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172-PT

PROFICIENCY TESTING 2012

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: 16 APRIL 2012

DATE REPORT: 28 JUNE 2012

I. Introduction

Details relevant to the proficiency test are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle'.

II. Aim

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 5 reference serum samples either free from detectable QFV-specific antibodies (n = 2; coded 'PT2012QFVSERNS1' and 'PT2012QFVSERNS2') or containing detectable QFV-specific antibodies (n = 3; coded 'PT2012QFVSERPS1', 'PT2012QFVSERPS2' and 'PT2012QFVSERPS3') were used. In total, 60 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 4 aliquots of each of the 5 reference serum samples (i.e., 20 aliquots per participant). The positions of the reference serum samples in each of the sent blocks were randomized for each participant (Table 3).

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 before the proficiency test (status and homogeneity of the samples), 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 addition, 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 5 reference milk samples either free from detectable QFV-specific antibodies (n = 2; coded 'PT2012QFVSERNM1 and 'PT2012QFVSERNM2') or containing detectable QFV-specific antibodies (n = 3; coded 'PT2012QFVSERPM1', 'PT2012QFVSERPM2' and 'PT2012QFVSERPM3') were used. In total, 60 aliquots, prepared by the reference laboratory for QFV of CODA-CERVA, were distributed to the participating laboratories. All participating laboratories were given 4 aliquots of each of the 5 reference milk samples (i.e., 20 aliquots per participant). The positions of the reference milk samples in each of the sent blocks were randomized for each participant (Table 4).

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 before the proficiency test (status and homogeneity of the samples), 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 addition, all reference milk samples were also tested once after the proficiency test in order to confirm their stability and status (post-verification).

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as success (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or failure (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or 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 20 reference serum samples (aliquots) and/or for all 20 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 40 aliquots for LAB 1 and LAB 3 (20 serum and 20 milk samples), the 20 aliquots for LAB 2 (20 serum samples) and the 20 aliquots for LAB 4 (milk samples) were sent to the corresponding participating laboratory on 16th of April 2012 (120 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 17th (LAB 1, LAB 3) and 23rd (LAB 2) of April 2012. LAB 4 did not communicate the date of analysis.
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IV.2. Dates at which results were returned to the CVD-ERA

Results from the participating laboratories have been received on 18th (LAB 3), 23rd (LAB 4), 26th (LAB 2) of April 2012 and the 3rd of May 2012 (LAB 1). LAB 1 hereby exceeded the deadline of 27th of April 2012 for the delivery of the results.

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 2 out of 3 laboratories (LAB 1 and LAB 2) reached 100% of agreement for the detection of QFV-specific antibodies in reference serum samples (Table 1). LAB 3 reached only 40% of agreement for the detection of QFV-specific antibodies in reference serum samples (Table 1). All participating laboratories reached 100% of agreement for the detection of QFV-specific antibodies in reference milk samples (Table 2).

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

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 20 reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0)	0 (0)	12 (60)
success	20 (100)	20 (100)	8 (40)

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 20 reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**3**	**4**
failure	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)

IV.4.2 Variability among participating laboratories

Since all participants reached 100% of agreement for the detection of QFV-specific antibodies in reference milk samples no variability between laboratories could be observed at the qualitative data level.

For the detection of QFV-specific antibodies in reference serum samples LAB 3 misclassified each aliquot of the sample PT2012QFVSERNS1 as positive while negative results were reported for each aliquot of samples PT2012QFVSERPS2 and PT2012QFVSERPS3.

