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172-PT

PROFICIENCY TESTING 2012

BLUE TONGUE (BT)

Detection of BT virus (BTV) RNA in blood by

Real-time Reverse Transcriptase Polymerase Chain Reaction

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 26 MARCH 2012

DATE REPORT: 1 JUNE 2012

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I. Introduction

Details relevant to the proficiency test 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'.

II. Aim

The aim of this proficiency test was to evaluate the ability of the participating laboratories to identify the absence or presence of BT virus (BTV) RNA in blood of bovine origin by real-time reverse transcriptase Polymerase Chain Reaction (Rt RT-PCR).

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this proficiency test, predefined reference blood samples must be tested by means of Rt RT-PCR. The procedures for the Rt RT-PCR tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 5 reference blood samples of bovine origin, either free from detectable BTV RNA (n = 2; coded 'PT2012BLTVIRNB1' and 'PT2012BLTVIRNB2') or containing detectable BTV RNA (n = 3; coded 'PT2012BLTVIRPB1', 'PT2012BLTVIRPB2' and, 'PT2012BLTVIRPB3') were used. The 3 reference blood samples with detectable BTV RNA were obtained from 3 different animals 98 days post experimental infections with BTV serotype 8. The 2 reference blood samples free from detectable BTV RNA were obtained from 2 different negative animals within the same experiment.
In total, 100 aliquots, prepared by the reference laboratory for BTV of the Veterinary and Agrochemical Research Center (CODA-CERVA), were distributed to the participating laboratories. All five participants were given 4 aliquots of the five reference blood samples (i.e., 20 aliquots per participant). The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 2).

For each reference blood sample, a certificate containing the assigned value was made by the reference laboratory for BTV of CODA-CERVA (status of the sample = 'golden standard'). The assigned value of each reference blood sample was obtained by testing each sample 10 times before the proficiency test (testing the homogeneity of the samples), hereby obtaining each time the same qualitative result. Consequently, these 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 bovine blood. In addition, all reference blood samples were also tested once after the proficiency test in order to confirm their stability and status (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* (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive).

III.3.2. Level of agreement

The level of agreement achieved by a participating laboratory is expressed as the percentage *success* for all 20 reference blood samples (aliquots).

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III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if at least 90% of the reference blood samples (aliquots) are analysed correctly, i.e., when the reported result corresponds with the assigned value.

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 the CODA-CERVA.

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

The 20 aliquots of reference blood samples were sent to each of the 5 participating laboratories on 26th of March 2012 (100 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 26th of March (LAB 3), 27th of March (LAB 1 and LAB 4), 28th of March (LAB 2) and the 4th of April (LAB 5) 2012.

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

Results from the participating laboratories have been received on 28th (LAB 4), 29th of March 2012 (LAB 1) and 6th of April (LAB 2, 3 and 5) 2012.

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 all participating laboratories reached 100% of agreement for the detection of BTV RNA in reference blood samples (Table 1).

A quantitative data analysis by boxplots is shown for educational purposes in Annex 1.

Table 1. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference blood samples assigned by the reference laboratory for BTV of CODA-CERVA. All participating laboratories received 20 reference blood samples (aliquots). Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**2**	**3**	**4**	**5**
succes	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

IV.4.2. Variability among participating laboratories

Since all participants reached 100% of agreement for the detection of BTV RNA in reference blood samples, no variability between laboratories could be observed at the qualitative data level. For each participating laboratory, the obtained qualitative responses and the assigned status for the reference blood samples are shown in Table 2.

