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172-TEST

PROFICIENCY TESTING 2011

BLUE TONGUE (BLT)

Detection of BLT virus 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: 28 MARCH 2011

DATE REPORT: 26 APRIL 2011

I. Introduction

Details relevant to the proficiency test are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen/Gestion des essais d'aptitude'.

II. Aim

The aim of this proficiency test was to evaluate the ability of the participating laboratories to identify the absence or presence of blue tongue virus (BLTV) RNA in blood of bovine origin by real-time reverse transcriptase PCR (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 six reference blood samples of bovine origin, either free from detectable BLTV RNA (n = 2; coded 'PT2011BLTVIRNB1' and 'PT2011BLTVIRNB2') or containing detectable BLTV RNA (n = 4; coded 'PT2011BLTVIRPB1', 'PT2011BLTVIRPB2', 'PT2011BLTVIRPB3', and 'PT2011BLTVIRPB4'), were used. In total, 120 aliquots, prepared by the reference laboratory for BLT of the Veterinary and Agrochemical Research Center (CODA-CERVA), were distributed to the participating laboratories. All four participants were given five aliquots of the six reference blood samples (i.e., 30 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 BLT 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 at different days before the proficiency test (pre-verification), 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 BLTV RNA in bovine blood. In addition, the reference blood samples were also tested once after the proficiency test in order to confirm their stability and status (post-verification).

III.3. Qualitative data analysis

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 30 reference blood samples (aliquots).

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 status assigned by the reference laboratory for BLT of CODA-CERVA.

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 30 aliquots of reference blood samples were sent to each of the four participating laboratories on 28th of March 2011 (120 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 29th (LAB1 and LAB4) and 31st (LAB2) of March 2011 and on 1st of April 2011 (LAB3).

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

Results from the participating laboratories have been received on 4th (LAB3), 5th (LAB4), 6th (LAB1), and 8th (LAB2) of April 2011.

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 BLTV RNA in reference blood samples (Table 1).

A quantitative data analysis (including boxplots) is shown for educational purposes in Annex 1 and Annex 2.

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 BLT of CODA-CERVA. All participating laboratories received 30 reference blood samples (aliquots). 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	30 (100.0)	30 (100.0)	30 (100.0)	30 (100.0)

IV.4.2 Variability among participating laboratories

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

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 BLT of the CODA-CERVA (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2011BLTVIRPB1	POS	POS	1
2	1	2	PT2011BLTVIRPB3	POS	POS	1
3	1	3	PT2011BLTVIRNB1	NEG	NEG	1
4	1	4	PT2011BLTVIRNB2	NEG	NEG	1
5	1	5	PT2011BLTVIRNB2	NEG	NEG	1
6	1	6	PT2011BLTVIRNB2	NEG	NEG	1
7	1	7	PT2011BLTVIRPB2	POS	POS	1
8	1	8	PT2011BLTVIRPB4	POS	POS	1
9	1	9	PT2011BLTVIRPB1	POS	POS	1
10	1	10	PT2011BLTVIRPB4	POS	POS	1
11	1	11	PT2011BLTVIRPB2	POS	POS	1
12	1	12	PT2011BLTVIRNB1	NEG	NEG	1
13	1	13	PT2011BLTVIRPB3	POS	POS	1
14	1	14	PT2011BLTVIRPB4	POS	POS	1
15	1	15	PT2011BLTVIRPB3	POS	POS	1
16	1	16	PT2011BLTVIRPB1	POS	POS	1
17	1	17	PT2011BLTVIRPB4	POS	POS	1
18	1	18	PT2011BLTVIRNB2	NEG	NEG	1
19	1	19	PT2011BLTVIRPB1	POS	POS	1
20	1	20	PT2011BLTVIRNB1	NEG	NEG	1
21	1	21	PT2011BLTVIRNB1	NEG	NEG	1
22	1	22	PT2011BLTVIRPB2	POS	POS	1
23	1	23	PT2011BLTVIRPB3	POS	POS	1
24	1	24	PT2011BLTVIRPB4	POS	POS	1
25	1	25	PT2011BLTVIRNB2	NEG	NEG	1
26	1	26	PT2011BLTVIRPB1	POS	POS	1
27	1	27	PT2011BLTVIRNB1	NEG	NEG	1
28	1	28	PT2011BLTVIRPB2	POS	POS	1
29	1	29	PT2011BLTVIRPB3	POS	POS	1
30	1	30	PT2011BLTVIRPB2	POS	POS	1