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	PT2012QFVSERPS1	POS	POS	1
2	1	2	PT2012QFVSERPS3	POS	POS	1
3	1	3	PT2012QFVSERPS3	POS	POS	1
4	1	4	PT2012QFVSERNS1	NEG	NEG	1
5	1	5	PT2012QFVSERPS2	POS	POS	1
6	1	6	PT2012QFVSERNS1	NEG	NEG	1
7	1	7	PT2012QFVSERNS2	NEG	NEG	1
8	1	8	PT2012QFVSERNS1	NEG	NEG	1
9	1	9	PT2012QFVSERPS2	POS	POS	1
10	1	10	PT2012QFVSERNS1	NEG	NEG	1
11	1	11	PT2012QFVSERPS3	POS	POS	1
12	1	12	PT2012QFVSERPS2	POS	POS	1
13	1	13	PT2012QFVSERPS1	POS	POS	1
14	1	14	PT2012QFVSERPS1	POS	POS	1
15	1	15	PT2012QFVSERPS2	POS	POS	1
16	1	16	PT2012QFVSERNS2	NEG	NEG	1
17	1	17	PT2012QFVSERNS2	NEG	NEG	1
18	1	18	PT2012QFVSERNS2	NEG	NEG	1
19	1	19	PT2012QFVSERPS3	POS	POS	1
20	1	20	PT2012QFVSERPS1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	PT2012QFVSERNS2	NEG	NEG	1
22	2	2	PT2012QFVSERPS3	POS	POS	1
23	2	3	PT2012QFVSERPS1	POS	POS	1
24	2	4	PT2012QFVSERPS1	POS	POS	1
25	2	5	PT2012QFVSERPS3	POS	POS	1
26	2	6	PT2012QFVSERPS3	POS	POS	1
27	2	7	PT2012QFVSERNS1	NEG	NEG	1
28	2	8	PT2012QFVSERPS2	POS	POS	1
29	2	9	PT2012QFVSERNS1	NEG	NEG	1
30	2	10	PT2012QFVSERNS2	NEG	NEG	1
31	2	11	PT2012QFVSERNS1	NEG	NEG	1
32	2	12	PT2012QFVSERPS2	POS	POS	1
33	2	13	PT2012QFVSERNS1	NEG	NEG	1
34	2	14	PT2012QFVSERPS3	POS	POS	1
35	2	15	PT2012QFVSERPS2	POS	POS	1
36	2	16	PT2012QFVSERPS1	POS	POS	1
37	2	17	PT2012QFVSERPS1	POS	POS	1
38	2	18	PT2012QFVSERPS2	POS	POS	1
39	2	19	PT2012QFVSERNS2	NEG	NEG	1
40	2	20	PT2012QFVSERNS2	NEG	NEG	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	**PT2012QFVSERPS2**	**POS**	**NEG**	**0**
42	3	2	PT2012QFVSERNS2	NEG	NEG	1
43	3	3	PT2012QFVSERNS2	NEG	NEG	1
44	3	4	PT2012QFVSERNS2	NEG	NEG	1
45	3	5	**PT2012QFVSERPS3**	**POS**	**NEG**	**0**
46	3	6	PT2012QFVSERPS1	POS	POS	1
47	3	7	PT2012QFVSERPS1	POS	POS	1
48	3	8	**PT2012QFVSERPS3**	**POS**	**NEG**	**0**
49	3	9	**PT2012QFVSERPS3**	**POS**	**NEG**	**0**
50	3	10	**PT2012QFVSERNS1**	**NEG**	**POS**	**0**
51	3	11	**PT2012QFVSERPS2**	**POS**	**NEG**	**0**
52	3	12	**PT2012QFVSERNS1**	**NEG**	**POS**	**0**
53	3	13	PT2012QFVSERNS2	NEG	NEG	1
54	3	14	**PT2012QFVSERNS1**	**NEG**	**POS**	**0**
55	3	15	**PT2012QFVSERPS2**	**POS**	**NEG**	**0**
56	3	16	**PT2012QFVSERNS1**	**NEG**	**POS**	**0**
57	3	17	**PT2012QFVSERPS3**	**POS**	**NEG**	**0**
58	3	18	**PT2012QFVSERPS2**	**POS**	**NEG**	**0**
59	3	19	PT2012QFVSERPS1	POS	POS	1
60	3	20	PT2012QFVSERPS1	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	PT2012QFVSERNM2	NEG	NEG	1
2	1	2	PT2012QFVSERPM2	POS	POS	1
