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172-PT

PROFICIENCY TESTING 2014

CLASSICAL SWINE FEVER (CSF)

Detection of CSF-specific antibodies in serum by

Enzyme-Linked Immunosorbent Assay (ELISA)

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 12 MAY 2014

DATE REPORT: 22 JULY 2014

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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 CSF-specific antibodies in porcine serum by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be tested by means of a CSF 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 samples of porcine origin, either free from detectable CSF-specific antibodies (n=3; coded 'PT2014CSFSERNS1', 'PT2014CSFSERNS2' and 'PT2014CSFSERNS3') or containing detectable CSF-specific antibodies (n=3; coded 'PT2014CSFSERPS1', 'PT2014CSFSERPS2' and 'PT2014CSFSERPS3'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2014CSFSERNS1, PT2014CSFSERNS2, PT2014CSFSERNS3 and PT2014CSFSERPS3, and 4 aliquots of the reference serum samples PT2014CSFSERPS1 and PT2014CSFSERPS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 3).

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/or (ii) the results obtained during pre-verification, hereby using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX and a seroneutralisation assay (SN).
The reference serum samples PT2014CSFSERNS1, PT2014CSFSERNS2 and PT2014CSFSERNS3 were obtained from CSF free animals, whereas the reference serum sample PT2014CSFSERPS2 was obtained from a pig that became infected during an *in vivo* CSF infection experiment (contact animal; blood sample collected at 33 days post contact). The reference serum samples PT2014CSFSERPS1 and PT2014CSFSERPS3 were a 1/8 and a 1/64 dilution, respectively, of the same CSF hyperimmune serum. For all reference serum samples, the same qualitative result was obtained with ELISA and SN. Based on these results, the reference serum samples PT2014CSFSERNS1, PT2014CSFSERNS2 and PT2014CSFSERNS3 were considered as negative sera, the reference serum sample PT2014CSFSERPS1 as a strong positive serum and the reference serum samples PT2014CSFSERPS2 and PT2014CSFSERPS3 as weak/intermediate positive sera in CSF antibody ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX, hereby obtaining the same qualitative result 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 CSF-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX.

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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 or *failure* when the reported result does not match with the assigned status.

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 the reference serum samples were sent frozen (dry ice) to each of the 4 participating laboratories by national or international courier on 12th of May 2014 (80 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between the 12th and the 15th of May 2014 (Table 1).

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

Results from the participating laboratories were submitted to the operational unit CVD-ERA between 19th and 26th of May 2014 (Table 1). All participants hereby respected the deadline of 30th of May 2014.

Table 1. Overview of the dates on which (i) the reference serum 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	12/05/2014	15/05/2014	20/05/2014
LAB2	12/05/2014	14/05/2014	21/05/2014
LAB3	12/05/2014	12/05/2014	19/05/2014
LAB4	12/05/2014	13/05/2014	26/05/2014

IV.3. Compliance with the procedure

Except LAB3, all participating laboratories have provided a duly dated and signed copy of the results.

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IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that 3 out of 4 participating laboratories (LAB1, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement), whereas LAB2 misclassified 3 aliquots and hence obtained 85% of agreement (Table 2).

Table 2. Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by CODA-CERVA. 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**
failure	0 (0.0)	3 (15.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	**17 (85.0)**	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1, LAB3 and LAB4 since these participants correctly identified all reference serum samples. In contrast, LAB2 misclassified all 3 aliquots of the positive reference serum sample PT2014CSFSERPS3 (3x NEG instead of POS).