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Table 2. 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 reference laboratory for BTV of the CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012BLTVIRPB1	POS	POS	1
2	1	2	PT2012BLTVIRPB2	POS	POS	1
3	1	3	PT2012BLTVIRNB1	NEG	NEG	1
4	1	4	PT2012BLTVIRPB3	POS	POS	1
5	1	5	PT2012BLTVIRPB2	POS	POS	1
6	1	6	PT2012BLTVIRNB2	NEG	NEG	1
7	1	7	PT2012BLTVIRPB2	POS	POS	1
8	1	8	PT2012BLTVIRNB2	NEG	NEG	1
9	1	9	PT2012BLTVIRPB1	POS	POS	1
10	1	10	PT2012BLTVIRNB2	NEG	NEG	1
11	1	11	PT2012BLTVIRPB3	POS	POS	1
12	1	12	PT2012BLTVIRNB2	NEG	NEG	1
13	1	13	PT2012BLTVIRNB1	NEG	NEG	1
14	1	14	PT2012BLTVIRPB1	POS	POS	1
15	1	15	PT2012BLTVIRPB2	POS	POS	1
16	1	16	PT2012BLTVIRPB3	POS	POS	1
17	1	17	PT2012BLTVIRPB3	POS	POS	1
18	1	18	PT2012BLTVIRPB1	POS	POS	1
19	1	19	PT2012BLTVIRNB1	NEG	NEG	1
20	1	20	PT2012BLTVIRNB1	NEG	NEG	1

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(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2012BLTVIRPB3	POS	POS	1
22	2	2	PT2012BLTVIRPB3	POS	POS	1
23	2	3	PT2012BLTVIRPB1	POS	POS	1
24	2	4	PT2012BLTVIRNB1	NEG	NEG	1
25	2	5	PT2012BLTVIRNB1	NEG	NEG	1
26	2	6	PT2012BLTVIRPB1	POS	POS	1
27	2	7	PT2012BLTVIRPB2	POS	POS	1
28	2	8	PT2012BLTVIRNB1	NEG	NEG	1
29	2	9	PT2012BLTVIRPB3	POS	POS	1
30	2	10	PT2012BLTVIRPB2	POS	POS	1
31	2	11	PT2012BLTVIRNB2	NEG	NEG	1
32	2	12	PT2012BLTVIRPB2	POS	POS	1
33	2	13	PT2012BLTVIRNB2	NEG	NEG	1
34	2	14	PT2012BLTVIRPB1	POS	POS	1
35	2	15	PT2012BLTVIRNB2	NEG	NEG	1
36	2	16	PT2012BLTVIRPB3	POS	POS	1
37	2	17	PT2012BLTVIRNB2	NEG	NEG	1
38	2	18	PT2012BLTVIRNB1	NEG	NEG	1
39	2	19	PT2012BLTVIRPB1	POS	POS	1
40	2	20	PT2012BLTVIRPB2	POS	POS	1

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(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012BLTVIRPB3	POS	POS	1
42	3	2	PT2012BLTVIRNB2	NEG	NEG	1
43	3	3	PT2012BLTVIRNB1	NEG	NEG	1
44	3	4	PT2012BLTVIRPB1	POS	POS	1
45	3	5	PT2012BLTVIRPB2	POS	POS	1
46	3	6	PT2012BLTVIRPB3	POS	POS	1
47	3	7	PT2012BLTVIRPB3	POS	POS	1
48	3	8	PT2012BLTVIRPB1	POS	POS	1
49	3	9	PT2012BLTVIRNB1	NEG	NEG	1
50	3	10	PT2012BLTVIRNB1	NEG	NEG	1
51	3	11	PT2012BLTVIRPB1	POS	POS	1
52	3	12	PT2012BLTVIRPB2	POS	POS	1
53	3	13	PT2012BLTVIRNB1	NEG	NEG	1
54	3	14	PT2012BLTVIRPB3	POS	POS	1
55	3	15	PT2012BLTVIRPB2	POS	POS	1
56	3	16	PT2012BLTVIRNB2	NEG	NEG	1
57	3	17	PT2012BLTVIRPB2	POS	POS	1
58	3	18	PT2012BLTVIRNB2	NEG	NEG	1
59	3	19	PT2012BLTVIRPB1	POS	POS	1
60	3	20	PT2012BLTVIRNB2	NEG	NEG	1