3	1	3	PT2012QFVSERPM3	POS	POS	1
4	1	4	PT2012QFVSERNM1	NEG	NEG	1
5	1	5	PT2012QFVSERPM1	POS	POS	1
6	1	6	PT2012QFVSERPM2	POS	POS	1
7	1	7	PT2012QFVSERNM2	NEG	NEG	1
8	1	8	PT2012QFVSERPM1	POS	POS	1
9	1	9	PT2012QFVSERNM1	NEG	NEG	1
10	1	10	PT2012QFVSERPM3	POS	POS	1
11	1	11	PT2012QFVSERPM2	POS	POS	1
12	1	12	PT2012QFVSERNM2	NEG	NEG	1
13	1	13	PT2012QFVSERPM1	POS	POS	1
14	1	14	PT2012QFVSERNM2	NEG	NEG	1
15	1	15	PT2012QFVSERPM3	POS	POS	1
16	1	16	PT2012QFVSERPM3	POS	POS	1
17	1	17	PT2012QFVSERPM1	POS	POS	1
18	1	18	PT2012QFVSERNM1	NEG	NEG	1
19	1	19	PT2012QFVSERPM2	POS	POS	1
20	1	20	PT2012QFVSERNM1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2012QFVSERNM1	NEG	NEG	1
22	3	2	PT2012QFVSERPM2	POS	POS	1
23	3	3	PT2012QFVSERNM1	NEG	NEG	1
24	3	4	PT2012QFVSERNM2	NEG	NEG	1
25	3	5	PT2012QFVSERPM2	POS	POS	1
26	3	6	PT2012QFVSERPM3	POS	POS	1
27	3	7	PT2012QFVSERNM1	NEG	NEG	1
28	3	8	PT2012QFVSERPM1	POS	POS	1
29	3	9	PT2012QFVSERPM2	POS	POS	1
30	3	10	PT2012QFVSERNM2	NEG	NEG	1
31	3	11	PT2012QFVSERPM1	POS	POS	1
32	3	12	PT2012QFVSERNM1	NEG	NEG	1
33	3	13	PT2012QFVSERPM3	POS	POS	1
34	3	14	PT2012QFVSERPM2	POS	POS	1
35	3	15	PT2012QFVSERNM2	NEG	NEG	1
36	3	16	PT2012QFVSERPM1	POS	POS	1
37	3	17	PT2012QFVSERNM2	NEG	NEG	1
38	3	18	PT2012QFVSERPM3	POS	POS	1
39	3	19	PT2012QFVSERPM3	POS	POS	1
40	3	20	PT2012QFVSERPM1	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	4	1	PT2012QFVSERPM3	POS	POS	1
42	4	2	PT2012QFVSERPM3	POS	POS	1
43	4	3	PT2012QFVSERPM1	POS	POS	1
44	4	4	PT2012QFVSERNM1	NEG	NEG	1
45	4	5	PT2012QFVSERPM2	POS	POS	1
46	4	6	PT2012QFVSERNM1	NEG	NEG	1
47	4	7	PT2012QFVSERNM2	NEG	NEG	1
48	4	8	PT2012QFVSERPM2	POS	POS	1
49	4	9	PT2012QFVSERPM3	POS	POS	1
50	4	10	PT2012QFVSERNM1	NEG	NEG	1
51	4	11	PT2012QFVSERPM1	POS	POS	1
52	4	12	PT2012QFVSERPM2	POS	POS	1
53	4	13	PT2012QFVSERNM2	NEG	NEG	1
54	4	14	PT2012QFVSERPM1	POS	POS	1
55	4	15	PT2012QFVSERNM1	NEG	NEG	1
56	4	16	PT2012QFVSERPM3	POS	POS	1
57	4	17	PT2012QFVSERPM2	POS	POS	1
58	4	18	PT2012QFVSERNM2	NEG	NEG	1
59	4	19	PT2012QFVSERPM1	POS	POS	1
60	4	20	PT2012QFVSERNM2	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, three different batches were used: batch 2-ELISACOXLS-006 was used by LAB 1, batch 2-ELISACOXLS-004 was used by LAB 2 and batch 5-ELISACOXLS-005 was used by LAB 3. For the detection of QFV-specific antibodies in reference milk samples, two different batches were used: batch 2-ELISACOXLS-006 was used by LAB 1 and batch 5 or 2-ELISACOXLS-005 were used by LAB 3 and LAB 4 respectively.

