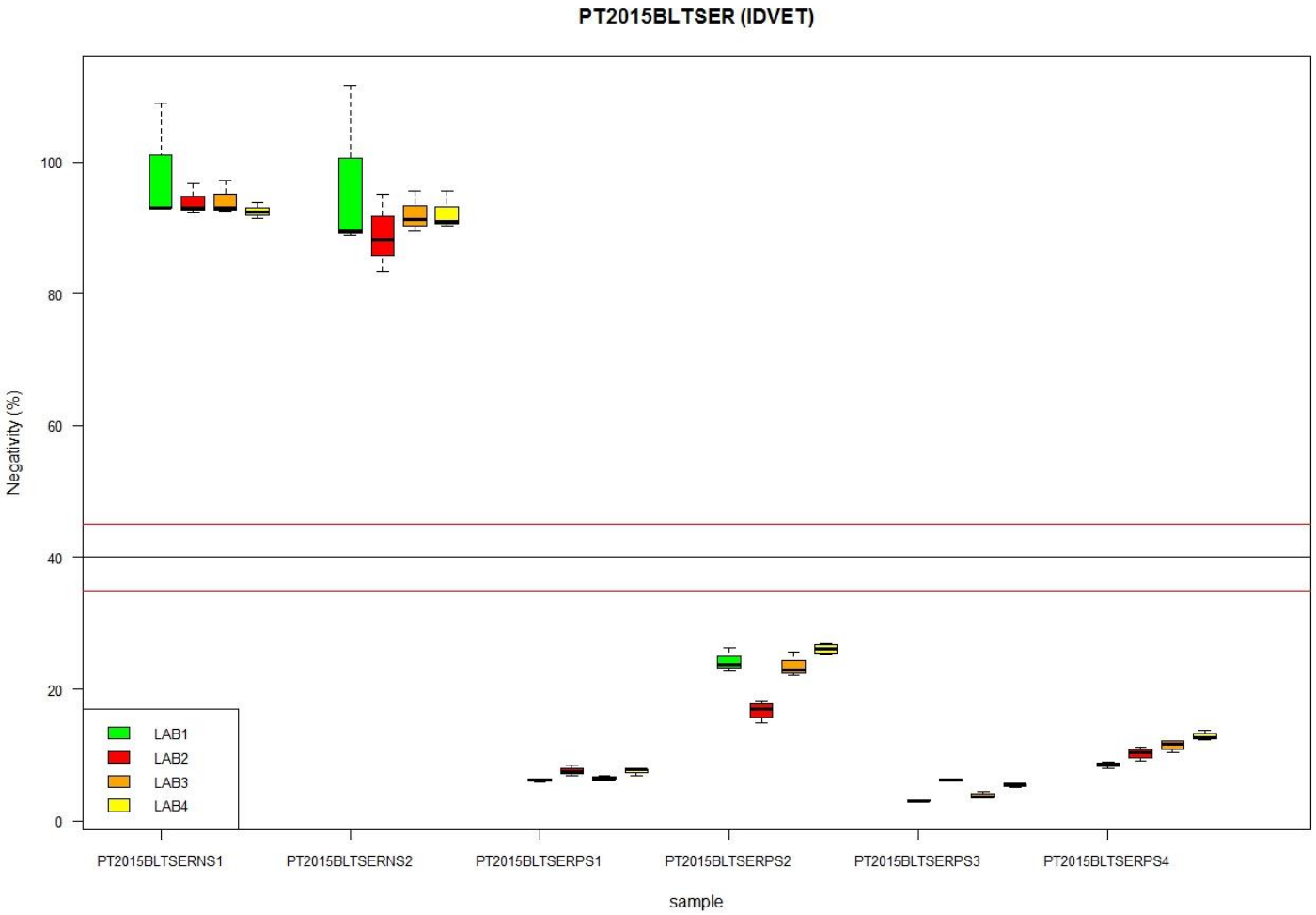

Figure 1b (ELISA IDVET). Box plots showing the percentage negativity per reference serum/plasma sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in black (40%; LAB2 and LAB4) and red (35%-45%; LAB1 and LAB3).

