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172-PT

PROFICIENCY TESTING 2013

Q-FEVER (QFV)

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

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 06 MAY 2013

DATE REPORT: 12 JULY 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 QFV-specific antibodies in serum and/or milk 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 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 of bovine origin, either free from detectable QFV-specific antibodies (n=2; coded 'PT2013QFVSERNS1' and 'PT2013QFVSERNS2') or containing detectable QFV-specific antibodies (n=3; coded 'PT2013QFVSERPS1', 'PT2013QFVSERPS2' and 'PT2013QFVSERPS3'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 4 aliquots of each reference serum sample, i.e. 20 aliquots in total. The identification numbers of the reference serum samples were randomized for all participants (Table 4).

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 the results obtained during pre-verification, hereby using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI, the CHEKIT QFV Antibody ELISA Test Kit from IDEXX and the ID Screen® QFV Indirect Multi-species Antibody ELISA Test Kit from IDVET. For the reference serum samples PT2013QFVSERNS1, PT2013QFVSERNS2, PT2013QFVSERPS1 and PT2013QFVSERPS2, the same qualitative result was obtained with all ELISA kits used. For the reference serum sample PT2013QFVSERPS3, a positive result was obtained using the ELISA kits from LSI and IDEXX, whereas a non-interpretable (doubtful) or negative result was obtained using the ELISA kit from IDVET. Moreover, some normalized data obtained for this reference serum sample using the ELISA kit from LSI were close to the cut-off. Taken together, the reference serum samples PT2013QFVSERNS1 and PT2013QFVSERNS2 were considered as negative samples, the reference serum samples PT2013QFVSERPS1 and PT2013QFVSERPS2 (= 2/3 dilution of PT2013QFVSERPS1) as positive samples, and the reference serum sample PT2013QFVSERPS3 as a cut-off sample in QFV antibody ELISA. Therefore, the reference serum sample PT2013QFVSERPS3 could be reported as positive, non-interpretable or negative.

A homogeneity check on the aliquoted reference serum samples had already been performed in the context of previous PTs Serology QFV. Indeed, 10 aliquots of each reference serum sample were analysed using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI, 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 the participating laboratories to correctly identify the absence or presence of QFV-specific antibodies in serum. In addition, all reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI.

III.2.2. Reference milk samples

Replicates of 5 reference milk samples of bovidae origin, either free from detectable QFV-specific antibodies (n=2; coded 'PT2013QFVSERNM1' and 'PT2013QFVSERNM2') or containing detectable QFV-specific antibodies (n=3; coded 'PT2013QFVSERPM1', 'PT2013QFVSERPM2' and 'PT2013QFVSERPM3'), were used. The reference milk samples PT2013QFVSERNM1, PT2013QFVSERNM2, PT2013QFVSERPM2 and PT2013QFVSERPM3 were bovine tank milk, whereas the reference milk sample PT2013QFVSERPM1 was caprine tank milk. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2013QFVSERNM2 and PT2013QFVSERPM1, 4 aliquots of the reference milk sample PT2013QFVSERNM1, and 5 aliquots of the reference milk samples PT2013QFVSERPM2 and PT2013QFVSERPM3. The identification numbers of the reference milk samples were randomized for all participants (Table 5).

For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference milk samples was based on the results obtained during pre-verification, hereby using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI, the CHEKIT QFV Antibody ELISA Test Kit from IDEXX and the ID Screen® QFV Indirect Multi-species Antibody ELISA Test Kit from IDVET. For all reference milk samples, the same qualitative result was obtained with all ELISA kits used. Taken together, the reference milk samples PT2013QFVSERNM1 and PT2013QFVSERNM2 were considered as negative samples and the reference milk samples PT2013QFVSERPM1, PT2013QFVSERPM2 and PT2013QFVSERPM3 as positive samples in QFV antibody ELISA.

A homogeneity check on the aliquoted reference milk samples had already been performed in the context of previous PTs Serology QFV. Indeed, 10 aliquots of each reference milk sample were analysed using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample. Consequently, all reference milk samples were considered as reliable samples in order to evaluate the ability of the participating laboratories to correctly identify the absence or presence of QFV-specific antibodies in milk. In addition, all reference milk samples were tested once after the PT in order to confirm their stability and status (post-verification) using the LSIVET ruminant milk/serum QFV Antibody ELISA Test Kit from LSI.

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 either 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 used for either PT 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

LAB1, LAB2, LAB3, LAB4 and LAB5 participated in both the PT serum and the PT milk and hence received 40 aliquots: 20 aliquots of reference serum samples and 20 aliquots of reference milk samples. In contrast, LAB6 and LAB7 only participated in the PT serum and hence received 20 aliquots of reference serum samples, whereas LAB8 only participated in the PT milk and thus received 20 aliquots of reference milk samples.

Lyophilized reference serum samples (140 aliquots in total) and lyophilized reference milk samples (120 aliquots in total) were sent at ambient temperature to the 8 participating laboratories by national or international courier on the 6th (all participants except LAB5) or 13th (LAB5) of May 2013. LAB1, LAB2, LAB3, LAB6 and LAB8 acknowledged receipt of the samples on 6th of May 2013, whereas the other participants acknowledged receipt of the samples on 10th (LAB4 and LAB7) and 15th (LAB5) of May 2013. Analyses were performed between 6th and 16th of May 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 10th and 21st of May 2013 (Table 1). All participants hereby respected the deadline of 17th (all participants except LAB5) and 22nd (LAB5) of May 2013 for submission of the results.

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 SERUM	Start of analysis MILK	Submission of the results (Excel file)
LAB1	06/05/2013	14/05/2013	15/05/2013	17/05/2013
LAB2	06/05/2013	13/05/2013	13/05/2013	14/05/2013
LAB3	06/05/2013	07/05/2013	06/05/2013	10/05/2013
LAB4	10/05/2013	15/05/2013	15/05/2013	16/05/2013
LAB5	15/05/2013▲	16/05/2013	16/05/2013	21/05/2013▲
LAB6	06/05/2013	08/05/2013	NA	17/05/2013
LAB7	10/05/2013	15/05/2013	NA	16/05/2013
LAB8	06/05/2013	NA	08/05/2013	10/05/2013

Legend: NA = not applicable; ▲ LAB5 was closed from 06/05/2013 until 10/05/2013 (holidays): for this participant, the PT samples were sent on 13/05/2013 and the deadline for submission of the results was extended until 22/05/2013

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:

(i) For the detection of QFV-specific antibodies in **serum**, all 7 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and thus achieved 100% of agreement (Table 2).