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 milk samples, while this was not the case for reference serum samples. LAB 3 misclassified all aliquots of samples PT2012QFVSERNS1, PT2012QFVSERPS2, and PT2012QFVSERPS3. The reason for this is not clear, but there seems to be a systematic error for the positive serum samples with lower values compared to the other participants (figure 1. box plots).

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 are 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.).

All participating laboratories provided qualitative results that were in full agreement with the true status of the reference milk samples. Therefore, all three participating laboratories achieved a satisfactory performance.

Two out of three participating laboratories (LAB 1 and LAB 2) provided qualitative results that were in full agreement with the true status of the reference serum samples. LAB 1 and LAB 2 achieved thus a satisfactory performance. LAB 3 did not achieve a satisfactory performance, because LAB 3 reached only 40% of agreement with the true status of the reference serum samples.

Head CVD-ERA

Yves Van der Stede

Appendix

Name of the participating Laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)
Veterinary and Agrochemical Research Center (CODA-CERVA), (Ukkel, Belgium)
Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)
Melk Controle Centrum Vlaanderen (MCC-Vlaanderen) (Lier, Belgium)

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.

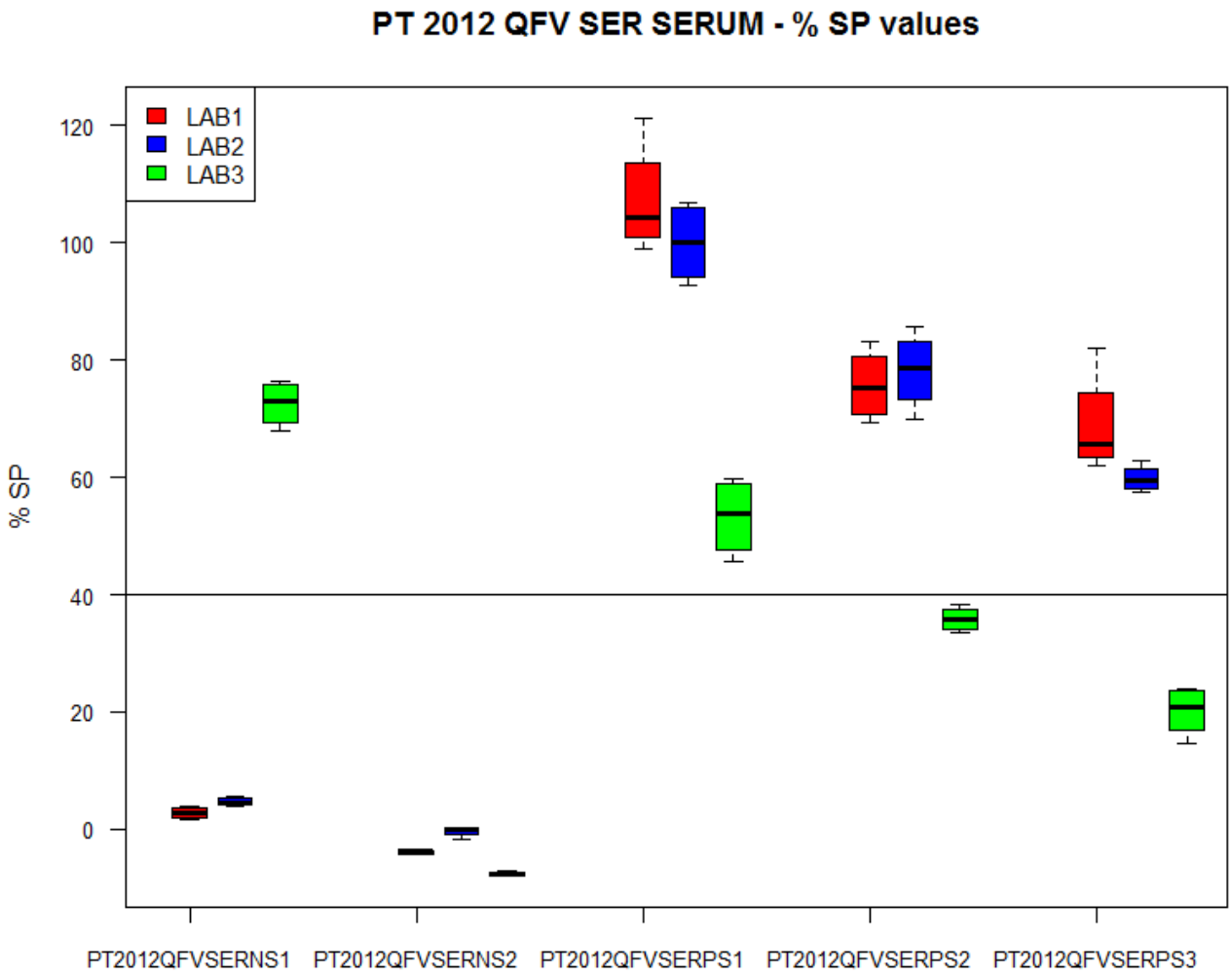

Figure 1. Box plots of the % S/P per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off value is 40%.

