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081-PT

PROFICIENCY TESTING 2019

BLUE TONGUE VIRUS (BTV)

Detection of BTV-specific antibodies in serum by

Enzyme Linked Immunosorbent Assay (ELISA)

and/or

Detection of BTV RNA in blood by real-time

Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS

SCIENSANO

DATE START PT: 17 JUNE 2019

DATE REPORT: 27 SEPTEMBER 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 BTV-specific antibodies in serum 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 samples were tested by means of a BTV antibody ELISA test and/or predefined reference blood samples were 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

III.2.1. Reference serum samples

Replicates of 6 reference serum samples of bovidae origin, either free from detectable BTV-specific antibodies (n=2; coded 'PT2019BLTSERNS1' and 'PT2019BLTSERNS2') or containing detectable BTV-specific antibodies (n=4; coded 'PT2019BLTSERPS1', 'PT2019BLTSERPS2', 'PT2019BLTSERPS3' and 'PT2019BLTSERPS4'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference samples PT2019BLTSERNS1, PT2019BLTSERNS2, PT2019BLTSERPS1 and PT2019BLTSERPS3, and 4 aliquots of the reference samples PT2019BLTSERPS2 and PT2019BLTSERPS4. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 6).

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 during pre-verification, hereby using the ID Screen® Bluetongue Competition kit from ID.VET.

Table 1. Overview of the reference serum samples

Reference serum sample	origine	background	status
PT2019BLTSERNS1	bovine	Uninfected/unvaccinated	negative
PT2019BLTSERNS2	bovine	Uninfected/unvaccinated	negative
PT2019BLTSERPS1	ovine	Vaccinated	positive
PT2019BLTSERPS2	ovine	Vaccinated	positive
PT2019BLTSERPS3	bovine	Infected (dilution 1/150)	positive
PT2019BLTSERPS4	bovine	Infected (dilution 1/16)	positive

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the ID Screen® Bluetongue Competition ELISA test from ID.VET. For all reference serum samples, the same qualitative result was obtained for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BTV-specific antibodies in serum. In addition, 3 aliquots of each serum sample were tested once after the PT in order to confirm their stability and status (post-verification) using the ID Screen® Bluetongue Competition ELISA test from ID.VET.

III.2.2. Reference blood samples

Replicates of 7 reference blood samples of bovidae origin, either free from detectable BTV RNA (n = 2; coded 'PT2019BLTVIRNB1' and 'PT2019BLTVIRNB2') or containing detectable BTV RNA (n = 5; coded 'PT2019BLTVIRPB1', 'PT2019BLTVIRPB2', 'PT2019BLTVIRPB4', 'PT2019BLTVIRPB5' and 'PT2019BLTVIRPB7') were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference blood samples 'PT2019BLTVIRNB2', 'PT2019BLTVIRPB1', 'PT2019BLTVIRPB2', 'PT2019BLTVIRPB4', 'PT2019BLTVIRPB5' and 'PT2019BLTVIRPB7' and 2 aliquots of the reference blood sample 'PT2019BLTVIRNB1'. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 7).

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 5 different in-house developed BTV RT-qPCR assays: one detecting all BTV serotypes, one detecting only BTV-1, one detecting only BTV-3, one detecting only BTV-4 and one detecting only BTV-8.

Table 2. Overview of the reference blood samples

Reference sample	origine	background	strain(s)	status
PT2019BLTVIRNB1	bovine	Uninfected	NA	negative
PT2019BLTVIRNB2	bovine	Uninfected	NA	negative
PT2019BLTVIRPB1	bovine	Uninfected/blood spiked	BTV-1	positive
PT2019BLTVIRPB2	bovine	Uninfected/blood spiked	BTV-1	positive
PT2019BLTVIRPB4	bovine	Uninfected/blood spiked	BTV-4	positive
PT2019BLTVIRPB5	bovine	Uninfected/blood spiked	BTV-8	positive
PT2019BLTVIRPB7	bovine	Uninfected/blood spiked	BTV-3	positive

In conclusion, the reference blood samples PT2019BLTVIRNB1 and PT2019BLTVIRNB2 were considered as BTV negative samples, and the reference blood samples PT2019BLTVIRPB1, PT2019BLTVIRPB2, PT2019BLTVIRPB4, PT2019BLTVIRPB5 and PT2019BLTVIRPB7 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 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

All participants received 20 aliquots of reference serum samples and 20 aliquots of reference blood samples. The 40 reference samples were sent frozen (dry ice) to each of the 6 participating laboratories by national or international courier on 17th of June 2019. LAB2, LAB3, LAB4, LAB5 and LAB6 acknowledged receipt of the samples on the same day whereas LAB1 received the samples on 18th of June 2019. Analyses were performed between 18th and 28th of June 2019 (Table 3). LAB2 could not performe the RT-qPCR within the deadline of submitting the results, results from LAB2 were submitted on September 20th.

