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172-PT

PROFICIENCY TESTING 2013

BLUE TONGUE VIRUS (BTV)

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

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 25 MARCH 2013

DATE REPORT: 31 MAY 2013

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 and/or plasma of bovidae origin by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

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

III.2. Reference samples

Replicates of 6 reference serum/plasma samples of bovidae origin, either free from detectable BTV-specific antibodies (n=2; coded 'PT2013BLTSERNS1' and 'PT2013BLTSERNS2') or containing detectable BTV-specific antibodies (n=4; coded 'PT2013BLTSERPS1', 'PT2013BLTSERPS2', 'PT2013BLTSERPS3' and 'PT2013BLTSERPS4'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference samples PT2013BLTSERPS1, PT2013BLTSERPS2, PT2013BLTSERPS3 and PT2013BLTSERPS4, and 4 aliquots of the reference samples PT2013BLTSERNS1 and PT2013BLTSERNS2. The positions of the reference serum/plasma samples in the sent blocks were randomized for each participant (Table 3).

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 PT2013BLTSERNS1, PT2013BLTSERNS2, PT2013BLTSERPS1, PT2013BLTSERPS2 and PT2013BLTSERPS3 were serum samples, whereas the reference sample PT2013BLTSERPS4 was a plasma sample. The reference serum samples PT2013BLTSERNS1 and PT2013BLTSERNS2 were obtained from BTV uninfected and non-vaccinated cattle (sentinel animals). The reference serum sample PT2013BLTSERPS1 was a 1/40 dilution of a BTV positive reference serum obtained from Cirad (Centre de coopération internationale en recherche agronomique pour le développement, France) (batch 357). The BTV positive reference serum from Cirad was obtained from goats vaccinated with a BTV-2 and BTV-4 vaccine (pooled sera). The reference serum samples PT2013BLTSERPS2 and PT2013BLTSERPS3 were a 1/16 and a 1/32 dilution, respectively, of 2 different sera derived from BTV infected cattle. The reference plasma sample PT2013BLTSERPS4 was a 1/4 dilution of a plasma sample derived from a BTV vaccinated sheep. For each reference serum/plasma sample, the same qualitative result was obtained with all ELISA tests performed. Taken together, the reference serum samples PT2013BLTSERNS1 and PT2013BLTSERNS2 were considered as negative samples, and the reference serum/plasma samples PT2013BLTSERPS1, PT2013BLTSERPS2, PT2013BLTSERPS3 and PT2013BLTSERPS4 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. A homogeneity check had already been performed in 2012 for the reference serum samples PT2013BLTSERNS1, PT2013BLTSERNS2 and PT2013BLTSERPS1 using the ID Screen® Bluetongue Competition ELISA test from ID VET. For the reference serum/plasma samples that were prepared and aliquoted in 2013 (PT2013BLTSERPS2, PT2013BLTSERPS3 and PT2013BLTSERPS4), the homogeneity check was performed using the Bluetongue Virus (BTV) VP7 Antibody Test Kit from IDEXX. For all reference serum/plasma samples, the same qualitative

result was obtained for all 10 aliquots of the same reference serum/plasma sample. 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, all reference serum/plasma samples 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.

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 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 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

The 20 aliquots of reference serum/plasma samples were sent frozen (dry ice) to each of the 7 participating laboratories by national or international courier on 25th of March 2013 (140 aliquots in total). LAB4, LAB5, LAB6 and LAB7 acknowledged receipt of the samples on the same day, whereas the other laboratories acknowledged receipt of the samples on 27th of March 2013. All participating laboratories confirmed that the reference samples were still frozen upon receipt. Analyses were performed between 25th of March and 2nd of April 2013 (Table 1).

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

Results were submitted to the operational unit CVD-ERA between 26th of March and 5th of April 2013 (Table 1). All participants hereby respected the deadline of 5th of April 2013 for submission of the results.

On 2nd of April 2013, LAB2 was asked to resubmit their results because a transcription error occurred for sample 3 (PT2013BLTSERNS1: the qualitative result did not correspond with the quantitative result). A corrected version was received on 3rd of April 2013.