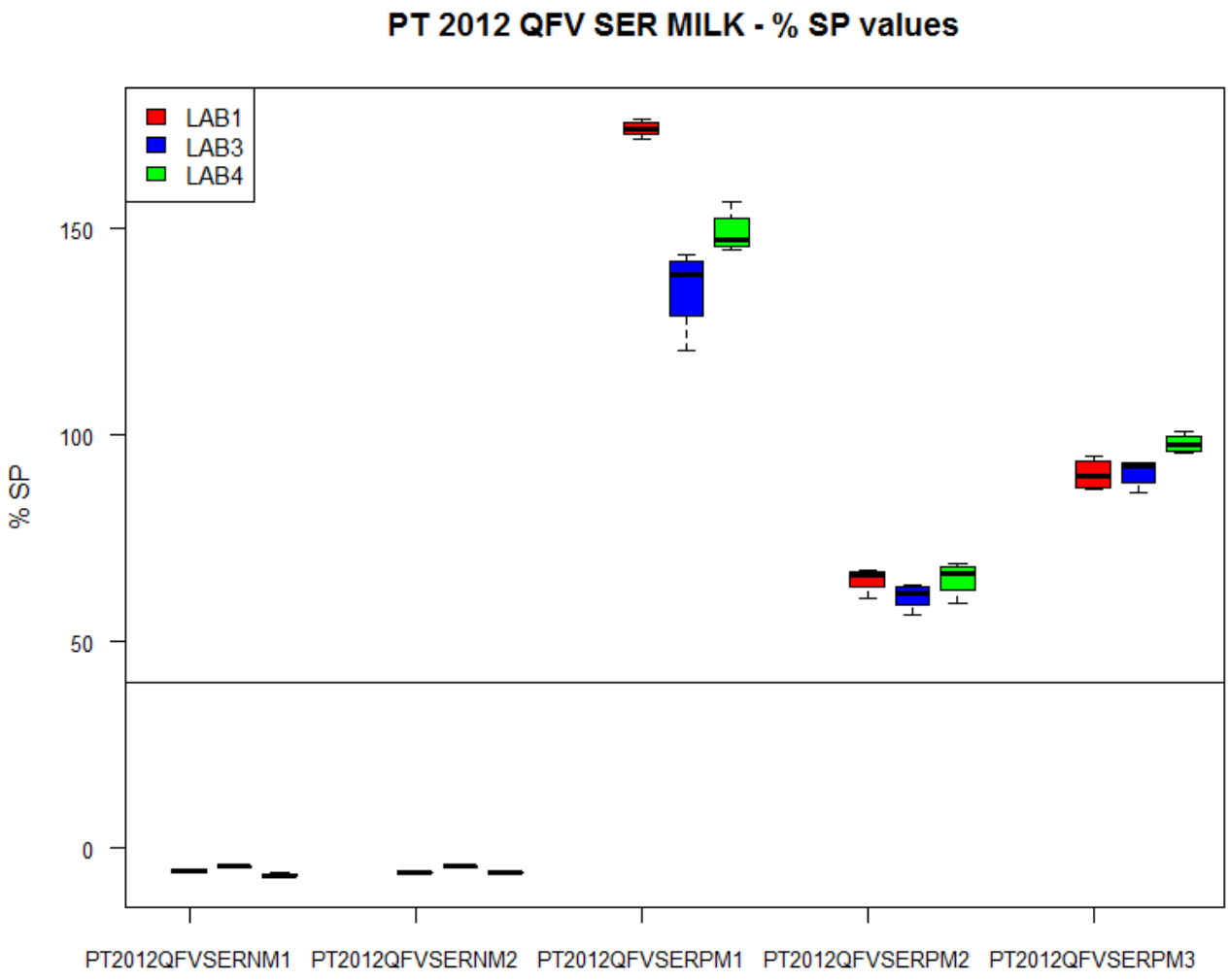

Figure 2. Box plots of the % S/P per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off value is 40%.

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 samples, a satisfactory between-lab consistency was observed for all three laboratories (h ≤ 1,15 – based on Table 1). However, borderline values were observed for LAB 3 in sample PT2012QFVSERNS1 and for all positive reference serum samples. For the reference serum samples, an unsatisfactory within-laboratory consistency was observed for LAB 3 (samples PT2012QFVSERNS1 and PT2012QFVSERPS3) and LAB 2 (sample PT2012QFVSERNS2) since for these samples k >1.45 (based on Table 1).

For all reference milk samples satisfactory between-lab consistency was observed for all three laboratories (h ≤ 1,15 – based on Table 1). However, borderline values were observed for LAB 3 in sample PT2012QFVSERNM2 and PT2012QFVSERPM2 and for LAB 4 in sample PT2012QFVSERPM3. For all reference milk samples a satisfactory k-value was found except for LAB 3 in sample PT2012QFVSERPM1.