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 21st of June and 20th of September 2019 (Table 3).

Table 3. 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 ELISA	Start of analysis RT-qPCR	Submission of the results (Excel file)
LAB1	18/06/2019	28/06/2019	20/06/2019	RT-qPCR 25/06/2019 ELISA 03/07/2019
LAB2	17/06/2019	20/06/2019	**19/09/2019**	**RT-qPCR 20/09/2019** ELISA 04/07/2019
LAB3	17/06/2019	21/06/2019	18/06/2019	21/06/2019
LAB4	17/06/2019	21/06/2019	18/06/2019	02/07/2019
LAB5	17/06/2019	19/06/2019	18-19/06/2019	28/06/2019
LAB6	17/06/2019	24/06/2019 IDVET 26/06/2019 IDEXX	27/06/2019	RT-qPCR 04/07/2019 ELISA 05/07/2019

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** : 3 out of 6 participating laboratories (LAB1, LAB4 and LAB6) provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% of agreement whereas LAB2, LAB3 and LAB5 misclassified 3 aliquots (85% of agreement) (Table 4).

(ii) For the detection of BTV RNA by **RT-qPCR in blood** : 5 out of 6 participating laboratories (LAB1, LAB2, LAB3, LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference blood samples (100% of agreement), whereas LAB6 misclassified 1 aliquot (95% of agreement) (Table 5).

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

Table 4. ELISA: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the BTV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1	**2**	**3**	**4**	**5**	**6.1**	**6.2**
failure	0 (0)	**3 (15)**	**3 (15)**	0 (0)	**3 (15)**	0 (0)	0 (0)
success	20 (100)	**17 (85)**	**17 (85)**	20 (100)	**17 (85)**	20 (100)	20 (100)

Table 5. 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR					
	1	**2**	**3**	**4**	**5**	**6**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**1 (5)**
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	**19 (95)**

IV.4.2. Variability among participating laboratories

(i) For detection of BTV-specific antibodies by **ELISA in serum**, no variability between LAB1, LAB4, and LAB6 (LAB6.1 Idexx ELISA kit and 6.2 IDVet ELISA kit) could be observed since these participants correctly identified all reference serum samples. In contrast, LAB2, LAB3 and LAB5 misclassified the 3 aliquots of the positive reference serum sample PT2019BLTSERPS3 (NEG instead of POS).

(ii) For the detection of BTV RNA by **RT-qPCR in blood**, no variability between LAB1, LAB2, LAB3, LAB4 and LAB5 could be observed since these laboratories correctly identified all reference samples. In contrast, LAB6 misclassified 1 out of the 3 aliquots of the negative reference blood sample PT2019BLTVIRNB2 (NI instead of NEG).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum and blood samples are shown in Table 6 (ELISA) and Table 7 (RT-qPCR).