Table 1. 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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	27/03/2013	28/03/2013	29/03/2013
LAB2	27/03/2013	27/03/2013	02/04/2013▲
LAB3	27/03/2013	29/03/2013	29/03/2013
LAB4	25/03/2013	02/04/2013	04/04/2013
LAB5	25/03/2013	28/03/2013	05/04/2013
LAB6	25/03/2013	26/03/2013	04/04/2013
LAB7	25/03/2013	25/03/2013	26/03/2013

Legend: ▲ LAB2 was asked on 02/04/2013 to resubmit their results because of a transcription error for sample 3 (a corrected version was received on 03/04/2013)

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 4 out of 7 participating laboratories (LAB1, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the assigned status of the reference serum/plasma samples (100% of agreement), whereas LAB2 misclassified 3 aliquots (85% of agreement), and LAB3 and LAB4 1 aliquot (95% of agreement) (Table 2).

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

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum/plasma samples assigned by CODA-CERVA. 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**	**5**	**6**	**7**
failure	0 (0.0)	**3 (15.0)**	1 (5.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	**17 (85.0)**	19 (95.0)	19 (95.0)	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1, LAB5, LAB6 and LAB7 since these participants correctly identified all reference serum/plasma samples. In contrast, LAB2 misclassified 3 out of 4 aliquots of the negative reference serum sample PT2013BLTSERNS1 (1x NI and 2x POS instead of NEG), whereas LAB3 misclassified 1 aliquot of the same reference serum sample (NI instead of NEG). In addition, LAB4 misclassified 1 out of 3 aliquots of the positive reference serum sample PT2013BLTSERPS3 (NI instead of POS).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum/plasma samples are shown in Table 3.