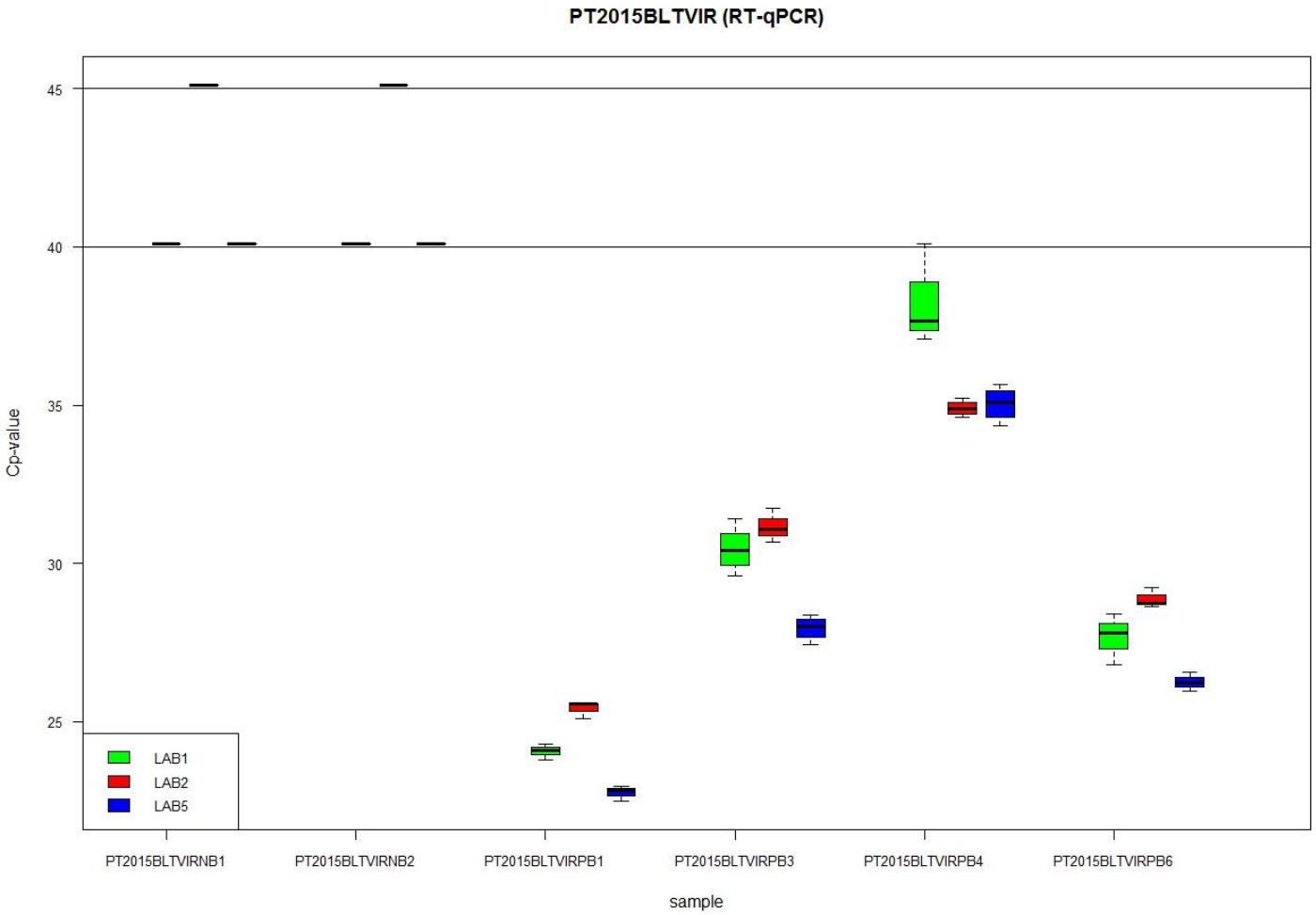

Figure 2. Box plots showing the Ct or Cp values per reference blood sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values for the different RT-qPCR methods are shown in black: 40 for LAB1, 40-45 for LAB2 and 45 for LAB5. A default Ct or Cp value of 40 or 45 was assigned to negative results, according to the corresponding RT-qPCR assay.