Table 6 ELISA: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the BTV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BLTSERPS1	POS	POS	1
2	1	2	PT2019BLTSERNS2	NEG	NEG	1
3	1	3	PT2019BLTSERPS2	POS	POS	1
4	1	4	PT2019BLTSERPS3	POS	POS	1
5	1	5	PT2019BLTSERPS1	POS	POS	1
6	1	6	PT2019BLTSERNS1	NEG	NEG	1
7	1	7	PT2019BLTSERPS4	POS	POS	1
8	1	8	PT2019BLTSERPS2	POS	POS	1
9	1	9	PT2019BLTSERPS4	POS	POS	1
10	1	10	PT2019BLTSERNS2	NEG	NEG	1
11	1	11	PT2019BLTSERNS1	NEG	NEG	1
12	1	12	PT2019BLTSERPS2	POS	POS	1
13	1	13	PT2019BLTSERPS3	POS	POS	1
14	1	14	PT2019BLTSERPS1	POS	POS	1
15	1	15	PT2019BLTSERPS2	POS	POS	1
16	1	16	PT2019BLTSERPS4	POS	POS	1
17	1	17	PT2019BLTSERNS1	NEG	NEG	1
18	1	18	PT2019BLTSERPS4	POS	POS	1
19	1	19	PT2019BLTSERNS2	NEG	NEG	1
20	1	20	PT2019BLTSERPS3	POS	POS	1
21	2	1	PT2019BLTSERNS2	NEG	NEG	1
22	2	2	PT2019BLTSERPS2	POS	POS	1
23	2	3	PT2019BLTSERNS1	NEG	NEG	1
24	2	4	PT2019BLTSERPS1	POS	POS	1
25	2	5	PT2019BLTSERPS3	**POS**	**NEG**	**0**
26	2	6	PT2019BLTSERPS1	POS	POS	1
27	2	7	PT2019BLTSERPS4	POS	POS	1
28	2	8	PT2019BLTSERNS1	NEG	NEG	1
29	2	9	PT2019BLTSERPS3	**POS**	**NEG**	**0**
30	2	10	PT2019BLTSERPS2	POS	POS	1
31	2	11	PT2019BLTSERNS2	NEG	NEG	1
32	2	12	PT2019BLTSERPS1	POS	POS	1
33	2	13	PT2019BLTSERPS3	**POS**	**NEG**	**0**
34	2	14	PT2019BLTSERPS4	POS	POS	1
35	2	15	PT2019BLTSERNS1	NEG	NEG	1
36	2	16	PT2019BLTSERPS2	POS	POS	1
37	2	17	PT2019BLTSERPS4	POS	POS	1
38	2	18	PT2019BLTSERNS2	NEG	NEG	1
39	2	19	PT2019BLTSERPS4	POS	POS	1
40	2	20	PT2019BLTSERPS2	POS	POS	1
41	3	1	PT2019BLTSERPS1	POS	POS	1
42	3	2	PT2019BLTSERNS2	NEG	NEG	1
43	3	3	PT2019BLTSERPS2	POS	POS	1
44	3	4	PT2019BLTSERPS3	**POS**	**NEG**	**0**
45	3	5	PT2019BLTSERPS1	POS	POS	1
46	3	6	PT2019BLTSERNS1	NEG	NEG	1
47	3	7	PT2019BLTSERPS4	POS	POS	1
48	3	8	PT2019BLTSERPS2	POS	POS	1
49	3	9	PT2019BLTSERPS4	POS	POS	1
50	3	10	PT2019BLTSERNS2	NEG	NEG	1
51	3	11	PT2019BLTSERNS1	NEG	NEG	1
52	3	12	PT2019BLTSERPS2	POS	POS	1
53	3	13	PT2019BLTSERPS3	**POS**	**NEG**	**0**
54	3	14	PT2019BLTSERPS1	POS	POS	1