(ii) For the detection of QFV-specific antibodies in **milk**, all 6 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples and thus achieved 100% of agreement (Table 3).

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

Table 2. Agreement between the 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**	**5**	**6**	**7**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference milk samples assigned by CODA-CERVA. All participating laboratories received 20 aliquots of reference milk samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR					
	1	**2**	**3**	**4**	**5**	**8**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

Variability in the qualitative laboratory results of the participating laboratories could only be observed for the reference serum sample PT2013QFVSERPS3, which was considered as a cut-off sample: LAB1 until LAB6 reported all 4 aliquots of this reference serum sample as positive, whereas LAB7 reported this reference serum sample 3 times as positive and once as negative.

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 4 for the PT serum and in Table 5 for the PT milk.

Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (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	PT2013QFVSERNS1	NEG	NEG	1
2	1	2	PT2013QFVSERPS2	POS	POS	1
3	1	3	PT2013QFVSERNS2	NEG	NEG	1
4	1	4	PT2013QFVSERPS1	POS	POS	1
5	1	5	PT2013QFVSERPS2	POS	POS	1
6	1	6	PT2013QFVSERNS1	NEG	NEG	1
7	1	7	PT2013QFVSERPS3	POS/NI/NEG	POS	1
8	1	8	PT2013QFVSERNS2	NEG	NEG	1
9	1	9	PT2013QFVSERPS1	POS	POS	1
10	1	10	PT2013QFVSERPS3	POS/NI/NEG	POS	1
11	1	11	PT2013QFVSERPS2	POS	POS	1
12	1	12	PT2013QFVSERNS1	NEG	NEG	1
13	1	13	PT2013QFVSERPS3	POS/NI/NEG	POS	1
14	1	14	PT2013QFVSERNS2	NEG	NEG	1
15	1	15	PT2013QFVSERPS1	POS	POS	1
16	1	16	PT2013QFVSERPS3	POS/NI/NEG	POS	1
17	1	17	PT2013QFVSERNS1	NEG	NEG	1
18	1	18	PT2013QFVSERPS1	POS	POS	1
19	1	19	PT2013QFVSERNS2	NEG	NEG	1
20	1	20	PT2013QFVSERPS2	POS	POS	1
21	2	1	PT2013QFVSERNS1	NEG	NEG	1
22	2	2	PT2013QFVSERPS1	POS	POS	1
23	2	3	PT2013QFVSERNS2	NEG	NEG	1
24	2	4	PT2013QFVSERPS2	POS	POS	1
25	2	5	PT2013QFVSERNS1	NEG	NEG	1
26	2	6	PT2013QFVSERPS2	POS	POS	1
27	2	7	PT2013QFVSERNS2	NEG	NEG	1
28	2	8	PT2013QFVSERPS1	POS	POS	1
29	2	9	PT2013QFVSERPS2	POS	POS	1
30	2	10	PT2013QFVSERNS1	NEG	NEG	1
31	2	11	PT2013QFVSERPS3	POS/NI/NEG	POS	1
32	2	12	PT2013QFVSERNS2	NEG	NEG	1
33	2	13	PT2013QFVSERPS1	POS	POS	1
34	2	14	PT2013QFVSERPS3	POS/NI/NEG	POS	1
35	2	15	PT2013QFVSERPS2	POS	POS	1
36	2	16	PT2013QFVSERNS1	NEG	NEG	1
37	2	17	PT2013QFVSERPS3	POS/NI/NEG	POS	1
38	2	18	PT2013QFVSERNS2	NEG	NEG	1
39	2	19	PT2013QFVSERPS1	POS	POS	1
40	2	20	PT2013QFVSERPS3	POS/NI/NEG	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013QFVSERPS3	POS/NI/NEG	POS	1
42	3	2	PT2013QFVSERNS2	NEG	NEG	1
43	3	3	PT2013QFVSERPS1	POS	POS	1
44	3	4	PT2013QFVSERPS3	POS/NI/NEG	POS	1
45	3	5	PT2013QFVSERNS1	NEG	NEG	1
46	3	6	PT2013QFVSERPS1	POS	POS	1
47	3	7	PT2013QFVSERNS2	NEG	NEG	1
48	3	8	PT2013QFVSERPS2	POS	POS	1
49	3	9	PT2013QFVSERNS1	NEG	NEG	1
50	3	10	PT2013QFVSERPS2	POS	POS	1
51	3	11	PT2013QFVSERNS2	NEG	NEG	1
52	3	12	PT2013QFVSERPS1	POS	POS	1
53	3	13	PT2013QFVSERPS2	POS	POS	1
54	3	14	PT2013QFVSERNS1	NEG	NEG	1
55	3	15	PT2013QFVSERPS3	POS/NI/NEG	POS	1
56	3	16	PT2013QFVSERNS2	NEG	NEG	1
57	3	17	PT2013QFVSERPS1	POS	POS	1
58	3	18	PT2013QFVSERPS3	POS/NI/NEG	POS	1
59	3	19	PT2013QFVSERPS2	POS	POS	1
60	3	20	PT2013QFVSERNS1	NEG	NEG	1
61	4	1	PT2013QFVSERPS1	POS	POS	1
62	4	2	PT2013QFVSERPS3	POS/NI/NEG	POS	1
63	4	3	PT2013QFVSERPS2	POS	POS	1
64	4	4	PT2013QFVSERNS1	NEG	NEG	1
65	4	5	PT2013QFVSERPS3	POS/NI/NEG	POS	1
66	4	6	PT2013QFVSERNS2	NEG	NEG	1