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

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Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum samples (SAMPLE), the positions of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014CSFSERNS2	NEG	NEG	1
2	1	2	PT2014CSFSERPS1	POS	POS	1
3	1	3	PT2014CSFSERNS1	NEG	NEG	1
4	1	4	PT2014CSFSERPS2	POS	POS	1
5	1	5	PT2014CSFSERNS3	NEG	NEG	1
6	1	6	PT2014CSFSERPS3	POS	POS	1
7	1	7	PT2014CSFSERNS2	NEG	NEG	1
8	1	8	PT2014CSFSERPS2	POS	POS	1
9	1	9	PT2014CSFSERNS1	NEG	NEG	1
10	1	10	PT2014CSFSERPS2	POS	POS	1
11	1	11	PT2014CSFSERNS3	NEG	NEG	1
12	1	12	PT2014CSFSERPS3	POS	POS	1
13	1	13	PT2014CSFSERNS1	NEG	NEG	1
14	1	14	PT2014CSFSERPS1	POS	POS	1
15	1	15	PT2014CSFSERPS2	POS	POS	1
16	1	16	PT2014CSFSERNS2	NEG	NEG	1
17	1	17	PT2014CSFSERPS1	POS	POS	1
18	1	18	PT2014CSFSERPS3	POS	POS	1
19	1	19	PT2014CSFSERNS3	NEG	NEG	1
20	1	20	PT2014CSFSERPS1	POS	POS	1
21	2	1	PT2014CSFSERPS2	POS	POS	1
22	2	2	PT2014CSFSERNS3	NEG	NEG	1
23	2	3	PT2014CSFSERPS3	**POS**	**NEG**	**0**
24	2	4	PT2014CSFSERNS2	NEG	NEG	1
25	2	5	PT2014CSFSERPS2	POS	POS	1
26	2	6	PT2014CSFSERNS1	NEG	NEG	1
27	2	7	PT2014CSFSERPS2	POS	POS	1
28	2	8	PT2014CSFSERNS3	NEG	NEG	1
29	2	9	PT2014CSFSERPS3	**POS**	**NEG**	**0**
30	2	10	PT2014CSFSERNS1	NEG	NEG	1
31	2	11	PT2014CSFSERPS1	POS	POS	1
32	2	12	PT2014CSFSERPS2	POS	POS	1
33	2	13	PT2014CSFSERNS2	NEG	NEG	1
34	2	14	PT2014CSFSERPS1	POS	POS	1
35	2	15	PT2014CSFSERPS3	**POS**	**NEG**	**0**
36	2	16	PT2014CSFSERNS3	NEG	NEG	1
37	2	17	PT2014CSFSERPS1	POS	POS	1
38	2	18	PT2014CSFSERNS2	NEG	NEG	1
39	2	19	PT2014CSFSERPS1	POS	POS	1
40	2	20	PT2014CSFSERNS1	NEG	NEG	1

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(Table 3 - continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2014CSFSERNS2	NEG	NEG	1
42	3	2	PT2014CSFSERPS1	POS	POS	1
43	3	3	PT2014CSFSERNS1	NEG	NEG	1
44	3	4	PT2014CSFSERPS2	POS	POS	1
45	3	5	PT2014CSFSERNS3	NEG	NEG	1
46	3	6	PT2014CSFSERPS3	POS	POS	1
47	3	7	PT2014CSFSERNS2	NEG	NEG	1
48	3	8	PT2014CSFSERPS2	POS	POS	1
49	3	9	PT2014CSFSERNS1	NEG	NEG	1
50	3	10	PT2014CSFSERPS2	POS	POS	1
51	3	11	PT2014CSFSERNS3	NEG	NEG	1
52	3	12	PT2014CSFSERPS3	POS	POS	1
53	3	13	PT2014CSFSERNS1	NEG	NEG	1
54	3	14	PT2014CSFSERPS1	POS	POS	1
55	3	15	PT2014CSFSERPS2	POS	POS	1
56	3	16	PT2014CSFSERNS2	NEG	NEG	1
57	3	17	PT2014CSFSERPS1	POS	POS	1
58	3	18	PT2014CSFSERPS3	POS	POS	1
59	3	19	PT2014CSFSERNS3	NEG	NEG	1
60	3	20	PT2014CSFSERPS1	POS	POS	1
61	4	1	PT2014CSFSERPS2	POS	POS	1
62	4	2	PT2014CSFSERNS3	NEG	NEG	1
63	4	3	PT2014CSFSERPS3	POS	POS	1
64	4	4	PT2014CSFSERNS2	NEG	NEG	1
65	4	5	PT2014CSFSERPS2	POS	POS	1
66	4	6	PT2014CSFSERNS1	NEG	NEG	1
67	4	7	PT2014CSFSERPS2	POS	POS	1
68	4	8	PT2014CSFSERNS3	NEG	NEG	1
69	4	9	PT2014CSFSERPS3	POS	POS	1
70	4	10	PT2014CSFSERNS1	NEG	NEG	1
71	4	11	PT2014CSFSERPS1	POS	POS	1
72	4	12	PT2014CSFSERPS2	POS	POS	1
73	4	13	PT2014CSFSERNS2	NEG	NEG	1
74	4	14	PT2014CSFSERPS1	POS	POS	1
75	4	15	PT2014CSFSERPS3	POS	POS	1
76	4	16	PT2014CSFSERNS3	NEG	NEG	1
77	4	17	PT2014CSFSERPS1	POS	POS	1
78	4	18	PT2014CSFSERNS2	NEG	NEG	1
79	4	19	PT2014CSFSERPS1	POS	POS	1
80	4	20	PT2014CSFSERNS1	NEG	NEG	1