55	3	15	PT2019BLTSERPS2	POS	POS	1
56	3	16	PT2019BLTSERPS4	POS	POS	1
57	3	17	PT2019BLTSERNS1	NEG	NEG	1
58	3	18	PT2019BLTSERPS4	POS	POS	1
59	3	19	PT2019BLTSERNS2	NEG	NEG	1
60	3	20	PT2019BLTSERPS3	**POS**	**NEG**	**0**
61	4	1	PT2019BLTSERNS2	NEG	NEG	1
62	4	2	PT2019BLTSERPS2	POS	POS	1
63	4	3	PT2019BLTSERNS1	NEG	NEG	1
64	4	4	PT2019BLTSERPS1	POS	POS	1
65	4	5	PT2019BLTSERPS3	POS	POS	1
66	4	6	PT2019BLTSERPS1	POS	POS	1
67	4	7	PT2019BLTSERPS4	POS	POS	1
68	4	8	PT2019BLTSERNS1	NEG	NEG	1
69	4	9	PT2019BLTSERPS3	POS	POS	1
70	4	10	PT2019BLTSERPS2	POS	POS	1
71	4	11	PT2019BLTSERNS2	NEG	NEG	1
72	4	12	PT2019BLTSERPS1	POS	POS	1
73	4	13	PT2019BLTSERPS3	POS	POS	1
74	4	14	PT2019BLTSERPS4	POS	POS	1
75	4	15	PT2019BLTSERNS1	NEG	NEG	1
76	4	16	PT2019BLTSERPS2	POS	POS	1
77	4	17	PT2019BLTSERPS4	POS	POS	1
78	4	18	PT2019BLTSERNS2	NEG	NEG	1
79	4	19	PT2019BLTSERPS4	POS	POS	1
80	4	20	PT2019BLTSERPS2	POS	POS	1
81	5	1	PT2019BLTSERPS1	POS	POS	1
82	5	2	PT2019BLTSERNS2	NEG	NEG	1
83	5	3	PT2019BLTSERPS2	POS	POS	1
84	5	4	PT2019BLTSERPS3	**POS**	**NEG**	**0**
85	5	5	PT2019BLTSERPS1	POS	POS	1
86	5	6	PT2019BLTSERNS1	NEG	NEG	1
87	5	7	PT2019BLTSERPS4	POS	POS	1
88	5	8	PT2019BLTSERPS2	POS	POS	1
89	5	9	PT2019BLTSERPS4	POS	POS	1
90	5	10	PT2019BLTSERNS2	NEG	NEG	1
91	5	11	PT2019BLTSERNS1	NEG	NEG	1
92	5	12	PT2019BLTSERPS2	POS	POS	1
93	5	13	PT2019BLTSERPS3	**POS**	**NEG**	**0**
94	5	14	PT2019BLTSERPS1	POS	POS	1
95	5	15	PT2019BLTSERPS2	POS	POS	1
96	5	16	PT2019BLTSERPS4	POS	POS	1
97	5	17	PT2019BLTSERNS1	NEG	NEG	1
98	5	18	PT2019BLTSERPS4	POS	POS	1
99	5	19	PT2019BLTSERNS2	NEG	NEG	1
100	5	20	PT2019BLTSERPS3	**POS**	**NEG**	**0**
101	6.1	1	PT2019BLTSERNS2	NEG	NEG	1
102	6.1	2	PT2019BLTSERPS2	POS	POS	1
103	6.1	3	PT2019BLTSERNS1	NEG	NEG	1
104	6.1	4	PT2019BLTSERPS1	POS	POS	1
105	6.1	5	PT2019BLTSERPS3	POS	POS	1
106	6.1	6	PT2019BLTSERPS1	POS	POS	1
107	6.1	7	PT2019BLTSERPS4	POS	POS	1
108	6.1	8	PT2019BLTSERNS1	NEG	NEG	1
109	6.1	9	PT2019BLTSERPS3	POS	POS	1
110	6.1	10	PT2019BLTSERPS2	POS	POS	1
111	6.1	11	PT2019BLTSERNS2	NEG	NEG	1
112	6.1	12	PT2019BLTSERPS1	POS	POS	1
113	6.1	13	PT2019BLTSERPS3	POS	POS	1

114	6.1	14	PT2019BLTSERPS4	POS	POS	1
115	6.1	15	PT2019BLTSERNS1	NEG	NEG	1
116	6.1	16	PT2019BLTSERPS2	POS	POS	1
117	6.1	17	PT2019BLTSERPS4	POS	POS	1
118	6.1	18	PT2019BLTSERNS2	NEG	NEG	1
119	6.1	19	PT2019BLTSERPS4	POS	POS	1
120	6.1	20	PT2019BLTSERPS2	POS	POS	1
121	6.2	1	PT2019BLTSERNS2	NEG	NEG	1
122	6.2	2	PT2019BLTSERPS2	POS	POS	1
123	6.2	3	PT2019BLTSERNS1	NEG	NEG	1
124	6.2	4	PT2019BLTSERPS1	POS	POS	1
125	6.2	5	PT2019BLTSERPS3	POS	POS	1
126	6.2	6	PT2019BLTSERPS1	POS	POS	1
127	6.2	7	PT2019BLTSERPS4	POS	POS	1
128	6.2	8	PT2019BLTSERNS1	NEG	NEG	1
129	6.2	9	PT2019BLTSERPS3	POS	POS	1
130	6.2	10	PT2019BLTSERPS2	POS	POS	1
131	6.2	11	PT2019BLTSERNS2	NEG	NEG	1
132	6.2	12	PT2019BLTSERPS1	POS	POS	1
133	6.2	13	PT2019BLTSERPS3	POS	POS	1
134	6.2	14	PT2019BLTSERPS4	POS	POS	1
135	6.2	15	PT2019BLTSERNS1	NEG	NEG	1
136	6.2	16	PT2019BLTSERPS2	POS	POS	1
137	6.2	17	PT2019BLTSERPS4	POS	POS	1
138	6.2	18	PT2019BLTSERNS2	NEG	NEG	1
139	6.2	19	PT2019BLTSERPS4	POS	POS	1
140	6.2	20	PT2019BLTSERPS2	POS	POS	1