Table 3. 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 (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013BLTSERNS1	NEG	NEG	1
2	1	2	PT2013BLTSERPS1	POS	POS	1
3	1	3	PT2013BLTSERNS2	NEG	NEG	1
4	1	4	PT2013BLTSERPS4	POS	POS	1
5	1	5	PT2013BLTSERPS3	POS	POS	1
6	1	6	PT2013BLTSERNS1	NEG	NEG	1
7	1	7	PT2013BLTSERPS1	POS	POS	1
8	1	8	PT2013BLTSERPS3	POS	POS	1
9	1	9	PT2013BLTSERNS2	NEG	NEG	1
10	1	10	PT2013BLTSERPS4	POS	POS	1
11	1	11	PT2013BLTSERPS2	POS	POS	1
12	1	12	PT2013BLTSERPS4	POS	POS	1
13	1	13	PT2013BLTSERNS1	NEG	NEG	1
14	1	14	PT2013BLTSERPS2	POS	POS	1
15	1	15	PT2013BLTSERPS3	POS	POS	1
16	1	16	PT2013BLTSERNS2	NEG	NEG	1
17	1	17	PT2013BLTSERNS1	NEG	NEG	1
18	1	18	PT2013BLTSERPS1	POS	POS	1
19	1	19	PT2013BLTSERNS2	NEG	NEG	1
20	1	20	PT2013BLTSERPS2	POS	POS	1
21	2	1	PT2013BLTSERPS4	POS	POS	1
22	2	2	PT2013BLTSERPS3	POS	POS	1
23	2	3	PT2013BLTSERNS1	**NEG**	**NI**	**0**
24	2	4	PT2013BLTSERPS1	POS	POS	1
25	2	5	PT2013BLTSERPS3	POS	POS	1
26	2	6	PT2013BLTSERNS2	NEG	NEG	1
27	2	7	PT2013BLTSERPS4	POS	POS	1
28	2	8	PT2013BLTSERPS2	POS	POS	1
29	2	9	PT2013BLTSERPS4	POS	POS	1
30	2	10	PT2013BLTSERNS1	**NEG**	**POS**	**0**
31	2	11	PT2013BLTSERPS2	POS	POS	1
32	2	12	PT2013BLTSERPS3	POS	POS	1
33	2	13	PT2013BLTSERNS2	NEG	NEG	1
34	2	14	PT2013BLTSERNS1	NEG	NEG	1
35	2	15	PT2013BLTSERPS1	POS	POS	1
36	2	16	PT2013BLTSERNS2	NEG	NEG	1
37	2	17	PT2013BLTSERPS2	POS	POS	1
38	2	18	PT2013BLTSERNS1	**NEG**	**POS**	**0**
39	2	19	PT2013BLTSERPS1	POS	POS	1
40	2	20	PT2013BLTSERNS2	NEG	NEG	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013BLTSERPS1	POS	POS	1
42	3	2	PT2013BLTSERPS3	POS	POS	1
43	3	3	PT2013BLTSERNS2	NEG	NEG	1
44	3	4	PT2013BLTSERPS4	POS	POS	1
45	3	5	PT2013BLTSERPS2	POS	POS	1
46	3	6	PT2013BLTSERPS4	POS	POS	1
47	3	7	PT2013BLTSERNS1	NEG	NEG	1
48	3	8	PT2013BLTSERPS2	POS	POS	1
49	3	9	PT2013BLTSERPS3	POS	POS	1
50	3	10	PT2013BLTSERNS2	NEG	NEG	1
51	3	11	PT2013BLTSERNS1	**NEG**	**NI**	**0**
52	3	12	PT2013BLTSERPS1	POS	POS	1
53	3	13	PT2013BLTSERNS2	NEG	NEG	1
54	3	14	PT2013BLTSERPS2	POS	POS	1
55	3	15	PT2013BLTSERNS1	NEG	NEG	1
56	3	16	PT2013BLTSERPS1	POS	POS	1
57	3	17	PT2013BLTSERNS2	NEG	NEG	1
58	3	18	PT2013BLTSERPS4	POS	POS	1
59	3	19	PT2013BLTSERPS3	POS	POS	1
60	3	20	PT2013BLTSERNS1	NEG	NEG	1
61	4	1	PT2013BLTSERPS4	POS	POS	1
62	4	2	PT2013BLTSERPS2	POS	POS	1
63	4	3	PT2013BLTSERPS4	POS	POS	1
64	4	4	PT2013BLTSERNS1	NEG	NEG	1
65	4	5	PT2013BLTSERPS2	POS	POS	1
66	4	6	PT2013BLTSERPS3	POS	POS	1
67	4	7	PT2013BLTSERNS2	NEG	NEG	1
68	4	8	PT2013BLTSERNS1	NEG	NEG	1
69	4	9	PT2013BLTSERPS1	POS	POS	1
70	4	10	PT2013BLTSERNS2	NEG	NEG	1
71	4	11	PT2013BLTSERPS2	POS	POS	1
72	4	12	PT2013BLTSERNS1	NEG	NEG	1
73	4	13	PT2013BLTSERPS1	POS	POS	1
74	4	14	PT2013BLTSERNS2	NEG	NEG	1
75	4	15	PT2013BLTSERPS4	POS	POS	1
76	4	16	PT2013BLTSERPS3	POS	POS	1
77	4	17	PT2013BLTSERNS1	NEG	NEG	1
78	4	18	PT2013BLTSERPS1	POS	POS	1
79	4	19	PT2013BLTSERPS3	**POS**	**NI**	**0**
80	4	20	PT2013BLTSERNS2	NEG	NEG	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2013BLTSERNS1	NEG	NEG	1
82	5	2	PT2013BLTSERPS2	POS	POS	1
83	5	3	PT2013BLTSERPS3	POS	POS	1
84	5	4	PT2013BLTSERNS2	NEG	NEG	1
85	5	5	PT2013BLTSERNS1	NEG	NEG	1
86	5	6	PT2013BLTSERPS1	POS	POS	1
87	5	7	PT2013BLTSERNS2	NEG	NEG	1
88	5	8	PT2013BLTSERPS2	POS	POS	1
89	5	9	PT2013BLTSERNS1	NEG	NEG	1
90	5	10	PT2013BLTSERPS1	POS	POS	1
91	5	11	PT2013BLTSERNS2	NEG	NEG	1
92	5	12	PT2013BLTSERPS4	POS	POS	1
93	5	13	PT2013BLTSERPS3	POS	POS	1
94	5	14	PT2013BLTSERNS1	NEG	NEG	1
95	5	15	PT2013BLTSERPS1	POS	POS	1
96	5	16	PT2013BLTSERPS3	POS	POS	1
97	5	17	PT2013BLTSERNS2	NEG	NEG	1
98	5	18	PT2013BLTSERPS4	POS	POS	1
99	5	19	PT2013BLTSERPS2	POS	POS	1
100	5	20	PT2013BLTSERPS4	POS	POS	1
101	6	1	PT2013BLTSERNS2	NEG	NEG	1
102	6	2	PT2013BLTSERNS1	NEG	NEG	1
103	6	3	PT2013BLTSERPS1	POS	POS	1
104	6	4	PT2013BLTSERNS2	NEG	NEG	1
105	6	5	PT2013BLTSERPS2	POS	POS	1
106	6	6	PT2013BLTSERNS1	NEG	NEG	1
107	6	7	PT2013BLTSERPS1	POS	POS	1
108	6	8	PT2013BLTSERNS2	NEG	NEG	1
109	6	9	PT2013BLTSERPS4	POS	POS	1
110	6	10	PT2013BLTSERPS3	POS	POS	1
111	6	11	PT2013BLTSERNS1	NEG	NEG	1
112	6	12	PT2013BLTSERPS1	POS	POS	1
113	6	13	PT2013BLTSERPS3	POS	POS	1
114	6	14	PT2013BLTSERNS2	NEG	NEG	1
115	6	15	PT2013BLTSERPS4	POS	POS	1
116	6	16	PT2013BLTSERPS2	POS	POS	1
117	6	17	PT2013BLTSERPS4	POS	POS	1
118	6	18	PT2013BLTSERNS1	NEG	NEG	1
119	6	19	PT2013BLTSERPS2	POS	POS	1
120	6	20	PT2013BLTSERPS3	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2013BLTSERNS2	NEG	NEG	1
122	7	2	PT2013BLTSERPS2	POS	POS	1
123	7	3	PT2013BLTSERNS1	NEG	NEG	1
124	7	4	PT2013BLTSERPS1	POS	POS	1
125	7	5	PT2013BLTSERNS2	NEG	NEG	1
126	7	6	PT2013BLTSERPS4	POS	POS	1
127	7	7	PT2013BLTSERPS3	POS	POS	1
128	7	8	PT2013BLTSERNS1	NEG	NEG	1
129	7	9	PT2013BLTSERPS1	POS	POS	1
130	7	10	PT2013BLTSERPS3	POS	POS	1
131	7	11	PT2013BLTSERNS2	NEG	NEG	1
132	7	12	PT2013BLTSERPS4	POS	POS	1
133	7	13	PT2013BLTSERPS2	POS	POS	1
134	7	14	PT2013BLTSERPS4	POS	POS	1
135	7	15	PT2013BLTSERNS1	NEG	NEG	1
136	7	16	PT2013BLTSERPS2	POS	POS	1
137	7	17	PT2013BLTSERPS3	POS	POS	1
138	7	18	PT2013BLTSERNS2	NEG	NEG	1
139	7	19	PT2013BLTSERNS1	NEG	NEG	1
140	7	20	PT2013BLTSERPS1	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.