67	4	7	PT2013QFVSERPS1	POS	POS	1
68	4	8	PT2013QFVSERPS3	POS/NI/NEG	POS	1
69	4	9	PT2013QFVSERNS1	NEG	NEG	1
70	4	10	PT2013QFVSERPS1	POS	POS	1
71	4	11	PT2013QFVSERNS2	NEG	NEG	1
72	4	12	PT2013QFVSERPS2	POS	POS	1
73	4	13	PT2013QFVSERNS1	NEG	NEG	1
74	4	14	PT2013QFVSERPS2	POS	POS	1
75	4	15	PT2013QFVSERNS2	NEG	NEG	1
76	4	16	PT2013QFVSERPS1	POS	POS	1
77	4	17	PT2013QFVSERPS2	POS	POS	1
78	4	18	PT2013QFVSERNS1	NEG	NEG	1
79	4	19	PT2013QFVSERPS3	POS/NI/NEG	POS	1
80	4	20	PT2013QFVSERNS2	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2013QFVSERPS2	POS	POS	1
82	5	2	PT2013QFVSERNS1	NEG	NEG	1
83	5	3	PT2013QFVSERPS3	POS/NI/NEG	POS	1
84	5	4	PT2013QFVSERNS2	NEG	NEG	1
85	5	5	PT2013QFVSERPS1	POS	POS	1
86	5	6	PT2013QFVSERPS3	POS/NI/NEG	POS	1
87	5	7	PT2013QFVSERPS2	POS	POS	1
88	5	8	PT2013QFVSERNS1	NEG	NEG	1
89	5	9	PT2013QFVSERPS3	POS/NI/NEG	POS	1
90	5	10	PT2013QFVSERNS2	NEG	NEG	1
91	5	11	PT2013QFVSERPS1	POS	POS	1
92	5	12	PT2013QFVSERPS3	POS/NI/NEG	POS	1
93	5	13	PT2013QFVSERNS1	NEG	NEG	1
94	5	14	PT2013QFVSERPS1	POS	POS	1
95	5	15	PT2013QFVSERNS2	NEG	NEG	1
96	5	16	PT2013QFVSERPS2	POS	POS	1
97	5	17	PT2013QFVSERNS1	NEG	NEG	1
98	5	18	PT2013QFVSERPS2	POS	POS	1
99	5	19	PT2013QFVSERNS2	NEG	NEG	1
100	5	20	PT2013QFVSERPS1	POS	POS	1
101	6	1	PT2013QFVSERNS1	NEG	NEG	1
102	6	2	PT2013QFVSERPS2	POS	POS	1
103	6	3	PT2013QFVSERNS2	NEG	NEG	1
104	6	4	PT2013QFVSERPS1	POS	POS	1
105	6	5	PT2013QFVSERPS2	POS	POS	1
106	6	6	PT2013QFVSERNS1	NEG	NEG	1
107	6	7	PT2013QFVSERPS3	POS/NI/NEG	POS	1
108	6	8	PT2013QFVSERNS2	NEG	NEG	1
109	6	9	PT2013QFVSERPS1	POS	POS	1
110	6	10	PT2013QFVSERPS3	POS/NI/NEG	POS	1
111	6	11	PT2013QFVSERPS2	POS	POS	1
112	6	12	PT2013QFVSERNS1	NEG	NEG	1
113	6	13	PT2013QFVSERPS3	POS/NI/NEG	POS	1
114	6	14	PT2013QFVSERNS2	NEG	NEG	1
115	6	15	PT2013QFVSERPS1	POS	POS	1
116	6	16	PT2013QFVSERPS3	POS/NI/NEG	POS	1
117	6	17	PT2013QFVSERNS1	NEG	NEG	1
118	6	18	PT2013QFVSERPS1	POS	POS	1
119	6	19	PT2013QFVSERNS2	NEG	NEG	1
120	6	20	PT2013QFVSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2013QFVSERNS1	NEG	NEG	1
122	7	2	PT2013QFVSERPS1	POS	POS	1
123	7	3	PT2013QFVSERNS2	NEG	NEG	1
124	7	4	PT2013QFVSERPS2	POS	POS	1
125	7	5	PT2013QFVSERNS1	NEG	NEG	1
126	7	6	PT2013QFVSERPS2	POS	POS	1
127	7	7	PT2013QFVSERNS2	NEG	NEG	1
128	7	8	PT2013QFVSERPS1	POS	POS	1
129	7	9	PT2013QFVSERPS2	POS	POS	1
130	7	10	PT2013QFVSERNS1	NEG	NEG	1
131	7	11	PT2013QFVSERPS3	POS/NI/NEG	POS	1
132	7	12	PT2013QFVSERNS2	NEG	NEG	1
133	7	13	PT2013QFVSERPS1	POS	POS	1
134	7	14	PT2013QFVSERPS3	POS/NI/NEG	NEG	1
135	7	15	PT2013QFVSERPS2	POS	POS	1
136	7	16	PT2013QFVSERNS1	NEG	NEG	1
137	7	17	PT2013QFVSERPS3	POS/NI/NEG	POS	1
138	7	18	PT2013QFVSERNS2	NEG	NEG	1
139	7	19	PT2013QFVSERPS1	POS	POS	1
140	7	20	PT2013QFVSERPS3	POS/NI/NEG	POS	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference milk samples (SAMPLE), the external identification of the reference milk samples (LABPOSIT), and the status assigned by CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013QFVSERPM1	POS	POS	1
2	1	2	PT2013QFVSERNM1	NEG	NEG	1
3	1	3	PT2013QFVSERPM2	POS	POS	1
4	1	4	PT2013QFVSERNM2	NEG	NEG	1
5	1	5	PT2013QFVSERPM2	POS	POS	1
6	1	6	PT2013QFVSERPM1	POS	POS	1
7	1	7	PT2013QFVSERPM3	POS	POS	1
8	1	8	PT2013QFVSERNM1	NEG	NEG	1
9	1	9	PT2013QFVSERPM3	POS	POS	1
10	1	10	PT2013QFVSERNM2	NEG	NEG	1
11	1	11	PT2013QFVSERNM1	NEG	NEG	1
12	1	12	PT2013QFVSERPM3	POS	POS	1
13	1	13	PT2013QFVSERPM2	POS	POS	1
14	1	14	PT2013QFVSERPM1	POS	POS	1
15	1	15	PT2013QFVSERPM3	POS	POS	1
16	1	16	PT2013QFVSERPM2	POS	POS	1
17	1	17	PT2013QFVSERNM1	NEG	NEG	1
18	1	18	PT2013QFVSERPM3	POS	POS	1
19	1	19	PT2013QFVSERNM2	NEG	NEG	1
20	1	20	PT2013QFVSERPM2	POS	POS	1
