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BELAC
172-TEST

PROFICIENCY TESTING 2011

Q Fever (QFV)

Detection of antibodies in serum and milk by

Enzyme Linked Immunosorbent Assay (ELISA)

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 24 JANUARY 2011

DATE REPORT: 25 FEBRUARY 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

This proficiency test focuses on the detection of QFV-specific antibodies in serum and/or milk by ELISA and aims to assess the analytical accuracy of the QFV antibody ELISA tests conducted by the participants.

III. Materials and methods

III.1. Conduct of diagnostic tests

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

III.2. Reference samples

III.2.1. Reference serum samples

Replicates of two reference serum samples either free from detectable QFV-specific antibodies (n = 1; coded 'PT2011QFVSERNS1') or containing detectable QFV-specific antibodies (n = 1; coded 'PT2011QFVSERPS1') were used. In total, 90 aliquots, prepared by the reference laboratory for QFV of the Veterinary and Agrochemical Research Center (CODA-CERVA), were distributed to the participating laboratories. All participating laboratories were given 15 aliquots of the reference serum sample free from detectable QFV-specific antibodies (PT2011QFVSERNS1; n = 15) and 15 aliquots of the reference serum sample containing detectable QFV-specific antibodies (PT2011QFVSERPS1; n = 15). The position of the reference serum samples in each of the sent blocks was randomized for each participant (Table 4).

For each reference serum sample, a certificate containing the assigned value was made by the reference laboratory for QFV of CODA-CERVA (status of the sample = 'golden standard'). The assigned value was obtained by testing each reference serum sample at least 10 times, hereby obtaining each time the same qualitative result. Consequently, these reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of QFV-specific antibodies in bovine serum. All reference serum samples were also tested once after the proficiency test in order to confirm their stability and status (post-verification).

III.2.2. Reference milk samples

Replicates of two reference milk samples either free from detectable QFV-specific antibodies (n = 1; coded 'PT2011QFVSERNM1') or containing detectable QFV-specific antibodies (n = 1; coded 'PT2011QFVSERPM1') were used. In total, 90 aliquots, prepared by the reference laboratory for QFV of CODA-CERVA, were distributed to the participating laboratories. All participating laboratories were given 15 aliquots of the reference milk sample free from detectable QFV-specific antibodies (PT2011QFVSERNM1; n = 15) and 15 aliquots of the reference milk sample containing detectable QFV-specific antibodies (PT2011QFVSERPM1; n = 15). The position of the reference milk samples in each of the sent blocks was randomized for each participant (Table 5).

For each reference milk sample, a certificate containing the assigned value was made by the reference laboratory for QFV of CODA-CERVA (status of the sample = 'golden standard'). The assigned value was obtained by testing each reference milk sample at least 10 times, hereby obtaining each time the same qualitative result. Consequently, these reference milk samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the

absence or presence of QFV-specific antibodies in goat milk. All reference milk 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 of success for all 30 reference serum samples (aliquots) and/or for all 30 reference milk samples (aliquots).

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for all reference serum samples and/or reference milk 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 the CODA-CERVA.

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

The 60 aliquots for LAB1 and LAB2 (30 serum and 30 milk samples) and the 30 aliquots for LAB3 and LAB4 (either serum or milk samples) were sent to the corresponding participating laboratory on 24th of January 2011 (180 aliquots in total). The laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 25th (LAB1), 27th (LAB2), and 28th (LAB3) of January 2011. LAB4 did not communicate the date of analysis.

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

Results from the participating laboratories have been received on 28th of January (LAB2, LAB3, and LAB4) and 8th of February (LAB1) 2011. LAB1 hereby exceeded the deadline of 28th of January 2011 for the delivery of the results.

IV.3. Compliance with the procedure

Three participating laboratories (LAB2, LAB3, and LAB4) 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 QFV-specific antibodies in reference serum (Table 1) and/or reference milk (Table 2) samples.

A quantitative data analysis (including boxplot) 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 serum samples assigned by the QFV reference laboratory of CODA-CERVA. All participating laboratories received 30 reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	30 (100.0)	30 (100.0)	30 (100.0)