For the detection of BTV-specific antibodies in reference serum/plasma samples, 4 out of 7 participating laboratories (LAB1, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the assigned status of the reference serum/plasma samples (100% of agreement). LAB2 and LAB3 misclassified 3 and 1 aliquot(s), respectively, of the negative reference serum sample PT2013BLTSERNS1 (85% and 95% of agreement, respectively), whereas LAB4 misclassified 1 aliquot of the positive reference serum sample PT2013BLTSERPS3 (95% of agreement) (Table 2 and Table 3).

BTV antibody ELISA kits from 3 different producers as well as different batches from the same ELISA kit were used: ID VET (1 batch: 410), IDEXX Montpellier SAS (3 batches: 1015, 2069 and 3003) and LSI (1 batch: 5-VETBTAII-002). LAB1, LAB3, LAB5, LAB6 and LAB7 used a BTV antibody ELISA kit from the same producer (LAB5, LAB6 and LAB7 used the same batch).

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 serum/plasma samples assigned by CODA-CERVA (see III.3.3.). Consequently, 6 out of 7 participants achieved a satisfactory performance for the detection of BTV-specific antibodies in reference serum/plasma samples by ELISA. Hereby, LAB2 did not reach the required 90% of agreement.

Head CVD-ERA
Yves Van der Stede

Appendix

Name of the participating laboratories

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Gezondheidsdienst voor dieren (GD) (Deventer, The Netherlands)

IDEXX Montpellier SAS (Montpellier, France)

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

Laboratoire Service International (LSI) (Lissieu, France)

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). All quantitative data analyses 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.

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

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 1.