21	2	1	PT2013QFVSERNM1	NEG	NEG	1
22	2	2	PT2013QFVSERPM3	POS	POS	1
23	2	3	PT2013QFVSERNM2	NEG	NEG	1
24	2	4	PT2013QFVSERPM2	POS	POS	1
25	2	5	PT2013QFVSERPM1	POS	POS	1
26	2	6	PT2013QFVSERNM1	NEG	NEG	1
27	2	7	PT2013QFVSERPM2	POS	POS	1
28	2	8	PT2013QFVSERNM2	NEG	NEG	1
29	2	9	PT2013QFVSERPM2	POS	POS	1
30	2	10	PT2013QFVSERPM1	POS	POS	1
31	2	11	PT2013QFVSERPM3	POS	POS	1
32	2	12	PT2013QFVSERNM1	NEG	NEG	1
33	2	13	PT2013QFVSERPM3	POS	POS	1
34	2	14	PT2013QFVSERNM2	NEG	NEG	1
35	2	15	PT2013QFVSERNM1	NEG	NEG	1
36	2	16	PT2013QFVSERPM3	POS	POS	1
37	2	17	PT2013QFVSERPM2	POS	POS	1
38	2	18	PT2013QFVSERPM1	POS	POS	1
39	2	19	PT2013QFVSERPM3	POS	POS	1
40	2	20	PT2013QFVSERPM2	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013QFVSERPM2	POS	POS	1
42	3	2	PT2013QFVSERPM1	POS	POS	1
43	3	3	PT2013QFVSERPM3	POS	POS	1
44	3	4	PT2013QFVSERPM2	POS	POS	1
45	3	5	PT2013QFVSERNM1	NEG	NEG	1
46	3	6	PT2013QFVSERPM3	POS	POS	1
47	3	7	PT2013QFVSERNM2	NEG	NEG	1
48	3	8	PT2013QFVSERPM2	POS	POS	1
49	3	9	PT2013QFVSERPM1	POS	POS	1
50	3	10	PT2013QFVSERNM1	NEG	NEG	1
51	3	11	PT2013QFVSERPM2	POS	POS	1
52	3	12	PT2013QFVSERNM2	NEG	NEG	1
53	3	13	PT2013QFVSERPM2	POS	POS	1
54	3	14	PT2013QFVSERPM1	POS	POS	1
55	3	15	PT2013QFVSERPM3	POS	POS	1
56	3	16	PT2013QFVSERNM1	NEG	NEG	1
57	3	17	PT2013QFVSERPM3	POS	POS	1
58	3	18	PT2013QFVSERNM2	NEG	NEG	1
59	3	19	PT2013QFVSERNM1	NEG	NEG	1
60	3	20	PT2013QFVSERPM3	POS	POS	1
61	4	1	PT2013QFVSERPM3	POS	POS	1
62	4	2	PT2013QFVSERNM2	NEG	NEG	1
63	4	3	PT2013QFVSERNM1	NEG	NEG	1
64	4	4	PT2013QFVSERPM3	POS	POS	1
65	4	5	PT2013QFVSERPM2	POS	POS	1
66	4	6	PT2013QFVSERPM1	POS	POS	1
67	4	7	PT2013QFVSERPM3	POS	POS	1
68	4	8	PT2013QFVSERPM2	POS	POS	1
69	4	9	PT2013QFVSERNM1	NEG	NEG	1
70	4	10	PT2013QFVSERPM3	POS	POS	1
71	4	11	PT2013QFVSERNM2	NEG	NEG	1
72	4	12	PT2013QFVSERPM2	POS	POS	1
73	4	13	PT2013QFVSERPM1	POS	POS	1
74	4	14	PT2013QFVSERNM1	NEG	NEG	1
75	4	15	PT2013QFVSERPM2	POS	POS	1
76	4	16	PT2013QFVSERNM2	NEG	NEG	1
77	4	17	PT2013QFVSERPM2	POS	POS	1
78	4	18	PT2013QFVSERPM1	POS	POS	1
79	4	19	PT2013QFVSERPM3	POS	POS	1
80	4	20	PT2013QFVSERNM1	NEG	NEG	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2013QFVSERPM2	POS	POS	1
82	5	2	PT2013QFVSERPM1	POS	POS	1
83	5	3	PT2013QFVSERPM3	POS	POS	1
84	5	4	PT2013QFVSERNM1	NEG	NEG	1
85	5	5	PT2013QFVSERPM3	POS	POS	1
86	5	6	PT2013QFVSERNM2	NEG	NEG	1
87	5	7	PT2013QFVSERNM1	NEG	NEG	1
88	5	8	PT2013QFVSERPM3	POS	POS	1
89	5	9	PT2013QFVSERPM2	POS	POS	1
90	5	10	PT2013QFVSERPM1	POS	POS	1
91	5	11	PT2013QFVSERPM3	POS	POS	1
92	5	12	PT2013QFVSERPM2	POS	POS	1
93	5	13	PT2013QFVSERNM1	NEG	NEG	1
94	5	14	PT2013QFVSERPM3	POS	POS	1
95	5	15	PT2013QFVSERNM2	NEG	NEG	1
96	5	16	PT2013QFVSERPM2	POS	POS	1
97	5	17	PT2013QFVSERPM1	POS	POS	1
98	5	18	PT2013QFVSERNM1	NEG	NEG	1
99	5	19	PT2013QFVSERPM2	POS	POS	1
100	5	20	PT2013QFVSERNM2	NEG	NEG	1
101	8	1	PT2013QFVSERPM1	POS	POS	1
102	8	2	PT2013QFVSERNM1	NEG	NEG	1
103	8	3	PT2013QFVSERPM2	POS	POS	1
104	8	4	PT2013QFVSERNM2	NEG	NEG	1
105	8	5	PT2013QFVSERPM2	POS	POS	1
106	8	6	PT2013QFVSERPM1	POS	POS	1
107	8	7	PT2013QFVSERPM3	POS	POS	1
108	8	8	PT2013QFVSERNM1	NEG	NEG	1
109	8	9	PT2013QFVSERPM3	POS	POS	1
110	8	10	PT2013QFVSERNM2	NEG	NEG	1
111	8	11	PT2013QFVSERNM1	NEG	NEG	1
112	8	12	PT2013QFVSERPM3	POS	POS	1
113	8	13	PT2013QFVSERPM2	POS	POS	1
114	8	14	PT2013QFVSERPM1	POS	POS	1
115	8	15	PT2013QFVSERPM3	POS	POS	1
116	8	16	PT2013QFVSERPM2	POS	POS	1
117	8	17	PT2013QFVSERNM1	NEG	NEG	1
118	8	18	PT2013QFVSERPM3	POS	POS	1
119	8	19	PT2013QFVSERNM2	NEG	NEG	1
120	8	20	PT2013QFVSERPM2	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum and/or milk samples of bovidae origin for the detection of QFV-specific antibodies by ELISA.