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the QFV reference laboratory of CODA-CERVA. All participating laboratories received 30 reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**4**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	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 QFV-specific antibodies in both reference serum and reference milk samples, no variability between laboratories could be observed at the qualitative data level. Per participating laboratory, the obtained responses are given in Table 3 for the reference serum samples and in Table 4 for the reference milk samples.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum samples (SAMPLE), the position of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by the QFV reference laboratory of the CODA-CERVA (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2011QFVSERNS1	NEG	NEG	1
2	1	2	PT2011QFVSERPS1	POS	POS	1
3	1	3	PT2011QFVSERPS1	POS	POS	1
4	1	4	PT2011QFVSERNS1	NEG	NEG	1
5	1	5	PT2011QFVSERPS1	POS	POS	1
6	1	6	PT2011QFVSERNS1	NEG	NEG	1
7	1	7	PT2011QFVSERPS1	POS	POS	1
8	1	8	PT2011QFVSERNS1	NEG	NEG	1
9	1	9	PT2011QFVSERPS1	POS	POS	1
10	1	10	PT2011QFVSERNS1	NEG	NEG	1
11	1	11	PT2011QFVSERNS1	NEG	NEG	1
12	1	12	PT2011QFVSERPS1	POS	POS	1
13	1	13	PT2011QFVSERNS1	NEG	NEG	1
14	1	14	PT2011QFVSERNS1	NEG	NEG	1
15	1	15	PT2011QFVSERPS1	POS	POS	1
16	1	16	PT2011QFVSERNS1	NEG	NEG	1
17	1	17	PT2011QFVSERNS1	NEG	NEG	1
18	1	18	PT2011QFVSERPS1	POS	POS	1
19	1	19	PT2011QFVSERPS1	POS	POS	1
20	1	20	PT2011QFVSERPS1	POS	POS	1
21	1	21	PT2011QFVSERNS1	NEG	NEG	1
22	1	22	PT2011QFVSERPS1	POS	POS	1
23	1	23	PT2011QFVSERPS1	POS	POS	1
24	1	24	PT2011QFVSERPS1	POS	POS	1
25	1	25	PT2011QFVSERPS1	POS	POS	1
26	1	26	PT2011QFVSERNS1	NEG	NEG	1
27	1	27	PT2011QFVSERNS1	NEG	NEG	1
28	1	28	PT2011QFVSERNS1	NEG	NEG	1
29	1	29	PT2011QFVSERNS1	NEG	NEG	1
30	1	30	PT2011QFVSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2011QFVSERNS1	NEG	NEG	1
32	2	2	PT2011QFVSERNS1	NEG	NEG	1
33	2	3	PT2011QFVSERNS1	NEG	NEG	1
34	2	4	PT2011QFVSERNS1	NEG	NEG	1
35	2	5	PT2011QFVSERPS1	POS	POS	1
36	2	6	PT2011QFVSERNS1	NEG	NEG	1
37	2	7	PT2011QFVSERPS1	POS	POS	1
38	2	8	PT2011QFVSERPS1	POS	POS	1
39	2	9	PT2011QFVSERNS1	NEG	NEG	1
40	2	10	PT2011QFVSERPS1	POS	POS	1
41	2	11	PT2011QFVSERNS1	NEG	NEG	1
42	2	12	PT2011QFVSERPS1	POS	POS	1
43	2	13	PT2011QFVSERNS1	NEG	NEG	1
44	2	14	PT2011QFVSERPS1	POS	POS	1
45	2	15	PT2011QFVSERNS1	NEG	NEG	1
46	2	16	PT2011QFVSERNS1	NEG	NEG	1
47	2	17	PT2011QFVSERPS1	POS	POS	1
48	2	18	PT2011QFVSERNS1	NEG	NEG	1
49	2	19	PT2011QFVSERNS1	NEG	NEG	1
50	2	20	PT2011QFVSERPS1	POS	POS	1
51	2	21	PT2011QFVSERNS1	NEG	NEG	1
52	2	22	PT2011QFVSERNS1	NEG	NEG	1
53	2	23	PT2011QFVSERPS1	POS	POS	1
54	2	24	PT2011QFVSERPS1	POS	POS	1
55	2	25	PT2011QFVSERPS1	POS	POS	1
56	2	26	PT2011QFVSERNS1	NEG	NEG	1
57	2	27	PT2011QFVSERPS1	POS	POS	1
58	2	28	PT2011QFVSERPS1	POS	POS	1
59	2	29	PT2011QFVSERPS1	POS	POS	1
60	2	30	PT2011QFVSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	PT2011QFVSERNS1	NEG	NEG	1
62	3	2	PT2011QFVSERPS1	POS	POS	1
63	3	3	PT2011QFVSERPS1	POS	POS	1
64	3	4	PT2011QFVSERPS1	POS	POS	1
65	3	5	PT2011QFVSERPS1	POS	POS	1
66	3	6	PT2011QFVSERNS1	NEG	NEG	1
67	3	7	PT2011QFVSERNS1	NEG	NEG	1
68	3	8	PT2011QFVSERNS1	NEG	NEG	1
69	3	9	PT2011QFVSERNS1	NEG	NEG	1
70	3	10	PT2011QFVSERPS1	POS	POS	1
71	3	11	PT2011QFVSERNS1	NEG	NEG	1
72	3	12	PT2011QFVSERPS1	POS	POS	1
73	3	13	PT2011QFVSERPS1	POS	POS	1
74	3	14	PT2011QFVSERNS1	NEG	NEG	1
75	3	15	PT2011QFVSERPS1	POS	POS	1
76	3	16	PT2011QFVSERNS1	NEG	NEG	1
77	3	17	PT2011QFVSERPS1	POS	POS	1
78	3	18	PT2011QFVSERNS1	NEG	NEG	1
79	3	19	PT2011QFVSERPS1	POS	POS	1
80	3	20	PT2011QFVSERNS1	NEG	NEG	1
81	3	21	PT2011QFVSERNS1	NEG	NEG	1
82	3	22	PT2011QFVSERPS1	POS	POS	1
83	3	23	PT2011QFVSERNS1	NEG	NEG	1
84	3	24	PT2011QFVSERNS1	NEG	NEG	1
85	3	25	PT2011QFVSERPS1	POS	POS	1
86	3	26	PT2011QFVSERNS1	NEG	NEG	1
87	3	27	PT2011QFVSERNS1	NEG	NEG	1
88	3	28	PT2011QFVSERPS1	POS	POS	1
89	3	29	PT2011QFVSERPS1	POS	POS	1
90	3	30	PT2011QFVSERPS1	POS	POS	1

Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference milk samples (SAMPLE), the position of the reference milk samples as placed in the block (LABPOSIT), and the status assigned by the QFV reference laboratory of the CODA-CERVA (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2011QFVSERNM1	NEG	NEG	1
2	1	2	PT2011QFVSERPM1	POS	POS	1
3	1	3	PT2011QFVSERPM1	POS	POS	1
4	1	4	PT2011QFVSERNM1	NEG	NEG	1
5	1	5	PT2011QFVSERPM1	POS	POS	1
6	1	6	PT2011QFVSERNM1	NEG	NEG	1
7	1	7	PT2011QFVSERPM1	POS	POS	1
8	1	8	PT2011QFVSERNM1	NEG	NEG	1
9	1	9	PT2011QFVSERPM1	POS	POS	1
10	1	10	PT2011QFVSERNM1	NEG	NEG	1
11	1	11	PT2011QFVSERPM1	POS	POS	1
12	1	12	PT2011QFVSERNM1	NEG	NEG	1
13	1	13	PT2011QFVSERPM1	POS	POS	1
14	1	14	PT2011QFVSERNM1	NEG	NEG	1
15	1	15	PT2011QFVSERPM1	POS	POS	1
16	1	16	PT2011QFVSERNM1	NEG	NEG	1
17	1	17	PT2011QFVSERNM1	NEG	NEG	1
18	1	18	PT2011QFVSERPM1	POS	POS	1
19	1	19	PT2011QFVSERPM1	POS	POS	1
20	1	20	PT2011QFVSERNM1	NEG	NEG	1
21	1	21	PT2011QFVSERPM1	POS	POS	1
22	1	22	PT2011QFVSERPM1	POS	POS	1
23	1	23	PT2011QFVSERNM1	NEG	NEG	1
24	1	24	PT2011QFVSERNM1	NEG	NEG	1
25	1	25	PT2011QFVSERNM1	NEG	NEG	1
26	1	26	PT2011QFVSERPM1	POS	POS	1
27	1	27	PT2011QFVSERNM1	NEG	NEG	1
28	1	28	PT2011QFVSERPM1	POS	POS	1
29	1	29	PT2011QFVSERNM1	NEG	NEG	1
30	1	30	PT2011QFVSERPM1	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2011QFVSERPM1	POS	POS	1
32	2	2	PT2011QFVSERNM1	NEG	NEG	1
33	2	3	PT2011QFVSERPM1	POS	POS	1
34	2	4	PT2011QFVSERNM1	NEG	NEG	1
35	2	5	PT2011QFVSERPM1	POS	POS	1
36	2	6	PT2011QFVSERNM1	NEG	NEG	1
37	2	7	PT2011QFVSERPM1	POS	POS	1
38	2	8	PT2011QFVSERPM1	POS	POS	1
39	2	9	PT2011QFVSERNM1	NEG	NEG	1
40	2	10	PT2011QFVSERPM1	POS	POS	1
41	2	11	PT2011QFVSERNM1	NEG	NEG	1
42	2	12	PT2011QFVSERPM1	POS	POS	1
43	2	13	PT2011QFVSERNM1	NEG	NEG	1
44	2	14	PT2011QFVSERPM1	POS	POS	1
45	2	15	PT2011QFVSERNM1	NEG	NEG	1
46	2	16	PT2011QFVSERPM1	POS	POS	1
47	2	17	PT2011QFVSERNM1	NEG	NEG	1
48	2	18	PT2011QFVSERPM1	POS	POS	1
49	2	19	PT2011QFVSERNM1	NEG	NEG	1
50	2	20	PT2011QFVSERPM1	POS	POS	1
51	2	21	PT2011QFVSERNM1	NEG	NEG	1
52	2	22	PT2011QFVSERNM1	NEG	NEG	1
53	2	23	PT2011QFVSERPM1	POS	POS	1
54	2	24	PT2011QFVSERPM1	POS	POS	1
55	2	25	PT2011QFVSERNM1	NEG	NEG	1
56	2	26	PT2011QFVSERPM1	POS	POS	1
57	2	27	PT2011QFVSERPM1	POS	POS	1
58	2	28	PT2011QFVSERNM1	NEG	NEG	1
59	2	29	PT2011QFVSERNM1	NEG	NEG	1
60	2	30	PT2011QFVSERNM1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	PT2011QFVSERPM1	POS	POS	1
62	4	2	PT2011QFVSERPM1	POS	POS	1
63	4	3	PT2011QFVSERNM1	NEG	NEG	1
64	4	4	PT2011QFVSERNM1	NEG	NEG	1
65	4	5	PT2011QFVSERNM1	NEG	NEG	1
66	4	6	PT2011QFVSERPM1	POS	POS	1
67	4	7	PT2011QFVSERNM1	NEG	NEG	1
68	4	8	PT2011QFVSERPM1	POS	POS	1
69	4	9	PT2011QFVSERNM1	NEG	NEG	1
70	4	10	PT2011QFVSERPM1	POS	POS	1
71	4	11	PT2011QFVSERNM1	NEG	NEG	1
72	4	12	PT2011QFVSERPM1	POS	POS	1
73	4	13	PT2011QFVSERPM1	POS	POS	1
74	4	14	PT2011QFVSERNM1	NEG	NEG	1
75	4	15	PT2011QFVSERPM1	POS	POS	1
76	4	16	PT2011QFVSERNM1	NEG	NEG	1
77	4	17	PT2011QFVSERPM1	POS	POS	1
78	4	18	PT2011QFVSERNM1	NEG	NEG	1
79	4	19	PT2011QFVSERPM1	POS	POS	1
80	4	20	PT2011QFVSERNM1	NEG	NEG	1
81	4	21	PT2011QFVSERPM1	POS	POS	1
82	4	22	PT2011QFVSERNM1	NEG	NEG	1
83	4	23	PT2011QFVSERPM1	POS	POS	1
84	4	24	PT2011QFVSERNM1	NEG	NEG	1
85	4	25	PT2011QFVSERPM1	POS	POS	1
86	4	26	PT2011QFVSERNM1	NEG	NEG	1
87	4	27	PT2011QFVSERNM1	NEG	NEG	1
88	4	28	PT2011QFVSERPM1	POS	POS	1
89	4	29	PT2011QFVSERPM1	POS	POS	1
90	4	30	PT2011QFVSERNM1	NEG	NEG	1