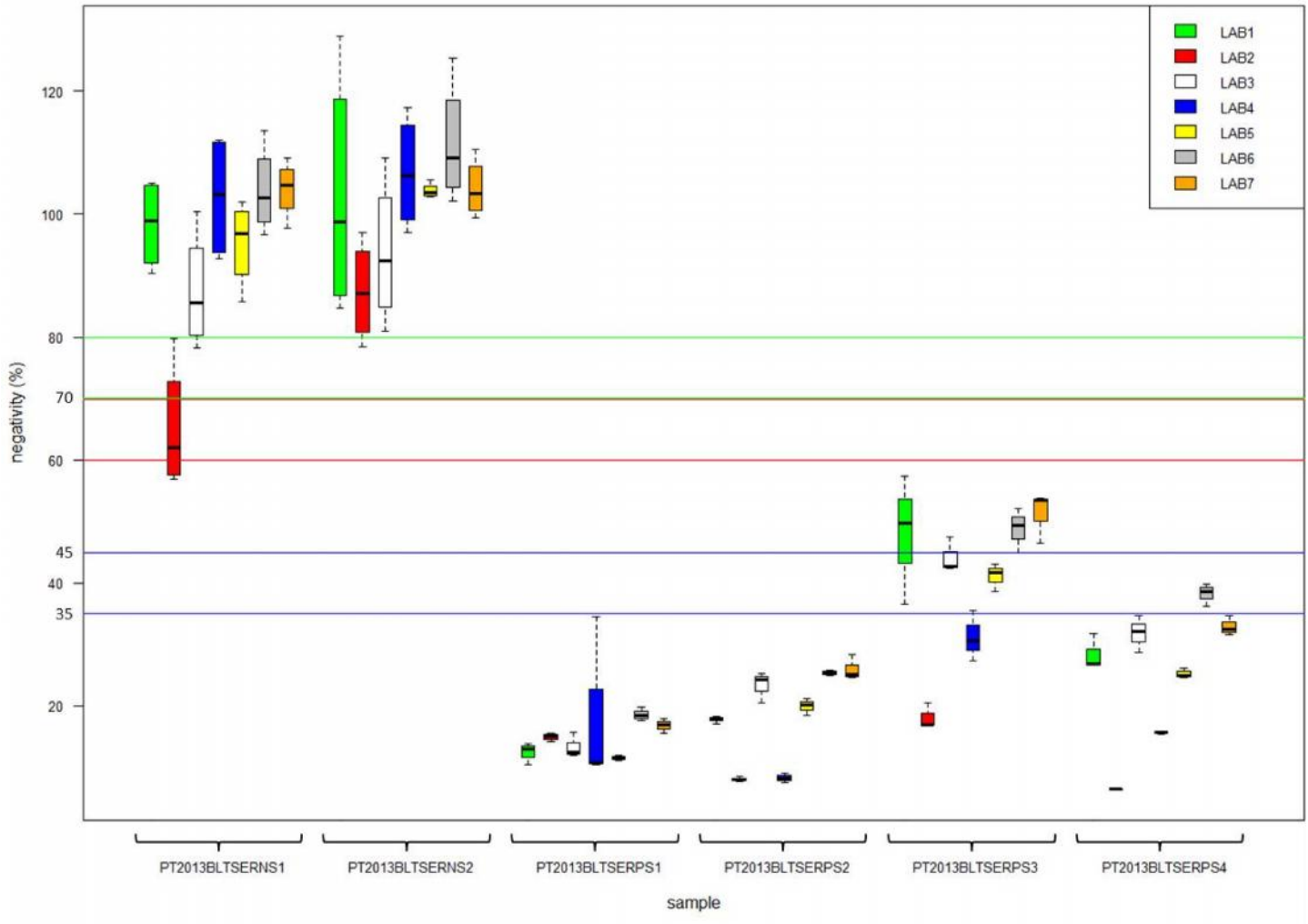

Figure 1. Box plots showing the percentage negativity per reference serum/plasma sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in blue (35-45%; LAB4), red (60-70%; LAB2) and green (70-80%; LAB1, LAB3, LAB5, LAB6, LAB7), respectively. LAB1, LAB3, LAB5, LAB6 and LAB7 used a BTV antibody ELISA kit from the same producer (LAB5, LAB6 and LAB7 used the same batch).

II. Mandel's 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 Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated based on percentages negativity per reference serum/plasma sample and per participating laboratory.