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V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of porcine origin for the detection of CSF-specific antibodies by ELISA.

For the detection of CSF-specific antibodies in reference serum samples, 3 out of 4 participating laboratories (LAB1, LAB3 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement), whereas LAB2 misclassified all 3 aliquots of the positive reference serum sample PT2014CSFSERPS3 (85% of agreement) (Table 2 and Table 3).

All participating laboratories used the same CSF antibody ELISA kit from IDEXX but 2 different batches were used: B781 and B981. Hereby, LAB1, LAB3 and LAB4 used the same batch. In addition, all participants performed the long incubation protocol.

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 samples assigned by CODA-CERVA (see III.3.3.). Consequently, 3 out of 4 participants achieved a satisfactory performance for the detection of CSF-specific antibodies in reference serum samples of porcine origin by ELISA. Hereby, LAB2 did not reach the required 90% of agreement.

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) (Loncin, 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)

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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) and SAS 9.2. (summary statistics). All quantitative data analyses were performed on the normalized data, namely the percentages blocking calculated according to the instructions for this PT: [1- (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 blocking per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

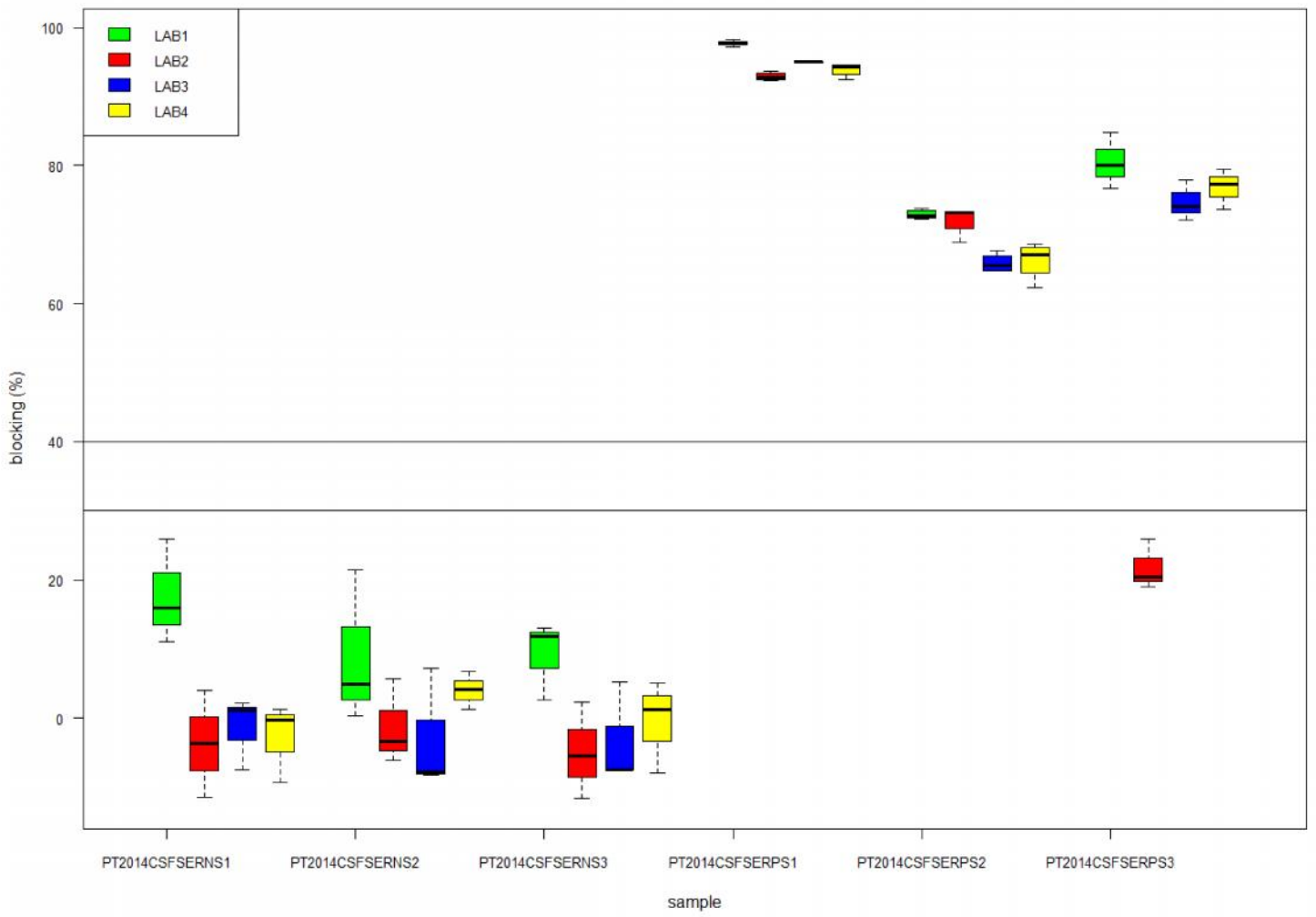

Figure 1. Box plots showing the percentage blocking per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in black (30-40%). All participating laboratories performed the same incubation protocol of the same CSF antibody ELISA kit (LAB1, LAB3 and LAB4 used the same batch).

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II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-laboratory variability (reproducibility) and within-laboratory 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 the normalized OD values per reference 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 1,42 (p=4), whereas the maximum value for Mandel's k-statistic is 1,59 for the reference samples PT2014CSFSERNS1, PT2014CSFSERNS2, PT2014CSFSERNS3 and PT2014CSFSERPS3 (p=4 and n=3) and 1,50 for the reference samples PT2014CSFSERPS1 and PT2014CSFSERPS2 (p=4 and n=4).

LAB3 and LAB4 obtained a satisfactory between-laboratory consistency for all reference serum samples. In contrast, the other participants showed an increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB1 for the reference serum samples PT2014CSFSERNS1 (h=1,49) and PT2014CSFSERNS3 (h=1,44), and LAB2 for the positive reference serum sample PT2014CSFSERPS3 (h=-1,49). All participating laboratories performed the same incubation protocol of the same CSF antibody ELISA kit. In addition, LAB1, LAB3 and LAB4 used the same batch.

Furthermore, 3 out of 4 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB4, which showed an increased value for Mandel's k-statistics for the positive reference serum sample PT2014CSFSERPS1 (k=1,61).