V. Discussion

The purpose of this proficiency test was to assess performances of the participating laboratories when analyzing reference serum and/or milk samples of bovine or goat origin, respectively, for the detection of QFV-specific antibodies by ELISA.

For this proficiency test, the participating laboratories used different batches of QFV antibody ELISA kits from the same producer (LSI). For the detection of QFV-specific antibodies in reference serum samples, two different batches were used: batch 2-FQLS-017 was used by LAB3 and batch 2-FQLS-022 was used by LAB1 and LAB2. No differences in the qualitative results for the reference serum samples were observed between these three participating laboratories. For the detection of QFV-specific antibodies in the reference milk samples, all laboratories used LSI batch 2-FQLS-022.

Data obtained in this proficiency test showed that all participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum and/or milk samples, even when QFV antibody ELISA kits from different batches from the same producer were used.

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 and/or milk samples assigned by the reference laboratory for QFV of the CODA-CERVA (see III.3.3.). Since all participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum and/or milk samples, all participating laboratories achieved a satisfactory performance.

Head CVD-ERA

Yves Van der Stede

Appendix

Name of the participating Laboratories

ARSIA (Loncin)

CODA-CERVA

DGZ (Torhout)

MCC

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 % S/P (calculated according to the instructions) per reference serum or milk sample and per participating laboratory were made using the statistical software R, and are shown in Figure 1 and Figure 2, respectively.