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 PT, 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 PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's 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	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	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	1,40	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	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic for this PT is 1,71 (p=7), whereas the maximum value for Mandel's k-statistic is 1,66 for the reference samples PT2013BLTSERPS1, PT2013BLTSERPS2, PT2013BLTSERPS3 and PT2013BLTSERPS4 (p=7 and n=3), and 1,55 for the reference samples PT2013BLTSERNS1 and PT2013BLTSERNS2 (p=7 and n=4).

LAB1, LAB3, LAB4, LAB5, LAB6 and LAB7 obtained a satisfactory between-laboratory consistency for all reference samples. In contrast, LAB2 showed an increased value for Mandel's h-statistic for the negative reference samples PT2013BLTSERNS1 (h=-2,05) and PT2013BLTSERNS2 (h=-1,72), and for the positive reference samples PT2013BLTSERPS3 (h=-1,88) and PT2013BLTSERPS4 (h=-1,74). LAB1, LAB3, LAB5, LAB6 and LAB7 used a BTV antibody ELISA kit from the same producer (LAB5, LAB6 and LAB7 used the same batch).

LAB2, LAB5, LAB6 and LAB7 obtained a satisfactory within-laboratory consistency for all reference samples. In contrast, the other participants showed an increased value for Mandel's k-statistic for at least 1 reference sample: LAB1 for the negative reference sample PT2013BLTSERNS2 (k=1,86) and the positive reference sample PT2013BLTSERPS3 (k=2,11), LAB3 the positive reference sample PT2013BLTSERPS2 (k=1,82), and LAB4 for the positive reference sample PT2013BLTSERPS1 (k=2,57).

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

III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values (in this case the percentage negativity calculated according to the instructions for this PT) as a dependent variable, it was investigated whether statistically significant differences exist (α=0,05) between participating laboratories. Comparisons were made at the global level (all reference serum/plasma samples were analysed together), status level (all reference serum/plasma samples with the same status were analysed together) and sample level (all reference serum/plasma samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

No statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level. At the status level, significant differences were observed for both the negative and positive reference samples. For the negative reference samples, LAB2 and LAB3 reported percentages negativity that were significantly lower than those reported by LAB6, and LAB2 reported percentages negativity that were significantly lower than those reported by LAB1, LAB4, LAB5, LAB6 and LAB7. For the positive reference samples, LAB2 reported percentages negativity that were significantly lower than those reported by LAB3, LAB6 and LAB7.

Annex 2: Calculations of Mandel's h- and k-statistics (based on % negativity)