Table 7 RT-qPCR: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the blood samples (SAMPLE), the external identification of the blood samples (LABPOSIT), and the status assigned by the BTV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BLTVIRNB2	NEG	NEG	1
2	1	2	PT2019BLTVIRPB1	POS	POS	1
3	1	3	PT2019BLTVIRPB4	POS	POS	1
4	1	4	PT2019BLTVIRPB1	POS	POS	1
5	1	5	PT2019BLTVIRPB5	POS	POS	1
6	1	6	PT2019BLTVIRNB1	NEG	NEG	1
7	1	7	PT2019BLTVIRPB4	POS	POS	1
8	1	8	PT2019BLTVIRPB2	POS	POS	1
9	1	9	PT2019BLTVIRPB5	POS	POS	1
10	1	10	PT2019BLTVIRPB7	POS	POS	1
11	1	11	PT2019BLTVIRNB1	NEG	NEG	1
12	1	12	PT2019BLTVIRPB7	POS	POS	1
13	1	13	PT2019BLTVIRPB4	POS	POS	1
14	1	14	PT2019BLTVIRNB2	NEG	NEG	1
15	1	15	PT2019BLTVIRPB2	POS	POS	1
16	1	16	PT2019BLTVIRPB5	POS	POS	1
17	1	17	PT2019BLTVIRPB1	POS	POS	1
18	1	18	PT2019BLTVIRPB7	POS	POS	1
19	1	19	PT2019BLTVIRNB2	NEG	NEG	1
20	1	20	PT2019BLTVIRPB2	POS	POS	1
21	2	1	PT2019BLTVIRPB2	POS	POS	1
22	2	2	PT2019BLTVIRNB2	NEG	NEG	1
23	2	3	PT2019BLTVIRPB4	POS	POS	1
24	2	4	PT2019BLTVIRNB1	NEG	NEG	1
25	2	5	PT2019BLTVIRPB7	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
26	2	6	PT2019BLTVIRPB7	POS	POS	1
27	2	7	PT2019BLTVIRPB1	POS	POS	1
28	2	8	PT2019BLTVIRPB5	POS	POS	1
29	2	9	PT2019BLTVIRNB2	NEG	NEG	1
30	2	10	PT2019BLTVIRPB1	POS	POS	1
31	2	11	PT2019BLTVIRNB1	NEG	NEG	1
32	2	12	PT2019BLTVIRPB4	POS	POS	1
33	2	13	PT2019BLTVIRNB2	NEG	NEG	1
34	2	14	PT2019BLTVIRPB5	POS	POS	1
35	2	15	PT2019BLTVIRPB7	POS	POS	1
36	2	16	PT2019BLTVIRPB2	POS	POS	1
37	2	17	PT2019BLTVIRPB5	POS	POS	1
38	2	18	PT2019BLTVIRPB1	POS	POS	1
39	2	19	PT2019BLTVIRPB2	POS	POS	1
40	2	20	PT2019BLTVIRPB4	POS	POS	1
41	3	1	PT2019BLTVIRNB2	NEG	NEG	1
42	3	2	PT2019BLTVIRPB1	POS	POS	1
43	3	3	PT2019BLTVIRPB4	POS	POS	1
44	3	4	PT2019BLTVIRPB1	POS	POS	1
45	3	5	PT2019BLTVIRPB5	POS	POS	1
46	3	6	PT2019BLTVIRNB1	NEG	NEG	1
47	3	7	PT2019BLTVIRPB4	POS	POS	1
48	3	8	PT2019BLTVIRPB2	POS	POS	1
49	3	9	PT2019BLTVIRPB5	POS	POS	1
50	3	10	PT2019BLTVIRPB7	POS	POS	1
51	3	11	PT2019BLTVIRNB1	NEG	NEG	1
52	3	12	PT2019BLTVIRPB7	POS	POS	1
53	3	13	PT2019BLTVIRPB4	POS	POS	1
54	3	14	PT2019BLTVIRNB2	NEG	NEG	1
55	3	15	PT2019BLTVIRPB2	POS	POS	1
56	3	16	PT2019BLTVIRPB5	POS	POS	1
57	3	17	PT2019BLTVIRPB1	POS	POS	1
58	3	18	PT2019BLTVIRPB7	POS	POS	1
59	3	19	PT2019BLTVIRNB2	NEG	NEG	1
60	3	20	PT2019BLTVIRPB2	POS	POS	1
61	4	1	PT2019BLTVIRPB2	POS	POS	1
62	4	2	PT2019BLTVIRNB2	NEG	NEG	1
63	4	3	PT2019BLTVIRPB4	POS	POS	1