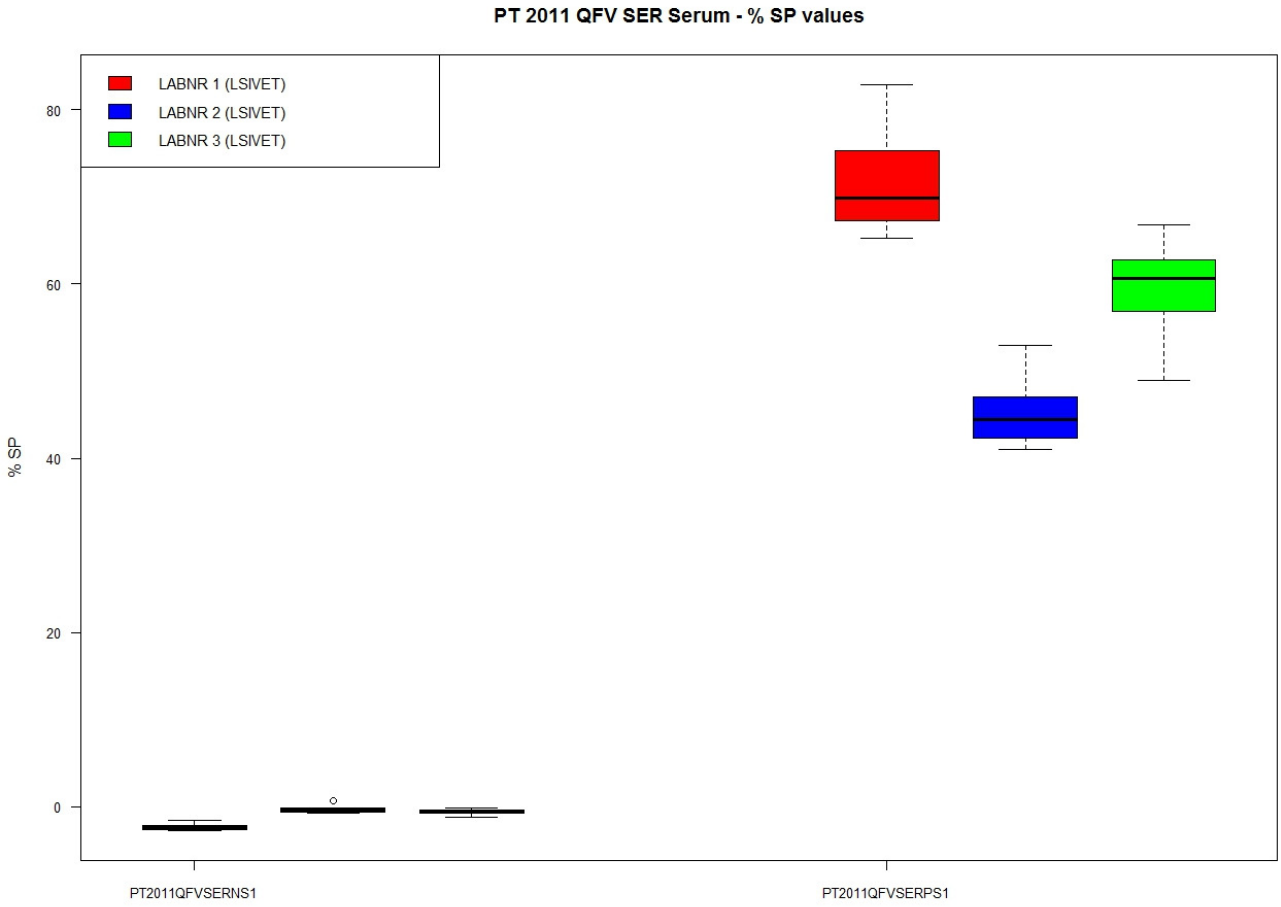

Figure 1. Box plots of the % S/P per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off value is 40%.