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013BLTSERNS1	1	4	55,10	98,29	93,84	0,30	8,35	9,96	5,44	0,32	0,89	7,55
PT2013BLTSERNS1	2	4	109,49	65,18	93,84	0,30	8,35	9,96	5,44	-2,05	1,25	16,05
PT2013BLTSERNS1	3	4	92,83	87,42	93,84	0,30	8,35	9,96	5,44	-0,46	1,15	11,02
PT2013BLTSERNS1	4	4	106,90	102,73	93,84	0,30	8,35	9,96	5,44	0,64	1,24	10,06
PT2013BLTSERNS1	5	4	49,62	95,31	93,84	0,30	8,35	9,96	5,44	0,11	0,84	7,39
PT2013BLTSERNS1	6	4	51,61	103,87	93,84	0,30	8,35	9,96	5,44	0,72	0,86	6,92
PT2013BLTSERNS1	7	4	22,40	104,05	93,84	0,30	8,35	9,96	5,44	0,73	0,57	4,55
PT2013BLTSERNS2	1	4	414,96	102,71	101,41	0,05	10,94	11,22	2,47	0,16	1,86	19,83
PT2013BLTSERNS2	2	4	67,53	87,38	101,41	0,05	10,94	11,22	2,47	-1,72	0,75	9,41
PT2013BLTSERNS2	3	4	144,46	93,76	101,41	0,05	10,94	11,22	2,47	-0,94	1,10	12,82
PT2013BLTSERNS2	4	4	86,28	106,71	101,41	0,05	10,94	11,22	2,47	0,65	0,85	8,70
PT2013BLTSERNS2	5	4	1,38	103,77	101,41	0,05	10,94	11,22	2,47	0,29	0,11	1,13
PT2013BLTSERNS2	6	4	100,76	111,45	101,41	0,05	10,94	11,22	2,47	1,23	0,92	9,01
PT2013BLTSERNS2	7	4	23,06	104,12	101,41	0,05	10,94	11,22	2,47	0,33	0,44	4,61
PT2013BLTSERPS1	1	3	3,17	12,44	15,17	0,00	5,37	5,37	0,00	-0,94	0,33	14,31
PT2013BLTSERPS1	2	3	0,52	14,91	15,17	0,00	5,37	5,37	0,00	-0,09	0,13	4,84
PT2013BLTSERPS1	3	3	4,26	13,35	15,17	0,00	5,37	5,37	0,00	-0,63	0,38	15,45
PT2013BLTSERPS1	4	3	190,65	18,60	15,17	0,00	5,37	5,37	0,00	1,18	2,57	74,25
PT2013BLTSERPS1	5	3	0,26	11,49	15,17	0,00	5,37	5,37	0,00	-1,27	0,09	4,40
PT2013BLTSERPS1	6	3	1,21	18,61	15,17	0,00	5,37	5,37	0,00	1,18	0,20	5,90
PT2013BLTSERPS1	7	3	1,53	16,82	15,17	0,00	5,37	5,37	0,00	0,57	0,23	7,35
PT2013BLTSERPS2	1	3	0,41	17,74	18,39	0,82	1,42	3,38	3,06	-0,09	0,45	3,59
PT2013BLTSERPS2	2	3	0,21	8,09	18,39	0,82	1,42	3,38	3,06	-1,37	0,32	5,68
PT2013BLTSERPS2	3	3	6,72	23,37	18,39	0,82	1,42	3,38	3,06	0,66	1,82	11,09
PT2013BLTSERPS2	4	3	0,58	8,29	18,39	0,82	1,42	3,38	3,06	-1,34	0,53	9,15
PT2013BLTSERPS2	5	3	1,89	19,96	18,39	0,82	1,42	3,38	3,06	0,21	0,97	6,89
PT2013BLTSERPS2	6	3	0,19	25,33	18,39	0,82	1,42	3,38	3,06	0,92	0,30	1,71
PT2013BLTSERPS2	7	3	4,17	25,98	18,39	0,82	1,42	3,38	3,06	1,01	1,44	7,86

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013BLTSERPS3	**1**	3	112,15	47,89	40,36	0,47	5,03	6,87	4,69	0,64	**2,11**	22,11
PT2013BLTSERPS3	**2**	3	4,50	18,10	40,36	0,47	5,03	6,87	4,69	**-1,88**	0,42	11,72
PT2013BLTSERPS3	3	3	8,05	44,14	40,36	0,47	5,03	6,87	4,69	0,32	0,56	6,43
PT2013BLTSERPS3	4	3	16,85	31,18	40,36	0,47	5,03	6,87	4,69	-0,77	0,82	13,16
PT2013BLTSERPS3	5	3	4,92	41,11	40,36	0,47	5,03	6,87	4,69	0,06	0,44	5,39
PT2013BLTSERPS3	6	3	13,37	48,81	40,36	0,47	5,03	6,87	4,69	0,71	0,73	7,49
PT2013BLTSERPS3	7	3	17,06	51,26	40,36	0,47	5,03	6,87	4,69	0,92	0,82	8,06
PT2013BLTSERPS4	1	3	8,66	28,36	25,53	0,85	1,87	4,84	4,46	0,26	1,57	10,38
PT2013BLTSERPS4	**2**	3	0,02	6,46	25,53	0,85	1,87	4,84	4,46	**-1,74**	0,07	1,95
PT2013BLTSERPS4	3	3	9,03	31,82	25,53	0,85	1,87	4,84	4,46	0,57	1,61	9,44
PT2013BLTSERPS4	4	3	0,05	15,70	25,53	0,85	1,87	4,84	4,46	-0,90	0,12	1,47
PT2013BLTSERPS4	5	3	0,67	25,27	25,53	0,85	1,87	4,84	4,46	-0,02	0,44	3,25
PT2013BLTSERPS4	6	3	3,31	38,24	25,53	0,85	1,87	4,84	4,46	1,16	0,97	4,76
PT2013BLTSERPS4	7	3	2,78	32,89	25,53	0,85	1,87	4,84	4,46	0,67	0,89	5,07

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalised data (% negativity); **x_i_m** = mean of normalized data (% negativity); **x_g_m** = mean of normalized data (% negativity) 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 %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).