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(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2012BLTVIRNB2	NEG	NEG	1
62	4	2	PT2012BLTVIRPB2	POS	POS	1
63	4	3	PT2012BLTVIRNB2	NEG	NEG	1
64	4	4	PT2012BLTVIRPB1	POS	POS	1
65	4	5	PT2012BLTVIRNB2	NEG	NEG	1
66	4	6	PT2012BLTVIRPB3	POS	POS	1
67	4	7	PT2012BLTVIRNB2	NEG	NEG	1
68	4	8	PT2012BLTVIRNB1	NEG	NEG	1
69	4	9	PT2012BLTVIRPB1	POS	POS	1
70	4	10	PT2012BLTVIRPB2	POS	POS	1
71	4	11	PT2012BLTVIRPB3	POS	POS	1
72	4	12	PT2012BLTVIRPB3	POS	POS	1
73	4	13	PT2012BLTVIRPB1	POS	POS	1
74	4	14	PT2012BLTVIRNB1	NEG	NEG	1
75	4	15	PT2012BLTVIRNB1	NEG	NEG	1
76	4	16	PT2012BLTVIRPB1	POS	POS	1
77	4	17	PT2012BLTVIRPB2	POS	POS	1
78	4	18	PT2012BLTVIRNB1	NEG	NEG	1
79	4	19	PT2012BLTVIRPB3	POS	POS	1
80	4	20	PT2012BLTVIRPB2	POS	POS	1

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(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2012BLTVIRPB1	POS	POS	1
82	5	2	PT2012BLTVIRPB2	POS	POS	1
83	5	3	PT2012BLTVIRNB1	NEG	NEG	1
84	5	4	PT2012BLTVIRPB3	POS	POS	1
85	5	5	PT2012BLTVIRPB2	POS	POS	1
86	5	6	PT2012BLTVIRNB2	NEG	NEG	1
87	5	7	PT2012BLTVIRPB2	POS	POS	1
88	5	8	PT2012BLTVIRNB2	NEG	NEG	1
89	5	9	PT2012BLTVIRPB1	POS	POS	1
90	5	10	PT2012BLTVIRNB2	NEG	NEG	1
91	5	11	PT2012BLTVIRPB3	POS	POS	1
92	5	12	PT2012BLTVIRNB2	NEG	NEG	1
93	5	13	PT2012BLTVIRNB1	NEG	NEG	1
94	5	14	PT2012BLTVIRPB1	POS	POS	1
95	5	15	PT2012BLTVIRPB2	POS	POS	1
96	5	16	PT2012BLTVIRPB3	POS	POS	1
97	5	17	PT2012BLTVIRPB3	POS	POS	1
98	5	18	PT2012BLTVIRPB1	POS	POS	1
99	5	19	PT2012BLTVIRNB1	NEG	NEG	1
100	5	20	PT2012BLTVIRNB1	NEG	NEG	1

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V. Discussion

The purpose of this proficiency test was to assess the performances of the participating laboratories when analyzing reference blood samples of bovine origin by Rt RT-PCR for the detection of BTV RNA.

Different Rt RT-PCR protocols were used by the participating laboratories in order to analyse the reference blood samples: LAB 2 and LAB 3 used an in house Rt RT-PCR while LAB 1, LAB 4 and LAB5 used the ADIAVET BTV REALTIME kit (LAB 5 used another batch compared to LAB 1 and LAB 4). All used protocols were developed to detect RNA of all BTV serotypes.

Data obtained in this proficiency test showed that all participating laboratories, even using different Rt RT-PCR protocols, provided qualitative results that were in full agreement with the true status of the reference blood samples.

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 blood samples assigned by the reference laboratory for BTV of the CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance since they provided qualitative results that were in full agreement with the true status of the reference blood samples.