64	4	4	PT2019BLTVIRNB1	NEG	NEG	1
65	4	5	PT2019BLTVIRPB7	POS	POS	1
66	4	6	PT2019BLTVIRPB7	POS	POS	1
67	4	7	PT2019BLTVIRPB1	POS	POS	1
68	4	8	PT2019BLTVIRPB5	POS	POS	1
69	4	9	PT2019BLTVIRNB2	NEG	NEG	1
70	4	10	PT2019BLTVIRPB1	POS	POS	1
71	4	11	PT2019BLTVIRNB1	NEG	NEG	1
72	4	12	PT2019BLTVIRPB4	POS	POS	1
73	4	13	PT2019BLTVIRNB2	NEG	NEG	1
74	4	14	PT2019BLTVIRPB5	POS	POS	1
75	4	15	PT2019BLTVIRPB7	POS	POS	1
76	4	16	PT2019BLTVIRPB2	POS	POS	1
77	4	17	PT2019BLTVIRPB5	POS	POS	1
78	4	18	PT2019BLTVIRPB1	POS	POS	1
79	4	19	PT2019BLTVIRPB2	POS	POS	1
80	4	20	PT2019BLTVIRPB4	POS	POS	1
81	5	1	PT2019BLTVIRNB2	NEG	NEG	1
82	5	2	PT2019BLTVIRPB1	POS	POS	1
83	5	3	PT2019BLTVIRPB4	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
84	5	4	PT2019BLTVIRPB1	POS	POS	1
85	5	5	PT2019BLTVIRPB5	POS	POS	1
86	5	6	PT2019BLTVIRNB1	NEG	NEG	1
87	5	7	PT2019BLTVIRPB4	POS	POS	1
88	5	8	PT2019BLTVIRPB2	POS	POS	1
89	5	9	PT2019BLTVIRPB5	POS	POS	1
90	5	10	PT2019BLTVIRPB7	POS	POS	1
91	5	11	PT2019BLTVIRNB1	NEG	NEG	1
92	5	12	PT2019BLTVIRPB7	POS	POS	1
93	5	13	PT2019BLTVIRPB4	POS	POS	1
94	5	14	PT2019BLTVIRNB2	NEG	NEG	1
95	5	15	PT2019BLTVIRPB2	POS	POS	1
96	5	16	PT2019BLTVIRPB5	POS	POS	1
97	5	17	PT2019BLTVIRPB1	POS	POS	1
98	5	18	PT2019BLTVIRPB7	POS	POS	1
99	5	19	PT2019BLTVIRNB2	NEG	NEG	1
100	5	20	PT2019BLTVIRPB2	POS	POS	1
101	6	1	PT2019BLTVIRPB2	POS	POS	1
102	6	2	PT2019BLTVIRNB2	**NEG**	**NI**	**0**
103	6	3	PT2019BLTVIRPB4	POS	POS	1
104	6	4	PT2019BLTVIRNB1	NEG	NEG	1
105	6	5	PT2019BLTVIRPB7	POS	POS	1
106	6	6	PT2019BLTVIRPB7	POS	POS	1
107	6	7	PT2019BLTVIRPB1	POS	POS	1
108	6	8	PT2019BLTVIRPB5	POS	POS	1
109	6	9	PT2019BLTVIRNB2	NEG	NEG	1
110	6	10	PT2019BLTVIRPB1	POS	POS	1
111	6	11	PT2019BLTVIRNB1	NEG	NEG	1
112	6	12	PT2019BLTVIRPB4	POS	POS	1
113	6	13	PT2019BLTVIRNB2	NEG	NEG	1
114	6	14	PT2019BLTVIRPB5	POS	POS	1
115	6	15	PT2019BLTVIRPB7	POS	POS	1
116	6	16	PT2019BLTVIRPB2	POS	POS	1
117	6	17	PT2019BLTVIRPB5	POS	POS	1
118	6	18	PT2019BLTVIRPB1	POS	POS	1
119	6	19	PT2019BLTVIRPB2	POS	POS	1
120	6	20	PT2019BLTVIRPB4	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum 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 samples, 3 out of 6 participating laboratories (LAB1, LAB4 and LAB6) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB2, LAB3 and LAB5 misclassified the 3 aliquots of the positive reference serum sample PT2019BLTSERPS3 (85% of agreement) (Table 4 and Table 6).