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 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 samples were analysed together), status level (all reference samples with the same status were analysed together) and sample level (all reference samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits,

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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 between laboratories were observed for the negative, but not for the positive reference serum samples. For the negative reference serum samples, LAB1 reported percentages blocking that were significantly higher than those reported by the other participants.

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

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2014CSFSERNS1	**1**	3	56,90	17,69	2,47	0,40	6,68	8,62	5,44	**1,49**	1,13	42,64
PT2014CSFSERNS1	2	3	60,67	-3,64	2,47	0,40	6,68	8,62	5,44	-0,60	1,17	-213,71
PT2014CSFSERNS1	3	3	28,04	-1,38	2,47	0,40	6,68	8,62	5,44	-0,38	0,79	-383,56
PT2014CSFSERNS1	4	3	32,89	-2,78	2,47	0,40	6,68	8,62	5,44	-0,52	0,86	-206,21
PT2014CSFSERNS2	1	3	122,78	8,97	2,21	0,04	7,83	8,01	1,70	1,25	1,41	123,59
PT2014CSFSERNS2	2	3	38,47	-1,24	2,21	0,04	7,83	8,01	1,70	-0,64	0,79	-499,29
PT2014CSFSERNS2	3	3	76,69	-2,93	2,21	0,04	7,83	8,01	1,70	-0,95	1,12	-298,47
PT2014CSFSERNS2	4	3	7,45	4,04	2,21	0,04	7,83	8,01	1,70	0,34	0,35	67,63
PT2014CSFSERNS3	**1**	3	31,96	9,20	0,15	0,16	6,70	7,29	2,87	**1,44**	0,84	61,46
PT2014CSFSERNS3	2	3	48,78	-4,89	0,15	0,16	6,70	7,29	2,87	-0,80	1,04	-142,90
PT2014CSFSERNS3	3	3	54,26	-3,25	0,15	0,16	6,70	7,29	2,87	-0,54	1,10	-226,35
PT2014CSFSERNS3	4	3	44,70	-0,47	0,15	0,16	6,70	7,29	2,87	-0,10	1,00	-1409,56
PT2014CSFSERPS1	1	4	0,17	97,77	94,91	0,78	0,64	1,36	1,19	1,37	0,65	0,43
PT2014CSFSERPS1	2	4	0,39	92,93	94,91	0,78	0,64	1,36	1,19	-0,95	0,98	0,67
PT2014CSFSERPS1	3	4	0,01	95,04	94,91	0,78	0,64	1,36	1,19	0,06	0,16	0,11
PT2014CSFSERPS1	**4**	4	1,06	93,91	94,91	0,78	0,64	1,36	1,19	-0,48	**1,61**	1,10
PT2014CSFSERPS2	1	4	0,48	72,90	69,30	0,54	1,92	2,84	2,09	0,96	0,36	0,95
PT2014CSFSERPS2	2	4	4,85	72,14	69,30	0,54	1,92	2,84	2,09	0,76	1,15	3,05
PT2014CSFSERPS2	3	4	1,87	65,90	69,30	0,54	1,92	2,84	2,09	-0,91	0,71	2,08
PT2014CSFSERPS2	4	4	7,56	66,24	69,30	0,54	1,92	2,84	2,09	-0,82	1,43	4,15
PT2014CSFSERPS3	1	3	16,81	80,50	63,45	0,96	3,43	16,42	16,06	0,61	1,20	5,09
PT2014CSFSERPS3	**2**	3	12,89	21,77	63,45	0,96	3,43	16,42	16,06	**-1,49**	1,05	16,49
PT2014CSFSERPS3	3	3	8,63	74,75	63,45	0,96	3,43	16,42	16,06	0,41	0,86	3,93
PT2014CSFSERPS3	4	3	8,70	76,78	63,45	0,96	3,43	16,42	16,06	0,48	0,86	3,84

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalized data (% blocking); **x_i_m** = mean of normalized data (% blocking); **x_g_m** = mean of normalized data (% blocking) 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).

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