For the detection of QFV-specific antibodies in serum, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2 and Table 4). Hereby, 27 out of 28 aliquots of the reference serum sample PT2013QFVSERPS3 (which was considered as a cut-off sample) were reported as positive, and 1 out of 28 aliquots as negative (Table 4). QFV antibody ELISA kits from 3 different producers as well as different batches from the same ELISA kit were used: Bio-X Diagnostics (1 batch: 13M13), IDEXX (1 batch: A971) and LSI (3 batches: ELISACOXLS-006, ELISACOXLS-010, ELISACOXLS-011). LAB1, LAB3, LAB5, LAB6 and LAB7 used a QFV antibody ELISA kit from the same producer. In addition, LAB1 and LAB7 on the one hand, and LAB3 and LAB5 on the other hand used the same batch.

For the detection of QFV-specific antibodies in milk, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples (100% of agreement) (Table 3 and Table 5). The participating laboratories used QFV antibody ELISA kits from 3 different producers as well as different batches from the same ELISA kit: Bio-X Diagnostics (1 batch: 13M13), IDEXX (1 batch: A971) and LSI (3 batches: ELISACOXLS-008, ELISACOXLS-010, ELISACOXLS-011). LAB1, LAB3, LAB5 and LAB8 used a QFV antibody ELISA kit from the same producer. Hereby, LAB3 and LAB5 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 samples assigned by CODA-CERVA (see III.3.3.). Consequently, all participants in the PT serum achieved a satisfactory performance for the detection of QFV-specific antibodies in reference serum samples and all participants in the PT milk achieved a satisfactory performance for the detection of QFV-specific antibodies in reference milk samples.

Head CVD-ERA
Yves Van der Stede

Appendix

Name of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) (Sophia-Antipolis, France)

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

Bio-X Diagnostics SPRL (Jemelle, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

IDEXX Switzerland AG (Liebefeld-Bern, Switzerland)

Laboratoire Service International (LSI) (Lissieu, France)

Melkcontrolecentrum Vlaanderen (MCC) (Lier, Belgium)

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 S/P ratio calculated according to the instructions for this PT: [(OD_{Sample} - mean $OD_{Negative\ Kit\ Controls}$) / (mean $OD_{Positive\ Kit\ Controls}$ - 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 S/P ratio per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

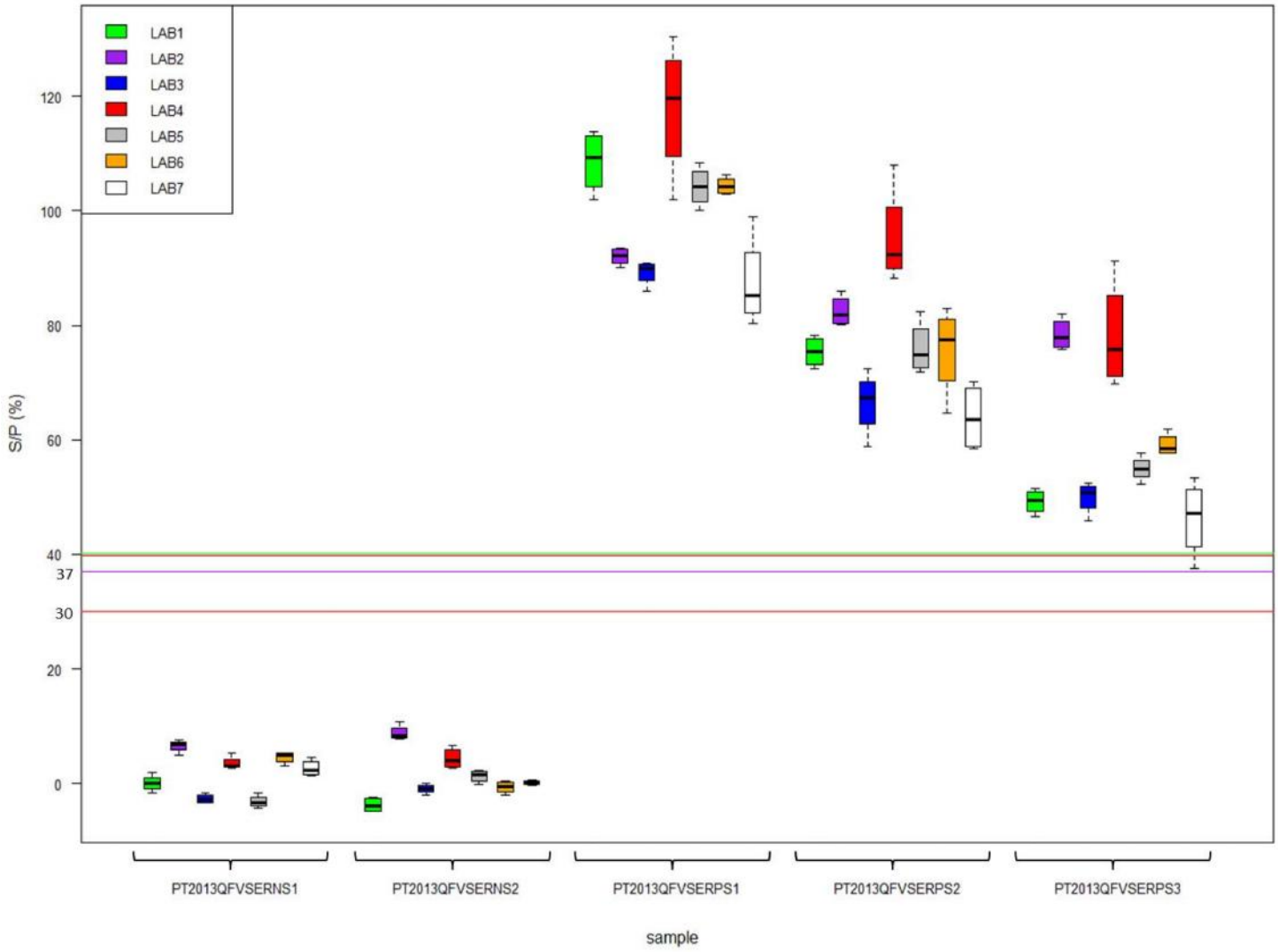

Figure 1. Box plots showing the percentages S/P ratio per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in red (30-40%, LAB4), purple (37%; LAB2) and green (40%; LAB1, LAB3, LAB5, LAB6 and LAB7), respectively. LAB1, LAB3, LAB5, LAB6 and LAB7 used a QFV antibody ELISA kit from the same producer (LAB1 and LAB7 on the one hand, and LAB3 and LAB5 on the other hand used the same batch).