BTV antibody ELISA kits from 2 different producers as well as different batches from the same ELISA kit were used: IDEXX (1 batch: 8031) and ID.VET (2 batches: C42 and E17). LAB6 used BTV antibody ELISA kits from the 2 producers and the other participating laboratories used the BTV antibody ELISA kit from ID.VET (same batch).

For the detection of BTV RNA in reference blood samples, 5 out of 6 participating laboratories (LAB1, LAB2, LAB3, LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference blood samples

(100% of agreement). LAB6 misclassified 1 out of the 3 aliquots of the negative reference blood sample PT2019BLTVIRNB2 (95% of agreement) (Table 5 and Table 7).

Five different RNA extraction kits were used: the Magvet Universal Isolation kit from Life Technologies by LAB1, the MagAttract Cador pathogen Kit from QIAGEN by LAB3, the Nucleospin RNA Virus kit from Machery-Nagel by LAB4, , the MagVet Universal Isolation kit from Thermofisher Scientific by LAB5 and the Magattract 96 cador pathogen kit from Indical Bioscience by LAB2 and LAB6.

Also different RT-qPCR assays were used by the participating laboratories: LAB1 used the LSI Vetmax Bluetongue Virus NS3-All genotype from Life Technologies, LAB2, LAB3, LAB5 and LAB6 used the ADIAVET BTV real time from ADIAGENE and LAB4 used an in-house developed BTV non serotype specific BTV 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 BTV reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of BTV RNA in reference blood samples and all participants except LAB2, LAB3 and LAB5 achieved a satisfactory performance for the detection of BTV-specific antibodies in reference serum samples.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

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

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)

Sciensano (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 R (box plots).

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

For the antibody ELISA box plots of the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory are shown in Figure 1