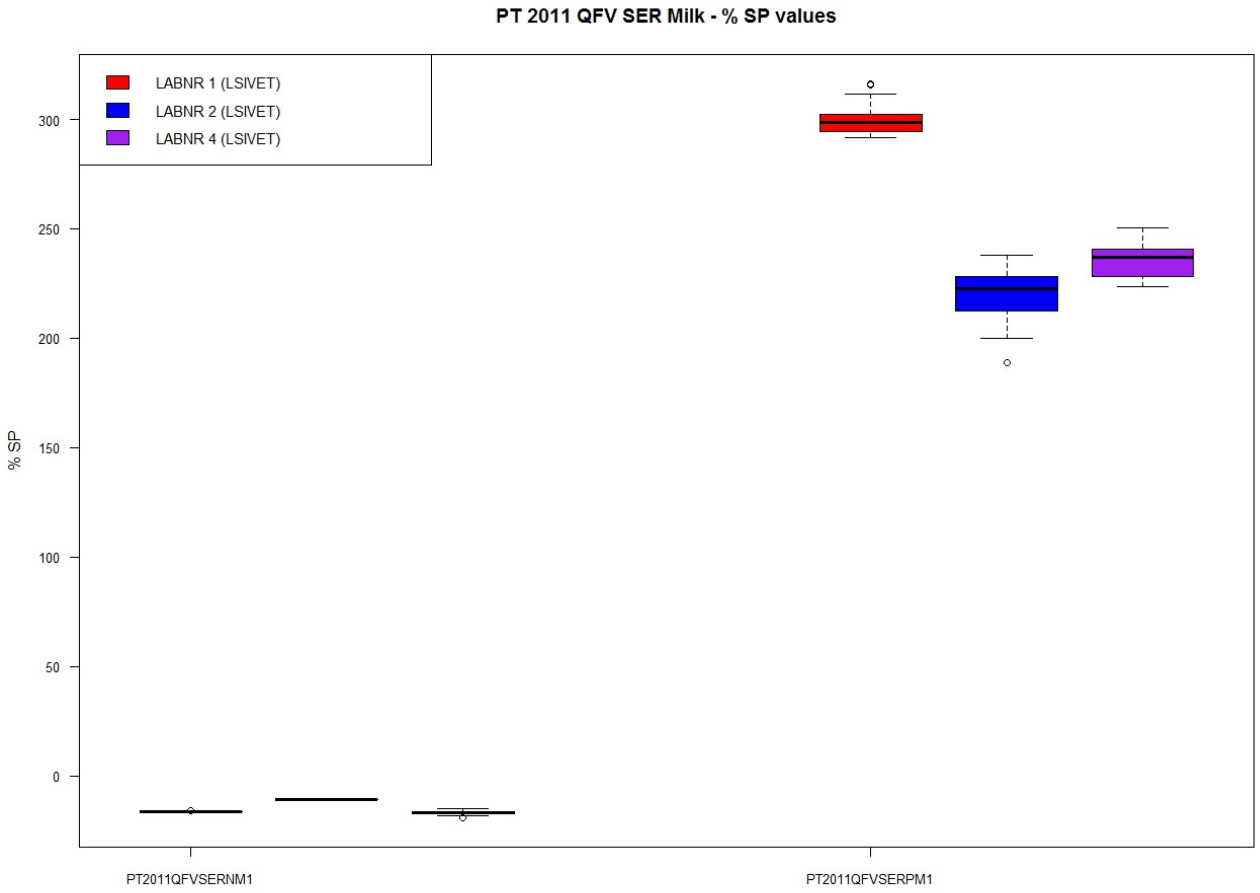

Figure 2. Box plots of the % S/P per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off value is 40%.

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.

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 often the case, other indicator values apply for Mandels h- and k-statistics (Table 1). 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.

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

For all reference serum and milk samples, a satisfactory between-lab consistency was observed for all three laboratories ($h \leq 1,15$ – based on Table 1) (Figure 3A and Figure 4A). For the reference serum samples, a satisfactory within-laboratory consistency was observed since $k < 1,29$ (based on Table 1) for all samples and for each laboratory (Figure 3B). In contrast, a questionable within-laboratory consistency was observed for sample PT2011QFVSERPM1 at LAB2 (borderline) and for sample PT2011QFVSERNM1 at LAB4 (Figure 4B). All data used for the calculations of Mandels h- and k-statistics can be found in Annex 2.