Head CVD-ERA

Yves Van der Stede

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Appendix

Name of the participating Laboratories

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

Veterinary and Agrochemical Research Center (CODA-CERVA), Department Vesicular and exotic diseases (Ukkel, Belgium)

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

Dierengezondheidszorg Vlaanderen (DGZ) (Lier, Belgium)

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

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Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive or negative status), also quantitative data analysis was performed using the statistical software programs SAS 9.2. (Summary statistics) and R (Box plots). The quantitative data analysis in this report was not used to evaluate the participants in this proficiency test, 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

Box plots of the Ct-values per reference blood sample and per participating laboratory were made using the statistical software R, and are shown in Figure 1. When comparing the results of the Rt RT-PCR, it should be noted that the presented values are not normalized with the internal controls. In addition, modifiable factors such as PCR machine and calculation of Ct-values are not taken into account.

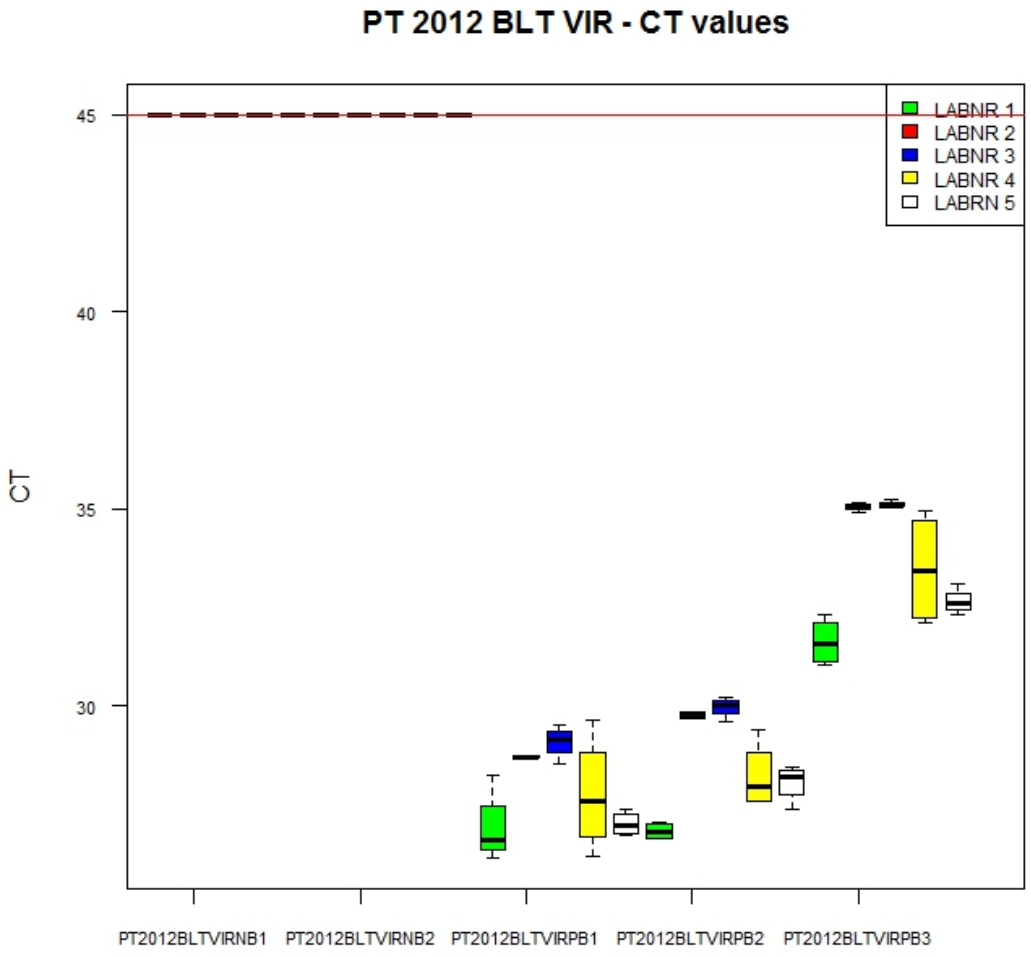

Figure 1. Box plots showing the Ct-values per reference blood sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. A default Ct-value of 45 was assigned for all negativeresults (Ct<40 is positive, 40≤Ct<45 is doubtful, Ct≥45 is negative).

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