Box plots of the percentages S/P ratio per reference milk sample and per participating laboratory were made using the statistical software R and are shown in Figure 2.

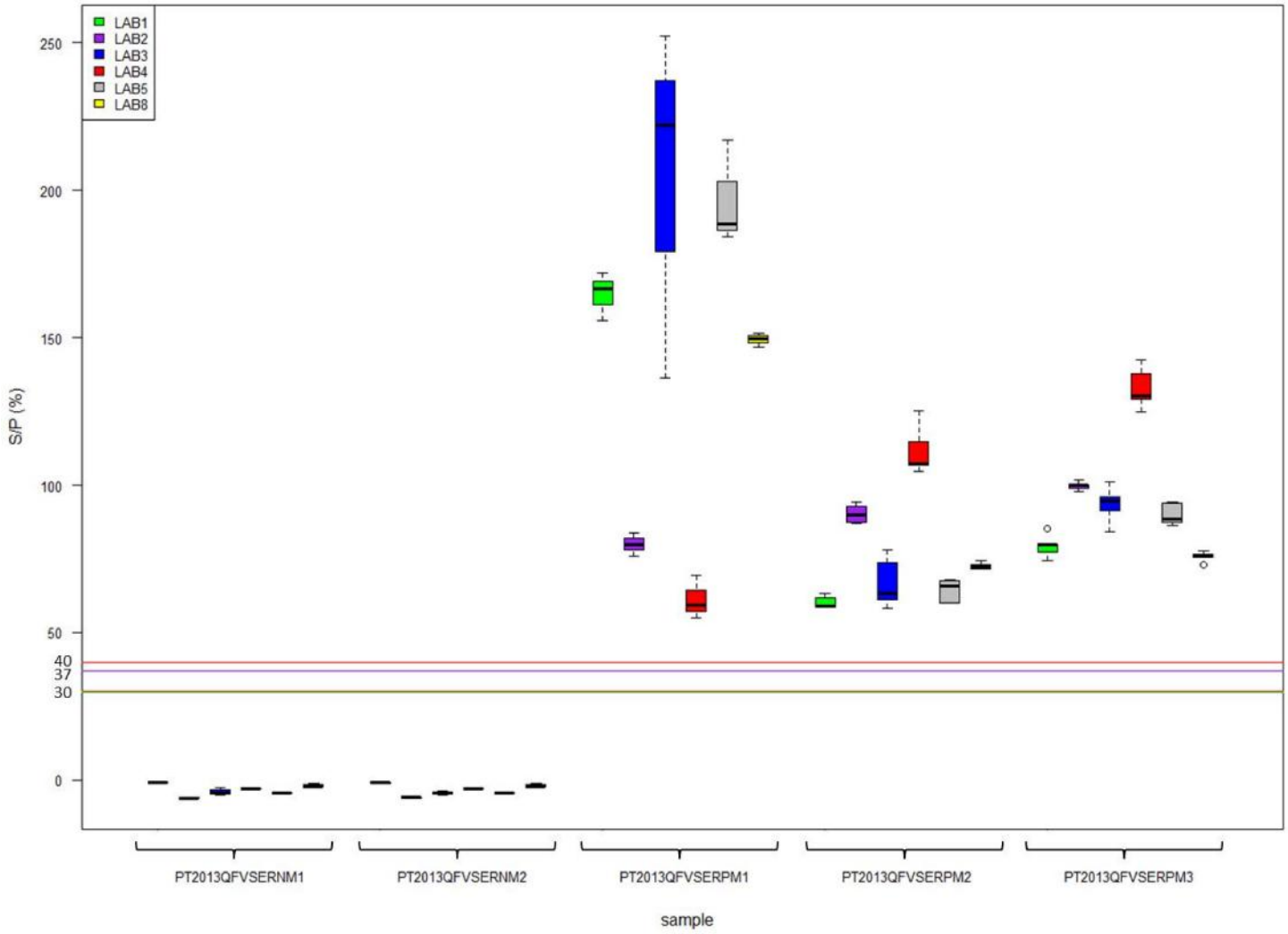

Figure 2. Box plots showing the percentages S/P ratio per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in green (30%; LAB1, LAB3, LAB5 and LAB8), red (30-40%, LAB4) and purple (37%; LAB2), respectively. LAB1, LAB3, LAB5 and LAB8 used a QFV antibody ELISA kit from the same producer (LAB3 and LAB5 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 S/P ratio 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 is 1,71 for the PT serum (p=7) and 1,66 for the PT milk (p=6). The maximum value for Mandel's k-statistic is 1,55 for all reference serum samples (p=7 and n=4), whereas the maximum value for Mandel's k-statistic is 1,64 for the reference milk samples PT2013QFVSERNM2 and PT2013QFVSERPM1 (p=6 and n=3), 1,54 for the reference milk sample PT2013QFVSERNM1 (p=6 and n=4), and 1,48 for the reference milk samples PT2013QFVSERPM2 and PT2013QFVSERPM3 (p=6 and n=5).