All 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 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
PT2012QFVSERNS1	1	4	0.87	2.76	26.66	0.99	2.36	28.20	28.10	-0.60	0.40	33.77
PT2012QFVSERNS1	2	4	0.50	4.66	26.66	0.99	2.36	28.20	28.10	-0.55	0.30	15.25
PT2012QFVSERNS1	3	4	15.38	72.56	26.66	0.99	2.36	28.20	28.10	1.15	1.66	5.41
PT2012QFVSERNS2	1	4	0.16	-3.88	-3.98	0.94	0.62	2.61	2.53	0.03	0.64	-10.23
PT2012QFVSERNS2	2	4	0.85	-0.43	-3.98	0.94	0.62	2.61	2.53	0.99	1.50	-212.62
PT2012QFVSERNS2	3	4	0.13	-7.62	-3.98	0.94	0.62	2.61	2.53	-1.01	0.59	-4.76
PT2012QFVSERPS1	1	4	96.28	107.23	86.84	0.87	7.99	22.00	20.50	0.70	1.23	9.15
PT2012QFVSERPS1	2	4	48.75	99.97	86.84	0.87	7.99	22.00	20.50	0.45	0.87	6.98
PT2012QFVSERPS1	3	4	46.30	53.31	86.84	0.87	7.99	22.00	20.50	-1.15	0.85	12.76
PT2012QFVSERPS2	1	4	37.54	75.74	63.25	0.91	5.40	17.59	16.75	0.52	1.13	8.09
PT2012QFVSERPS2	2	4	45.22	78.23	63.25	0.91	5.40	17.59	16.75	0.63	1.25	8.60
PT2012QFVSERPS2	3	4	4.70	35.76	63.25	0.91	5.40	17.59	16.75	-1.15	0.40	6.06
PT2012QFVSERPS3	1	4	81.47	68.87	49.59	0.90	5.91	19.13	18.19	0.74	1.53	13.11
PT2012QFVSERPS3	2	4	4.96	59.75	49.59	0.90	5.91	19.13	18.19	0.39	0.38	3.73
PT2012QFVSERPS3	3	4	18.41	20.16	49.59	0.90	5.91	19.13	18.19	-1.14	0.73	21.29

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
PT2012QFVSERNM1	1	4	0.15	-5.63	-5.58	0.81	0.38	0.87	0.78	-0.05	1.03	-6.96
PT2012QFVSERNM1	3	4	0.15	-4.44	-5.58	0.81	0.38	0.87	0.78	1.02	1.02	-8.73
PT2012QFVSERNM1	4	4	0.13	-6.67	-5.58	0.81	0.38	0.87	0.78	-0.98	0.94	-5.35
PT2012QFVSERNM2	1	4	0.02	-5.90	-5.54	0.96	0.12	0.61	0.59	-0.44	1.13	-2.27
PT2012QFVSERNM2	3	4	0.02	-4.57	-5.54	0.96	0.12	0.61	0.59	1.14	1.31	-3.40
PT2012QFVSERNM2	4	4	0.00	-6.13	-5.54	0.96	0.12	0.61	0.59	-0.71	0.00	0.00
PT2012QFVSERPM1	1	4	4.10	174.34	153.01	0.81	6.72	15.28	13.72	1.08	0.30	1.16
PT2012QFVSERPM1	3	4	105.36	135.52	153.01	0.81	6.72	15.28	13.72	-0.89	1.53	7.57
PT2012QFVSERPM1	4	4	25.90	149.18	153.01	0.81	6.72	15.28	13.72	-0.19	0.76	3.41
PT2012QFVSERPM2	1	4	9.32	65.01	63.79	0.11	3.48	3.69	1.20	0.50	0.88	4.70
PT2012QFVSERPM2	3	4	10.34	60.98	63.79	0.11	3.48	3.69	1.20	-1.15	0.92	5.27
PT2012QFVSERPM2	4	4	16.75	65.37	63.79	0.11	3.48	3.69	1.20	0.65	1.17	6.26
PT2012QFVSERPM3	1	4	15.87	90.53	93.18	0.40	3.33	4.30	2.71	-0.63	1.20	4.40
PT2012QFVSERPM3	3	4	12.37	91.01	93.18	0.40	3.33	4.30	2.71	-0.52	1.06	3.86
PT2012QFVSERPM3	4	4	5.09	98.00	93.18	0.40	3.33	4.30	2.71	1.15	0.68	2.30

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 %