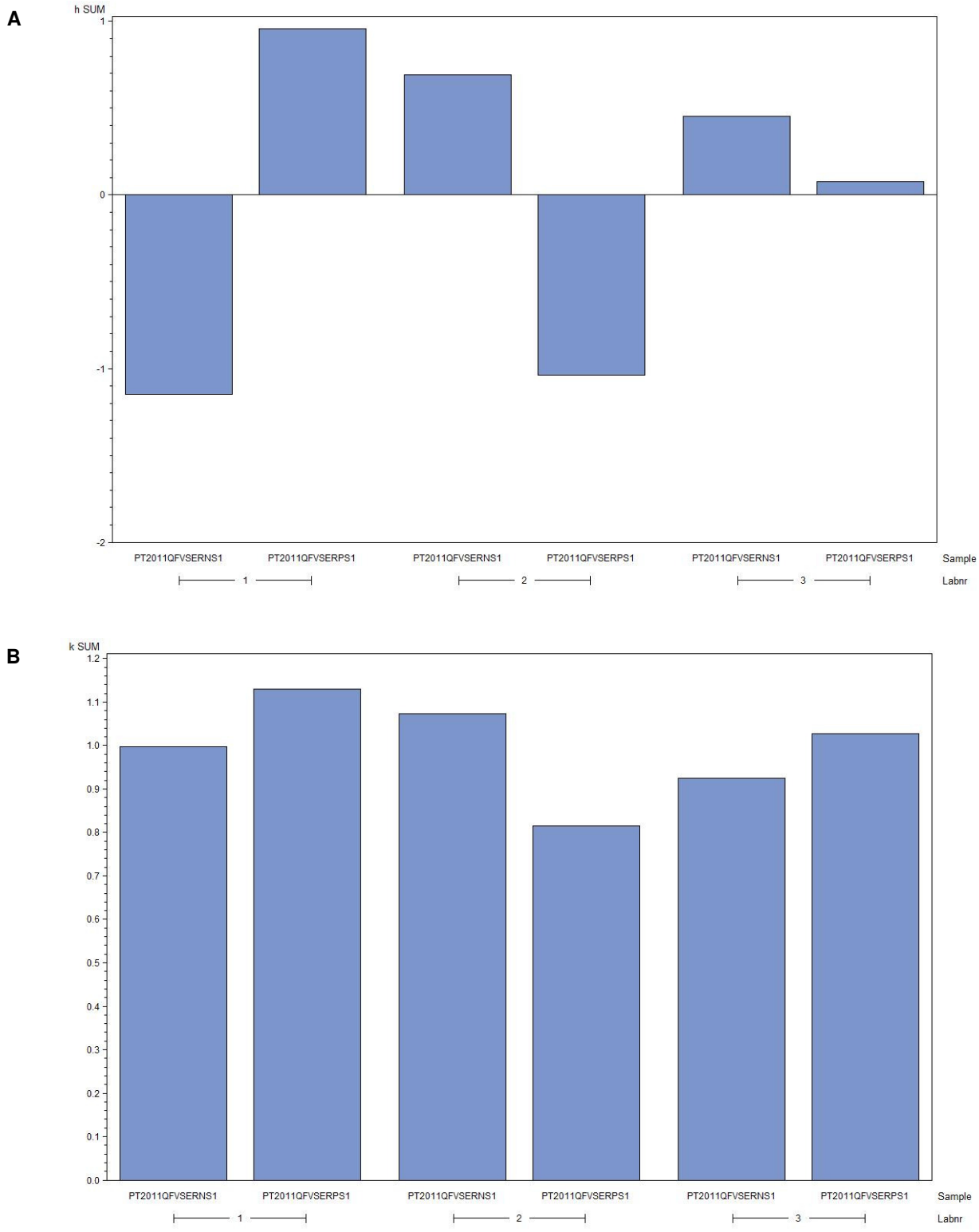

Figure 3. Graphs representing Mandels h- and k-statistics for the reference serum samples. (A) h-statistics give an indication about the between-laboratory consistency; (B) k-statistics give an indication about the within-laboratory consistency.