For the detection of QFV-specific antibodies in serum, 5 out of 7 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples: LAB1, LAB3, LAB5, LAB6 and LAB7. In contrast, the other participants showed an increased value for Mandel's h-statistic for 1 reference serum sample: LAB2 for the negative reference sample PT2013QFVSERNS2 (h=1,82) and LAB4 for the positive reference sample PT2013QFVSERPS2 (h=1,81). Noteworthy, LAB1, LAB3, LAB5, LAB6 and LAB7 used 3 different batches of the same QFV antibody ELISA kit.
Furthermore, 6 out of 7 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples: LAB1, LAB2, LAB3, LAB5, LAB6 and LAB7. In contrast, LAB4 showed an increased value for Mandel's k-statistic for the positive reference serum samples PT2013QFVSERPS1 (k=1,96) and PT2013QFVSERPS3 (k=1,96).

For the detection of QFV-specific antibodies in milk, 5 out of 6 participating laboratories obtained a satisfactory between-laboratory consistency for all reference milk samples: LAB1, LAB2, LAB3, LAB5 and LAB8. In contrast, LAB4 showed an increased value for Mandel's h-statistic for the positive reference milk samples PT2013QFVSERPM2 (h=1,73) and PT2013QFVSERPM3 (h=1,84). Noteworthy, LAB1, LAB3, LAB5 and LAB8 used 3 different batches of the same QFV antibody ELISA kit.
Furthermore, 4 out of 6 participating laboratories obtained a satisfactory within-laboratory consistency for all reference milk samples: LAB1, LAB2, LAB5 and LAB8. In contrast, the other participants showed an increased value for Mandel's k-statistic for at least 2 reference serum samples: LAB3 for the negative reference samples PT2013QFVSERNM1 (k=2,18) and PT2013QFVSERNM2 (k=1,74) as well as for the positive reference samples PT2013QFVSERPM1 (k=2,31) and PT2013QFVSERPM2 (k=1,58), and LAB4 for the positive reference samples PT2013QFVSERPM2 (k=1,56) and PT2013QFVSERPM3 (k=1,55).

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 percentages S/P ratio) 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/milk samples were analysed together), status level (all reference serum/milk samples with the same status were analysed together) and sample level (all reference serum/milk 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.

For the PT serum, no statistically significant differences were observed at a global level. Nevertheless, statistically significant differences exist at both sample level and status level. At the status level, significant differences between laboratories were observed for both the negative, the positive and the cut-off reference serum samples. For the negative reference serum samples, LAB2 reported percentages S/P ratio that were significantly higher than those reported by the other participants. In addition, LAB2, LAB4 and LAB6 reported percentages S/P ratio that were significantly higher than those reported by LAB1 and LAB3. For the positive reference serum samples, LAB4 reported percentages S/P ratio that were significantly higher than those reported by LAB3 and LAB7. For the cut-off reference serum sample (PT2013QFVSERPS3), LAB2 and LAB4 reported percentages S/P ratio that were significantly higher than those reported by the other participants, while LAB2, LAB4 and LAB6 reported percentages S/P ratio that were significantly higher than those reported by LAB7.

For the PT milk, no statistically significant differences were observed at a global level. Nevertheless, statistically significant differences exist at both sample level and status level. At the status level, significant differences between laboratories were observed for the negative reference milk samples but not for the positive reference milk samples. For the negative reference milk samples, only LAB3 and LAB5 reported percentages S/P ratio that were not significantly different.

Annex 2: Calculations of Mandel's h- and k-statistics (based on % S/P ratio)

A. Serum

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013QFVSERNS1	1	4	2,04	0,05	1,63	0,61	1,18	1,90	1,48	-0,43	1,21	2722,50
PT2013QFVSERNS1	2	4	1,24	6,53	1,63	0,61	1,18	1,90	1,48	1,33	0,94	17,09
PT2013QFVSERNS1	3	4	0,59	-2,70	1,63	0,61	1,18	1,90	1,48	-1,18	0,65	-28,45
PT2013QFVSERNS1	4	4	1,50	3,47	1,63	0,61	1,18	1,90	1,48	0,50	1,04	35,36
PT2013QFVSERNS1	5	4	1,21	-3,14	1,63	0,61	1,18	1,90	1,48	-1,30	0,93	-34,99
PT2013QFVSERNS1	6	4	1,19	4,59	1,63	0,61	1,18	1,90	1,48	0,80	0,92	23,75
PT2013QFVSERNS1	7	4	2,01	2,63	1,63	0,61	1,18	1,90	1,48	0,27	1,20	53,94
PT2013QFVSERNS2	1	4	1,60	-3,76	1,31	0,65	1,21	2,05	1,66	-1,24	1,04	-33,60
PT2013QFVSERNS2	2	4	1,92	8,79	1,31	0,65	1,21	2,05	1,66	1,82	1,14	15,76
PT2013QFVSERNS2	3	4	0,68	-0,91	1,31	0,65	1,21	2,05	1,66	-0,54	0,68	-90,93
PT2013QFVSERNS2	4	4	3,54	4,31	1,31	0,65	1,21	2,05	1,66	0,73	1,55	43,72
PT2013QFVSERNS2	5	4	1,18	1,30	1,31	0,65	1,21	2,05	1,66	0,00	0,89	83,56
PT2013QFVSERNS2	6	4	1,21	-0,68	1,31	0,65	1,21	2,05	1,66	-0,48	0,91	-162,07
PT2013QFVSERNS2	7	4	0,18	0,11	1,31	0,65	1,21	2,05	1,66	-0,29	0,35	403,62
PT2013QFVSERPS1	1	4	30,47	108,66	100,55	0,35	6,11	7,56	4,46	0,72	0,90	5,08
PT2013QFVSERPS1	2	4	2,28	92,03	100,55	0,35	6,11	7,56	4,46	-0,75	0,25	1,64
PT2013QFVSERPS1	3	4	4,80	89,20	100,55	0,35	6,11	7,56	4,46	-1,00	0,36	2,46
PT2013QFVSERPS1	4	4	143,23	117,94	100,55	0,35	6,11	7,56	4,46	1,53	1,96	10,15
PT2013QFVSERPS1	5	4	12,54	104,25	100,55	0,35	6,11	7,56	4,46	0,33	0,58	3,40
PT2013QFVSERPS1	6	4	2,41	104,38	100,55	0,35	6,11	7,56	4,46	0,34	0,25	1,49
PT2013QFVSERPS1	7	4	65,57	87,40	100,55	0,35	6,11	7,56	4,46	-1,16	1,33	9,27
PT2013QFVSERPS2	1	4	7,25	75,34	76,43	0,32	5,88	7,15	4,07	-0,10	0,46	3,57
PT2013QFVSERPS2	2	4	7,50	82,43	76,43	0,32	5,88	7,15	4,07	0,58	0,47	3,32
PT2013QFVSERPS2	3	4	31,43	66,48	76,43	0,32	5,88	7,15	4,07	-0,96	0,95	8,43
PT2013QFVSERPS2	4	4	76,34	95,25	76,43	0,32	5,88	7,15	4,07	1,81	1,49	9,17