(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2011BLTVIRPB1	POS	POS	1
32	2	2	PT2011BLTVIRNB1	NEG	NEG	1
33	2	3	PT2011BLTVIRPB2	POS	POS	1
34	2	4	PT2011BLTVIRPB3	POS	POS	1
35	2	5	PT2011BLTVIRPB2	POS	POS	1
36	2	6	PT2011BLTVIRPB1	POS	POS	1
37	2	7	PT2011BLTVIRPB3	POS	POS	1
38	2	8	PT2011BLTVIRNB1	NEG	NEG	1
39	2	9	PT2011BLTVIRNB2	NEG	NEG	1
40	2	10	PT2011BLTVIRNB2	NEG	NEG	1
41	2	11	PT2011BLTVIRNB2	NEG	NEG	1
42	2	12	PT2011BLTVIRPB2	POS	POS	1
43	2	13	PT2011BLTVIRPB4	POS	POS	1
44	2	14	PT2011BLTVIRPB1	POS	POS	1
45	2	15	PT2011BLTVIRPB4	POS	POS	1
46	2	16	PT2011BLTVIRPB2	POS	POS	1
47	2	17	PT2011BLTVIRNB1	NEG	NEG	1
48	2	18	PT2011BLTVIRPB3	POS	POS	1
49	2	19	PT2011BLTVIRPB4	POS	POS	1
50	2	20	PT2011BLTVIRPB3	POS	POS	1
51	2	21	PT2011BLTVIRPB1	POS	POS	1
52	2	22	PT2011BLTVIRPB4	POS	POS	1
53	2	23	PT2011BLTVIRNB2	NEG	NEG	1
54	2	24	PT2011BLTVIRPB1	POS	POS	1
55	2	25	PT2011BLTVIRNB1	NEG	NEG	1
56	2	26	PT2011BLTVIRNB1	NEG	NEG	1
57	2	27	PT2011BLTVIRPB2	POS	POS	1
58	2	28	PT2011BLTVIRPB3	POS	POS	1
59	2	29	PT2011BLTVIRPB4	POS	POS	1
60	2	30	PT2011BLTVIRNB2	NEG	NEG	1

(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	PT2011BLTVIRNB1	NEG	NEG	1
62	3	2	PT2011BLTVIRPB2	POS	POS	1
63	3	3	PT2011BLTVIRPB3	POS	POS	1
64	3	4	PT2011BLTVIRPB4	POS	POS	1
65	3	5	PT2011BLTVIRNB2	NEG	NEG	1
66	3	6	PT2011BLTVIRPB1	POS	POS	1
67	3	7	PT2011BLTVIRNB1	NEG	NEG	1
68	3	8	PT2011BLTVIRPB2	POS	POS	1
69	3	9	PT2011BLTVIRPB3	POS	POS	1
70	3	10	PT2011BLTVIRPB2	POS	POS	1
71	3	11	PT2011BLTVIRPB1	POS	POS	1
72	3	12	PT2011BLTVIRPB3	POS	POS	1
73	3	13	PT2011BLTVIRNB1	NEG	NEG	1
74	3	14	PT2011BLTVIRNB2	NEG	NEG	1
75	3	15	PT2011BLTVIRNB2	NEG	NEG	1
76	3	16	PT2011BLTVIRNB2	NEG	NEG	1
77	3	17	PT2011BLTVIRPB2	POS	POS	1
78	3	18	PT2011BLTVIRPB4	POS	POS	1
79	3	19	PT2011BLTVIRPB1	POS	POS	1
80	3	20	PT2011BLTVIRPB4	POS	POS	1
81	3	21	PT2011BLTVIRPB2	POS	POS	1
82	3	22	PT2011BLTVIRNB1	NEG	NEG	1
83	3	23	PT2011BLTVIRPB3	POS	POS	1
84	3	24	PT2011BLTVIRPB4	POS	POS	1
85	3	25	PT2011BLTVIRPB3	POS	POS	1
86	3	26	PT2011BLTVIRPB1	POS	POS	1
87	3	27	PT2011BLTVIRPB4	POS	POS	1
88	3	28	PT2011BLTVIRNB2	NEG	NEG	1
89	3	29	PT2011BLTVIRPB1	POS	POS	1
90	3	30	PT2011BLTVIRNB1	NEG	NEG	1