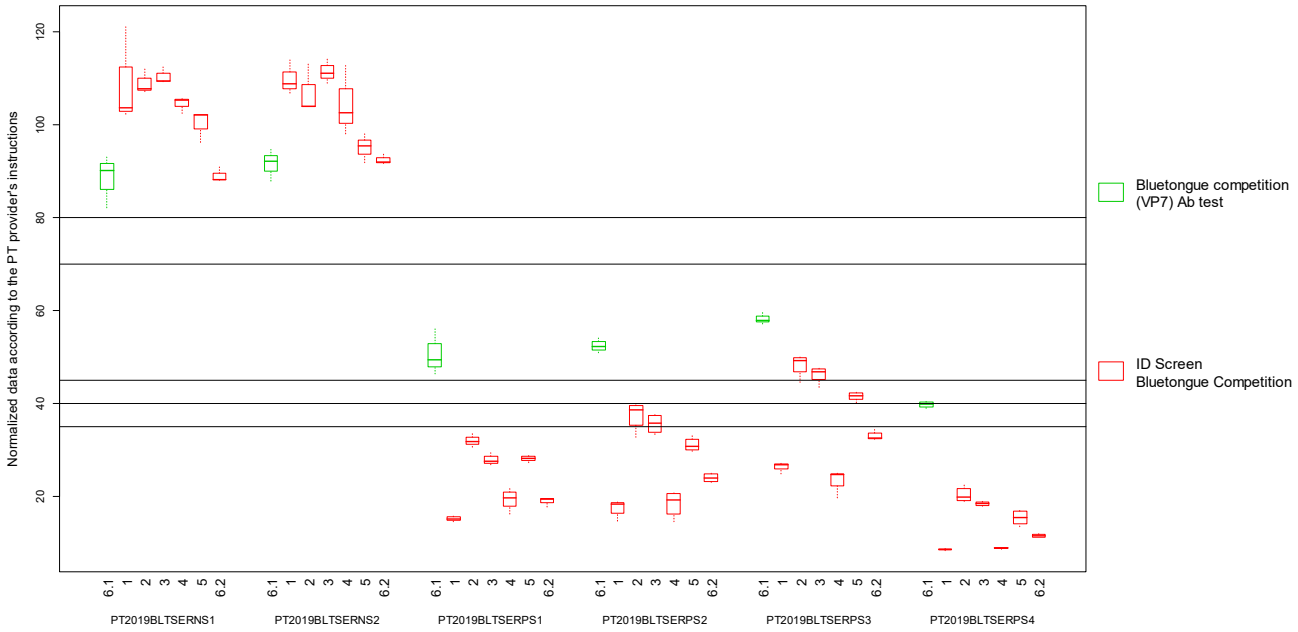

Figure 1. Box plots showing the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory.
LAB6 used BTV antibody ELISA kits from 2 producers [6.1 IDEXX (green plots) and 6.2 IDVet].
Cut-off values applied by the participating laboratories are shown by horizontal lines (IDVet 40% for LAB2, LAB3, LAB4 and LAB5 and 35%-45% for LAB1 and IDEXX 70-80%).

For RT-qPCR box plots of the Ct or Cp values per positive reference sample and per participating laboratory are shown in Figure 2. 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.

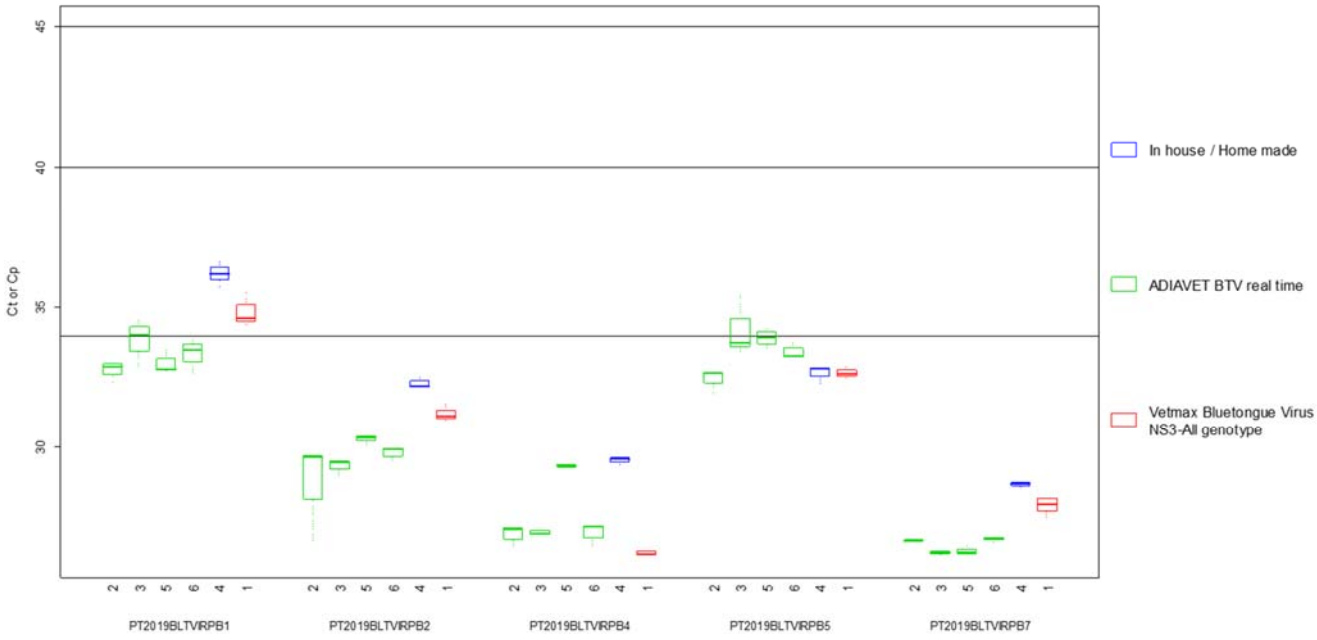

Figure 2. Box plots showing the Ct or Cp values per positive reference blood sample and per participating laboratory. Cut-off values applied by the participating laboratories are shown by horizontal lines (34-40 for LAB6, 40 for LAB1, LAB2 and LAB5, 40-45 for LAB4 and 45 for LAB3).