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013QFVSERPS2	5	4	21,87	75,93	76,43	0,32	5,88	7,15	4,07	-0,05	0,79	6,16
PT2013QFVSERPS2	6	4	62,00	75,66	76,43	0,32	5,88	7,15	4,07	-0,07	1,34	10,41
PT2013QFVSERPS2	7	4	35,90	63,90	76,43	0,32	5,88	7,15	4,07	-1,21	1,02	9,38
PT2013QFVSERPS3	1	4	4,49	49,24	59,44	0,55	4,87	7,29	5,43	-0,76	0,44	4,30
PT2013QFVSERPS3	2	4	8,10	78,42	59,44	0,55	4,87	7,29	5,43	1,40	0,58	3,63
PT2013QFVSERPS3	3	4	8,10	49,98	59,44	0,55	4,87	7,29	5,43	-0,70	0,58	5,69
PT2013QFVSERPS3	**4**	4	91,32	78,13	59,44	0,55	4,87	7,29	5,43	1,38	**1,96**	12,23
PT2013QFVSERPS3	5	4	4,90	54,95	59,44	0,55	4,87	7,29	5,43	-0,33	0,45	4,03
PT2013QFVSERPS3	6	4	3,58	59,11	59,44	0,55	4,87	7,29	5,43	-0,02	0,39	3,20
PT2013QFVSERPS3	7	4	45,44	46,29	59,44	0,55	4,87	7,29	5,43	-0,97	1,38	14,56

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

B. Milk

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013QFVSERNM1	1	4	0,01	-0,75	-3,30	0,71	0,53	0,97	0,82	1,38	0,20	-14,13
PT2013QFVSERNM1	2	4	0,01	-5,97	-3,30	0,71	0,53	0,97	0,82	-1,44	0,18	-1,62
PT2013QFVSERNM1	3	4	1,32	-3,96	-3,30	0,71	0,53	0,97	0,82	-0,35	2,18	-29,04
PT2013QFVSERNM1	4	4	0,02	-2,95	-3,30	0,71	0,53	0,97	0,82	0,19	0,28	-5,07
PT2013QFVSERNM1	5	4	0,00	-4,30	-3,30	0,71	0,53	0,97	0,82	-0,54	0,10	-1,28
PT2013QFVSERNM1	8	4	0,30	-1,91	-3,30	0,71	0,53	0,97	0,82	0,76	1,03	-28,58
PT2013QFVSERNM2	1	3	0,02	-0,90	-3,36	0,79	0,42	0,91	0,81	1,34	0,31	-14,48
PT2013QFVSERNM2	2	3	0,02	-5,88	-3,36	0,79	0,42	0,91	0,81	-1,37	0,30	-2,10
PT2013QFVSERNM2	3	3	0,53	-4,31	-3,36	0,79	0,42	0,91	0,81	-0,51	1,74	-16,86
PT2013QFVSERNM2	4	3	0,02	-2,95	-3,36	0,79	0,42	0,91	0,81	0,23	0,37	-5,17
PT2013QFVSERNM2	5	3	0,02	-4,33	-3,36	0,79	0,42	0,91	0,81	-0,53	0,35	-3,36
PT2013QFVSERNM2	8	3	0,44	-1,82	-3,36	0,79	0,42	0,91	0,81	0,84	1,59	-36,57
PT2013QFVSERPM1	1	3	69,92	164,69	142,57	0,49	26,07	36,65	25,76	0,37	0,32	5,08
PT2013QFVSERPM1	2	3	15,91	79,95	142,57	0,49	26,07	36,65	25,76	-1,05	0,15	4,99
PT2013QFVSERPM1	3	3	3613,08	203,56	142,57	0,49	26,07	36,65	25,76	1,02	2,31	29,53
PT2013QFVSERPM1	4	3	55,38	61,18	142,57	0,49	26,07	36,65	25,76	-1,37	0,29	12,16
PT2013QFVSERPM1	5	3	319,21	196,59	142,57	0,49	26,07	36,65	25,76	0,91	0,69	9,09
PT2013QFVSERPM1	8	3	5,54	149,43	142,57	0,49	26,07	36,65	25,76	0,12	0,09	1,58
PT2013QFVSERPM2	1	5	4,27	60,28	77,68	0,72	5,41	10,28	8,75	-0,88	0,38	3,43
PT2013QFVSERPM2	2	5	9,99	90,24	77,68	0,72	5,41	10,28	8,75	0,64	0,58	3,50
PT2013QFVSERPM2	3	5	72,82	66,92	77,68	0,72	5,41	10,28	8,75	-0,55	1,58	12,75
PT2013QFVSERPM2	4	5	71,53	111,76	77,68	0,72	5,41	10,28	8,75	1,73	1,56	7,57
PT2013QFVSERPM2	5	5	15,42	64,33	77,68	0,72	5,41	10,28	8,75	-0,68	0,73	6,10
PT2013QFVSERPM2	8	5	1,47	72,58	77,68	0,72	5,41	10,28	8,75	-0,26	0,22	1,67
PT2013QFVSERPM3	1	5	15,63	79,37	95,20	0,80	4,53	10,19	9,13	-0,77	0,87	4,98

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013QFVSERPM3	2	5	2,33	99,69	95,20	0,80	4,53	10,19	9,13	0,22	0,34	1,53
PT2013QFVSERPM3	3	5	39,32	93,43	95,20	0,80	4,53	10,19	9,13	-0,09	1,38	6,71
PT2013QFVSERPM3	**4**	5	49,44	132,94	95,20	0,80	4,53	10,19	9,13	**1,84**	**1,55**	5,29
PT2013QFVSERPM3	5	5	13,61	90,04	95,20	0,80	4,53	10,19	9,13	-0,25	0,81	4,10
PT2013QFVSERPM3	8	5	2,79	75,74	95,20	0,80	4,53	10,19	9,13	-0,95	0,37	2,21

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