(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
91	4	1	PT2011BLTVIRPB1	POS	POS	1
92	4	2	PT2011BLTVIRPB4	POS	POS	1
93	4	3	PT2011BLTVIRNB2	NEG	NEG	1
94	4	4	PT2011BLTVIRPB1	POS	POS	1
95	4	5	PT2011BLTVIRNB1	NEG	NEG	1
96	4	6	PT2011BLTVIRNB1	NEG	NEG	1
97	4	7	PT2011BLTVIRPB2	POS	POS	1
98	4	8	PT2011BLTVIRPB3	POS	POS	1
99	4	9	PT2011BLTVIRPB4	POS	POS	1
100	4	10	PT2011BLTVIRNB2	NEG	NEG	1
101	4	11	PT2011BLTVIRPB1	POS	POS	1
102	4	12	PT2011BLTVIRNB1	NEG	NEG	1
103	4	13	PT2011BLTVIRPB2	POS	POS	1
104	4	14	PT2011BLTVIRPB3	POS	POS	1
105	4	15	PT2011BLTVIRPB2	POS	POS	1
106	4	16	PT2011BLTVIRPB1	POS	POS	1
107	4	17	PT2011BLTVIRPB3	POS	POS	1
108	4	18	PT2011BLTVIRNB1	NEG	NEG	1
109	4	19	PT2011BLTVIRNB2	NEG	NEG	1
110	4	20	PT2011BLTVIRNB2	NEG	NEG	1
111	4	21	PT2011BLTVIRNB2	NEG	NEG	1
112	4	22	PT2011BLTVIRPB2	POS	POS	1
113	4	23	PT2011BLTVIRPB4	POS	POS	1
114	4	24	PT2011BLTVIRPB1	POS	POS	1
115	4	25	PT2011BLTVIRPB4	POS	POS	1
116	4	26	PT2011BLTVIRPB2	POS	POS	1
117	4	27	PT2011BLTVIRNB1	NEG	NEG	1
118	4	28	PT2011BLTVIRPB3	POS	POS	1
119	4	29	PT2011BLTVIRPB4	POS	POS	1
120	4	30	PT2011BLTVIRPB3	POS	POS	1

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 BLTV RNA.

Different Rt RT-PCR protocols were used by the participating laboratories in order to analyse the reference blood samples: LAB1 and LAB3 used the ADIAVET BTV REALTIME kit whereas LAB2 and LAB4 used an in house BLTV Rt RT-PCR. All used tests were developed to detect RNA of all BLTV 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 BLT 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

Appendix

Name of the participating Laboratories

ARSIA (Mons)

CODA-CERVA (Vesicular and exotic diseases)

CODA-CERVA (Virological Platform)

DGZ (Lier)

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.

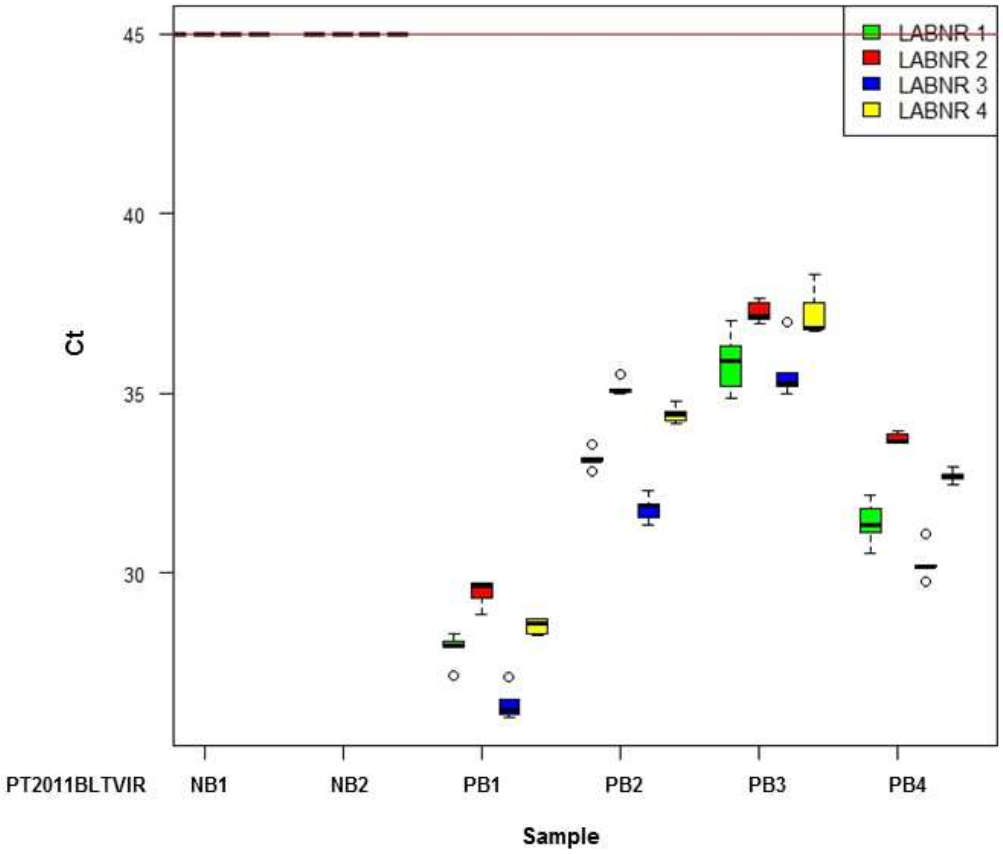

Figure 1. Box plots showing the Ct-values per reference blood sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. A default Ct-value of 45 was assigned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

II. Mandels h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-lab variability (reproducibility) and within-lab variability (repeatability) were estimated through Mandels h- and k-statistics, respectively, using the statistical software SAS 9.2.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a proficiency test, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2.