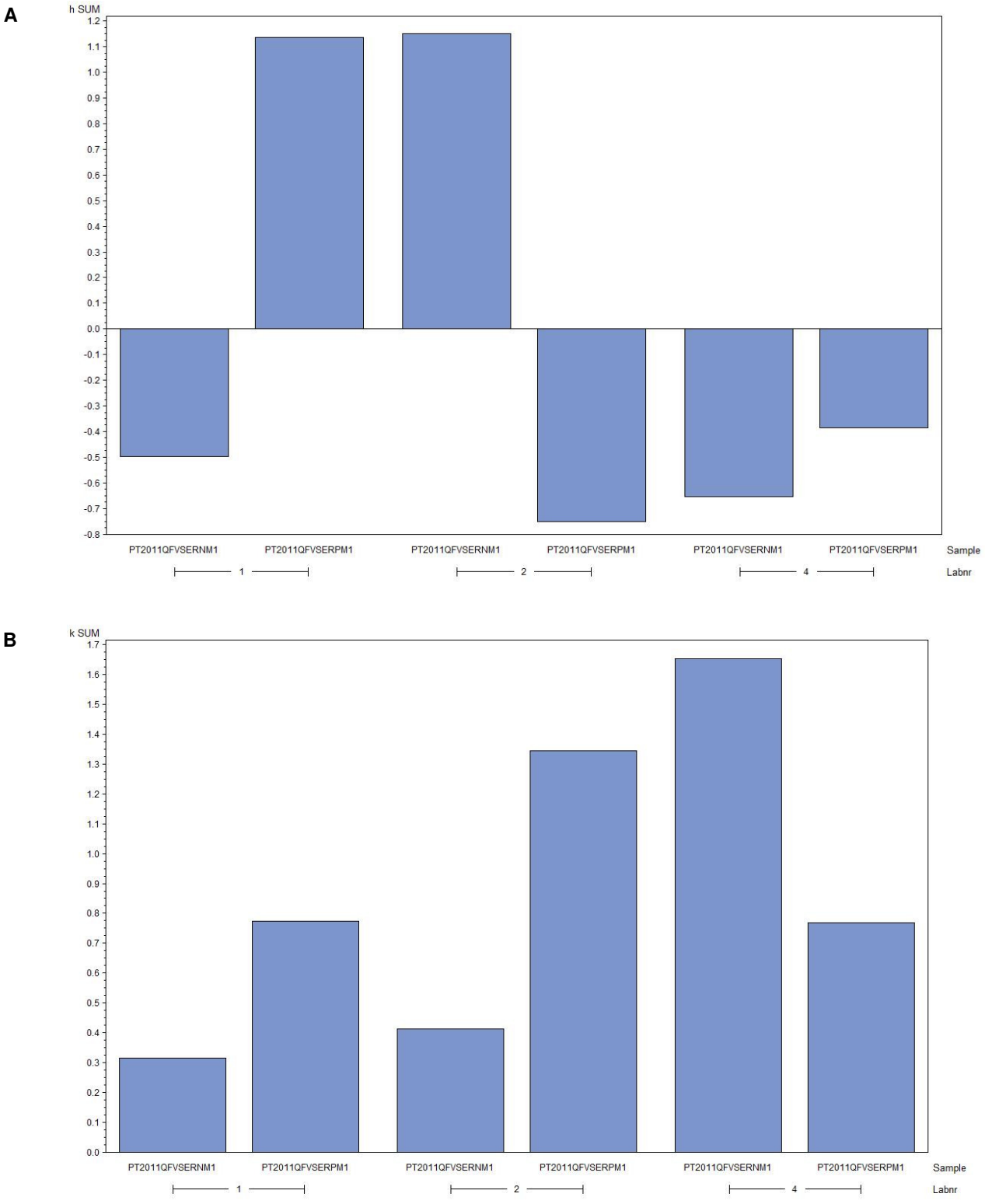

Figure 4. Graphs representing Mandels h- and k-statistics for the reference milk samples. (A) h-statistics give an indication about the between-laboratory consistency; (B) k-statistics give an indication about the within-laboratory consistency.

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 normalized OD values (in this case the percentage S/P calculated according to the instructions) as a dependent variable.

Taking all samples into account, no statistically significant differences were observed between laboratories.

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

Reference serum samples

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2011QFVSERNS1	1	15	0,11	-2,29	-1,06	0,83	0,34	0,83	0,75	-1,15	1,00	-14,76
PT2011QFVSERNS1	2	15	0,13	-0,32	-1,06	0,83	0,34	0,83	0,75	0,69	1,07	-113,41
PT2011QFVSERNS1	3	15	0,10	-0,58	-1,06	0,83	0,34	0,83	0,75	0,45	0,92	-54,12
PT2011QFVSERPS1	1	15	28,00	71,11	58,72	0,79	4,68	10,24	9,10	0,96	1,13	7,44
PT2011QFVSERPS1	2	15	14,58	45,31	58,72	0,79	4,68	10,24	9,10	-1,04	0,82	8,43
PT2011QFVSERPS1	3	15	23,16	59,74	58,72	0,79	4,68	10,24	9,10	0,08	1,03	8,05

Reference milk samples

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2011QFVSERNM1	1	15	0,04	-16,32	-14,63	0,94	0,60	2,47	2,39	-0,50	0,31	-1,15
PT2011QFVSERNM1	2	15	0,06	-10,73	-14,63	0,94	0,60	2,47	2,39	1,15	0,41	-2,30
PT2011QFVSERNM1	4	15	0,98	-16,84	-14,63	0,94	0,60	2,47	2,39	-0,65	1,65	-5,87
PT2011QFVSERPM1	1	15	64,98	300,53	252,08	0,89	10,43	31,87	30,11	1,14	0,77	2,68
PT2011QFVSERPM1	2	15	197,10	220,06	252,08	0,89	10,43	31,87	30,11	-0,75	1,35	6,38
PT2011QFVSERPM1	4	15	64,40	235,67	252,08	0,89	10,43	31,87	30,11	-0,38	0,77	3,41

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 %