An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this proficiency test), other indicator values apply for Mandels h- and k-statistics (Table 1).

Table 1. Indicators for Mandels h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,4	1,37	1,34	1,32	1,3	1,29
4	1,42	1,76	1,59	1,5	1,44	1,4	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,4	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,5	1,45	1,41	1,38	1,36	1,34
9	1,78	1,9	1,68	1,57	1,5	1,45	1,42	1,39	1,36	1,35
10	1,8	1,9	1,68	1,57	1,5	1,46	1,42	1,39	1,37	1,35

Since the negative results of all participants were assigned a default Ct-value of 45, Mandels h- and k-statistics could only be calculated for the BLTV positive reference blood samples. Based on Table 1, the maximum values for Mandels h- and k-statistics for this proficiency test are 1.42 and 1.44, respectively (p = 4 and n = 5). Hence, for all participating laboratories, a satisfactory between-lab consistency was observed for all positive reference blood samples. LAB2, LAB3 and LAB4 also obtained a satisfactory within-laboratory consistency for all positive reference blood samples, whereas LAB1 obtained a slightly increased within-laboratory consistency value for sample PT2011BLTVIRPB4 (k=1.51).

All data used for the calculations of Mandels h- and k-statistics can be found in Annex 2.

III. ANOVA

Statistically significant differences between the participating laboratories were studied using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the Ct-values as a dependent variable.

Although statistically significant differences exist between laboratories on a sample level, no statistically significant differences were observed between laboratories when all samples were taken into account.

Annex 2: Calculations of Mandels h- and k-statistics

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2011BLTVIRNB1	1	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB1	2	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB1	3	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB1	4	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB2	1	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB2	2	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB2	3	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRNB2	4	5	0,00	45,00	45,00		0,00	0,00	0,00			0,00
PT2011BLTVIRPB1	1	5	0,18	27,91	28,07	0,79	0,38	0,83	0,74	-0,12	1,13	1,54
PT2011BLTVIRPB1	2	5	0,15	29,45	28,07	0,79	0,38	0,83	0,74	1,07	1,01	1,30
PT2011BLTVIRPB1	3	5	0,20	26,38	28,07	0,79	0,38	0,83	0,74	-1,31	1,18	1,70
PT2011BLTVIRPB1	4	5	0,05	28,53	28,07	0,79	0,38	0,83	0,74	0,36	0,58	0,77
PT2011BLTVIRPB2	1	5	0,07	33,18	33,64	0,90	0,27	0,89	0,85	-0,31	0,97	0,80
PT2011BLTVIRPB2	2	5	0,04	35,15	33,64	0,90	0,27	0,89	0,85	1,03	0,77	0,60
PT2011BLTVIRPB2	3	5	0,13	31,80	33,64	0,90	0,27	0,89	0,85	-1,25	1,31	1,13
PT2011BLTVIRPB2	4	5	0,06	34,42	33,64	0,90	0,27	0,89	0,85	0,53	0,88	0,70
PT2011BLTVIRPB3	1	5	0,75	35,86	36,50	0,32	0,70	0,84	0,48	-0,72	1,24	2,42
PT2011BLTVIRPB3	2	5	0,09	37,27	36,50	0,32	0,70	0,84	0,48	0,88	0,44	0,81
PT2011BLTVIRPB3	3	5	0,64	35,61	36,50	0,32	0,70	0,84	0,48	-1,00	1,15	2,25
PT2011BLTVIRPB3	4	5	0,46	37,24	36,50	0,32	0,70	0,84	0,48	0,84	0,97	1,82
PT2011BLTVIRPB4	1	5	0,39	31,40	32,04	0,81	0,41	0,96	0,86	-0,42	1,51	1,98
PT2011BLTVIRPB4	2	5	0,02	33,75	32,04	0,81	0,41	0,96	0,86	1,14	0,37	0,45
PT2011BLTVIRPB4	3	5	0,24	30,30	32,04	0,81	0,41	0,96	0,86	-1,16	1,18	1,60
PT2011BLTVIRPB4	4	5	0,03	32,69	32,04	0,81	0,41	0,96	0,86	0,44	0,43	0,54

Legend: **Labnr** = number attributed to a laboratory during the PT test; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised data (% S/P ratio); **x_i_m** = mean of normalized data (% S/P ratio); **x_g_m** = mean of normalized data (% S/P ratio) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %