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172-PT

PROFICIENCY TESTING 2012

Paratuberculosis (PTU)

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

OPERATIONAL UNIT
COORDINATION OF VETERINARY DIAGNOSIS
EPIDEMIOLOGY AND RISK ASSESSMENT
(CVD-ERA)

DATE BEGIN PT: 26 NOVEMBER 2012
DATE REPORT: 21 JANUARY 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 PTU-specific antibodies in bovine serum and/or bovine milk by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum and/or reference milk samples must be analyzed by means of a PTU antibody ELISA test. The procedures for these 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 6 reference serum samples of bovine origin, either free from detectable PTU-specific antibodies (n = 3; coded 'PT2012PTUSERNS1', 'PT2012PTUSERNS2' and 'PT2012PTUSERNS3') or containing detectable PTU-specific antibodies (n = 3; coded 'PT2012PTUSERPS1', 'PT2012PTUSERPS2' and 'PT2012PTUSERPS3'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012PTUSERNS1, PT2012PTUSERNS3, PT2012PTUSERPS1 and PT2012PTUSERPS3, and 4 aliquots of the reference serum samples PT2012PTUSERNS2 and PT2012PTUSERPS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (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 (i) the historical background of the animals and (ii) the results obtained by the Pourquier® ELISA Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET (pre-verification). The reference serum samples PT2012PTUSERNS1, PT2012PTUSERNS2 and PT2012PTUSERNS3 were PTU antibody negative field sera, whereas the reference serum samples PT2012PTUSERPS1, PT2012PTUSERPS2 and PT2012PTUSERPS3 were a 1/8, 1/12 and 1/16 dilution, respectively, of a serum obtained from an animal that was PTU antibody positive. For each reference serum sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference serum samples PT2012PTUSERNS1, PT2012PTUSERNS2 and PT2012PTUSERNS3 were considered as negative sera, and the reference serum samples PT2012PTUSERPS1, PT2012PTUSERPS2 and PT2012PTUSERPS3 as variably positive sera in PTU antibody ELISA. However, since the reference serum sample PT2012PTUSERPS3 showed percentages S/P ratio that were relatively close to the cut-off for the IDVET kit, this reference serum sample could also be reported as non-interpretable.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the Pourquier® ELISA Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample with both ELISA kits. 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 PTU-specific antibodies in bovine 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 Pourquier® ELISA Paratuberculosis Screening Antibody Test Kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET.

III.2.2. Reference milk samples

Replicates of 6 reference milk samples of bovine origin, either free from detectable PTU-specific antibodies (n = 2; coded 'PT2012PTUSERNM1' and 'PT2012PTUSERNM2') or containing detectable PTU-specific antibodies (n = 4; coded 'PT2012PTUSERPM1', 'PT2012PTUSERPM2', 'PT2012PTUSERPM3' and 'PT2012PTUSERPM4'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference milk samples PT2012PTUSERNM2, PT2012PTUSERPM1, PT2012PTUSERPM2 and PT2012PTUSERPM3, and 4 aliquots of the reference milk samples PT2012PTUSERNM1 and PT2012PTUSERPM4. The positions of the reference milk samples in the sent blocks were randomized for each participant (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 (i) the historical background of the animals and (ii) the results obtained by the IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET (pre-verification). The reference milk samples PT2012PTUSERNM1 and PT2012PTUSERNM2 were derived from 2 different brands of commercial defatted milk, whereas the reference milk samples PT2012PTUSERPM1 and PT2012PTUSERPM4 were obtained from animals that were PTU antibody positive. The reference milk samples PT2012PTUSERPM2 and PT2012PTUSERPM3 were a 1/2 and 1/4 dilution, respectively, of the reference milk sample PT2012PTUSERPM1. For each reference milk sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference milk samples PT2012PTUSERNM1 and PT2012PTUSERNM2 were considered as negative milk samples, and the reference milk samples PT2012PTUSERPM1, PT2012PTUSERPM2, PT2012PTUSERPM3 and PT2012PTUSERPM4 as variably positive milk samples in PTU antibody ELISA.

After aliquoting the different reference milk samples, a homogeneity check was performed on 10 aliquots of each reference milk sample using the IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample with both ELISA kits. Consequently, all reference milk samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of PTU-specific antibodies in bovine 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 IDEXX Paratuberculosis Screening Ab Test kit from IDEXX Montpellier SAS and the ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET.

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 and LAB4 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, LAB5, LAB6, LAB7 and LAB8 only participated in the PT serum and hence received 20 aliquots of reference serum samples, whereas LAB9 and LAB10 only participated in the PT milk and thus received 20 aliquots of reference milk samples. The reference serum samples (160 aliquots in total) and reference milk samples (120 aliquots in total) were sent frozen (dry ice) to the 10 participating laboratories by national or international courier on the 26th of November 2012. LAB3, LAB4, LAB5, LAB8, LAB9 and LAB10 acknowledged receipt of the samples on the same day, whereas the other participants acknowledged receipt of the samples on 27th (LAB1, LAB2 and LAB6) and 28th (LAB7) of November 2012. Analyses were performed between 27th of November and 6th of December 2012, both for the PT serum and the PT milk (LAB10 did not communicate the date of analysis) (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 28th of November and 12th of December 2012 (Table 1). LAB4 and LAB10 hereby exceeded the deadline of 7th of December 2012 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	27/11/2012	30/11/2012	30/11/2012	03/12/2012
LAB2	27/11/2012	06/12/2012	06/12/2012	07/12/2012
LAB3	26/11/2012	03/12/2012	03/12/2012	04/12/2012
LAB4	26/11/2012	28/11/2012	28/11/2012	**10/12/2012** ¶
LAB5	26/11/2012	27/11/2012	NA	29/11/2012
LAB6	27/11/2012	03/12/2012	NA	04/12/2012
LAB7	28/11/2012	05/12/2012	NA	06/12/2012
LAB8	26/11/2012	28/11/2012	NA	07/12/2012
LAB9	26/11/2012	NA	27/11/2012	28/11/2012
LAB10	26/11/2012	NA	**NOT PROVIDED**	**12/12/2012**

Legend: NA = not applicable; ¶ LAB4 sent a dated and signed copy of its results on 30th of November 2012 but forgot to send the Excel file by email

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 PTU-specific antibodies in **serum**, 7 out of 8 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples and thus achieved 100% of agreement. LAB1 misclassified 9 reference serum samples and hence reached 55% of agreement (Table 2).

(ii) For the detection of PTU-specific antibodies in **milk**, all 6 participating laboratories provided qualitative results that were in full agreement with the true 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 results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the PTU reference laboratory of 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**	**8**
failure	**9 (45.0)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	**11 (55.0)**	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 results generated by the participating laboratories (LABNR) and the status of the reference milk samples assigned by the PTU reference laboratory of 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**	**9**	**10**
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

Only a small variability between laboratories could be observed at the qualitative data level:

(i) For the detection of PTU-specific antibodies in reference **serum** samples, no variability between qualitative laboratory results could be observed for LAB2 until LAB8 since these participants correctly identified all reference serum samples. In contrast, LAB1 misclassified 2 out of 3 aliquots of the positive reference serum sample PT2012PTUSERPS1 (2x NI instead of POS) and all aliquots of the positive reference serum samples PT2012PTUSERPS2 (1x NI and 3x NEG instead of POS) and PT2012PTUSERPS3 (3x NEG instead of POS/NI).

(ii) For the detection of PTU-specific antibodies in reference **milk** samples, no variability between qualitative laboratory results could be observed since all participating laboratories reached 100% of agreement.

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 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 the PTU reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive; NI: non-interpretable.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012PTUSERNS1	NEG	NEG	1
2	1	2	PT2012PTUSERNS2	NEG	NEG	1
3	1	3	PT2012PTUSERNS1	NEG	NEG	1
4	1	4	PT2012PTUSERNS2	NEG	NEG	1
5	1	5	PT2012PTUSERNS1	NEG	NEG	1
6	1	6	PT2012PTUSERNS2	NEG	NEG	1
7	1	7	PT2012PTUSERPS1	POS	POS	1
8	1	8	PT2012PTUSERNS2	NEG	NEG	1
9	1	9	PT2012PTUSERPS1	**POS**	**NI**	**0**
10	1	10	PT2012PTUSERNS3	NEG	NEG	1
11	1	11	PT2012PTUSERPS1	**POS**	**NI**	**0**
12	1	12	PT2012PTUSERNS3	NEG	NEG	1
13	1	13	PT2012PTUSERPS2	**POS**	**NI**	**0**
14	1	14	PT2012PTUSERNS3	NEG	NEG	1
15	1	15	PT2012PTUSERPS2	**POS**	**NEG**	**0**
16	1	16	PT2012PTUSERPS3	**POS/NI**	**NEG**	**0**
17	1	17	PT2012PTUSERPS2	**POS**	**NEG**	**0**
18	1	18	PT2012PTUSERPS3	**POS/NI**	**NEG**	**0**
19	1	19	PT2012PTUSERPS2	**POS**	**NEG**	**0**
20	1	20	PT2012PTUSERPS3	**POS/NI**	**NEG**	**0**
21	2	1	PT2012PTUSERPS3	POS/NI	POS	1
22	2	2	PT2012PTUSERNS1	NEG	NEG	1
23	2	3	PT2012PTUSERNS2	NEG	NEG	1
24	2	4	PT2012PTUSERNS1	NEG	NEG	1
25	2	5	PT2012PTUSERNS2	NEG	NEG	1
26	2	6	PT2012PTUSERNS1	NEG	NEG	1
27	2	7	PT2012PTUSERNS2	NEG	NEG	1
28	2	8	PT2012PTUSERPS1	POS	POS	1
29	2	9	PT2012PTUSERNS2	NEG	NEG	1
30	2	10	PT2012PTUSERPS1	POS	POS	1
31	2	11	PT2012PTUSERNS3	NEG	NEG	1
32	2	12	PT2012PTUSERPS1	POS	POS	1
33	2	13	PT2012PTUSERNS3	NEG	NEG	1
34	2	14	PT2012PTUSERPS2	POS	POS	1
35	2	15	PT2012PTUSERNS3	NEG	NEG	1
36	2	16	PT2012PTUSERPS2	POS	POS	1
37	2	17	PT2012PTUSERPS3	POS/NI	POS	1
38	2	18	PT2012PTUSERPS2	POS	POS	1
39	2	19	PT2012PTUSERPS3	POS/NI	POS	1
40	2	20	PT2012PTUSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012PTUSERPS2	POS	POS	1
42	3	2	PT2012PTUSERPS3	POS/NI	POS	1
43	3	3	PT2012PTUSERNS1	NEG	NEG	1
44	3	4	PT2012PTUSERNS2	NEG	NEG	1
45	3	5	PT2012PTUSERNS1	NEG	NEG	1
46	3	6	PT2012PTUSERNS2	NEG	NEG	1
47	3	7	PT2012PTUSERNS1	NEG	NEG	1
48	3	8	PT2012PTUSERNS2	NEG	NEG	1
49	3	9	PT2012PTUSERPS1	POS	POS	1
50	3	10	PT2012PTUSERNS2	NEG	NEG	1
51	3	11	PT2012PTUSERPS1	POS	POS	1
52	3	12	PT2012PTUSERNS3	NEG	NEG	1
53	3	13	PT2012PTUSERPS1	POS	POS	1
54	3	14	PT2012PTUSERNS3	NEG	NEG	1
55	3	15	PT2012PTUSERPS2	POS	POS	1
56	3	16	PT2012PTUSERNS3	NEG	NEG	1
57	3	17	PT2012PTUSERPS2	POS	POS	1
58	3	18	PT2012PTUSERPS3	POS/NI	POS	1
59	3	19	PT2012PTUSERPS2	POS	POS	1
60	3	20	PT2012PTUSERPS3	POS/NI	POS	1
61	4	1	PT2012PTUSERPS3	POS/NI	POS	1
62	4	2	PT2012PTUSERPS2	POS	POS	1
63	4	3	PT2012PTUSERPS3	POS/NI	POS	1
64	4	4	PT2012PTUSERNS1	NEG	NEG	1
65	4	5	PT2012PTUSERNS2	NEG	NEG	1
66	4	6	PT2012PTUSERNS1	NEG	NEG	1
67	4	7	PT2012PTUSERNS2	NEG	NEG	1
68	4	8	PT2012PTUSERNS1	NEG	NEG	1
69	4	9	PT2012PTUSERNS2	NEG	NEG	1
70	4	10	PT2012PTUSERPS1	POS	POS	1
71	4	11	PT2012PTUSERNS2	NEG	NEG	1
72	4	12	PT2012PTUSERPS1	POS	POS	1
73	4	13	PT2012PTUSERNS3	NEG	NEG	1
74	4	14	PT2012PTUSERPS1	POS	POS	1
75	4	15	PT2012PTUSERNS3	NEG	NEG	1
76	4	16	PT2012PTUSERPS2	POS	POS	1
77	4	17	PT2012PTUSERNS3	NEG	NEG	1
78	4	18	PT2012PTUSERPS2	POS	POS	1
79	4	19	PT2012PTUSERPS3	POS/NI	POS	1
80	4	20	PT2012PTUSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2012PTUSERPS2	POS	POS	1
82	5	2	PT2012PTUSERPS3	POS/NI	POS	1
83	5	3	PT2012PTUSERPS2	POS	POS	1
84	5	4	PT2012PTUSERPS3	POS/NI	POS	1
85	5	5	PT2012PTUSERNS1	NEG	NEG	1
86	5	6	PT2012PTUSERNS2	NEG	NEG	1
87	5	7	PT2012PTUSERNS1	NEG	NEG	1
88	5	8	PT2012PTUSERNS2	NEG	NEG	1
89	5	9	PT2012PTUSERNS1	NEG	NEG	1
90	5	10	PT2012PTUSERNS2	NEG	NEG	1
91	5	11	PT2012PTUSERPS1	POS	POS	1
92	5	12	PT2012PTUSERNS2	NEG	NEG	1
93	5	13	PT2012PTUSERPS1	POS	POS	1
94	5	14	PT2012PTUSERNS3	NEG	NEG	1
95	5	15	PT2012PTUSERPS1	POS	POS	1
96	5	16	PT2012PTUSERNS3	NEG	NEG	1
97	5	17	PT2012PTUSERPS2	POS	POS	1
98	5	18	PT2012PTUSERNS3	NEG	NEG	1
99	5	19	PT2012PTUSERPS2	POS	POS	1
100	5	20	PT2012PTUSERPS3	POS/NI	POS	1
101	6	1	PT2012PTUSERPS3	POS/NI	POS	1
102	6	2	PT2012PTUSERPS2	POS	POS	1
103	6	3	PT2012PTUSERPS3	POS/NI	POS	1
104	6	4	PT2012PTUSERPS2	POS	POS	1
105	6	5	PT2012PTUSERPS3	POS/NI	POS	1
106	6	6	PT2012PTUSERNS1	NEG	NEG	1
107	6	7	PT2012PTUSERNS2	NEG	NEG	1
108	6	8	PT2012PTUSERNS1	NEG	NEG	1
109	6	9	PT2012PTUSERNS2	NEG	NEG	1
110	6	10	PT2012PTUSERNS1	NEG	NEG	1
111	6	11	PT2012PTUSERNS2	NEG	NEG	1
112	6	12	PT2012PTUSERPS1	POS	POS	1
113	6	13	PT2012PTUSERNS2	NEG	NEG	1
114	6	14	PT2012PTUSERPS1	POS	POS	1
115	6	15	PT2012PTUSERNS3	NEG	NEG	1
116	6	16	PT2012PTUSERPS1	POS	POS	1
117	6	17	PT2012PTUSERNS3	NEG	NEG	1
118	6	18	PT2012PTUSERPS2	POS	POS	1
119	6	19	PT2012PTUSERNS3	NEG	NEG	1
120	6	20	PT2012PTUSERPS2	POS	POS	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2012PTUSERPS2	POS	POS	1
122	7	2	PT2012PTUSERPS3	POS/NI	POS	1
123	7	3	PT2012PTUSERPS2	POS	POS	1
124	7	4	PT2012PTUSERPS3	POS/NI	POS	1
125	7	5	PT2012PTUSERPS2	POS	POS	1
126	7	6	PT2012PTUSERPS3	POS/NI	POS	1
127	7	7	PT2012PTUSERNS1	NEG	NEG	1
128	7	8	PT2012PTUSERNS2	NEG	NEG	1
129	7	9	PT2012PTUSERNS1	NEG	NEG	1
130	7	10	PT2012PTUSERNS2	NEG	NEG	1
131	7	11	PT2012PTUSERNS1	NEG	NEG	1
132	7	12	PT2012PTUSERNS2	NEG	NEG	1
133	7	13	PT2012PTUSERPS1	POS	POS	1
134	7	14	PT2012PTUSERNS2	NEG	NEG	1
135	7	15	PT2012PTUSERPS1	POS	POS	1
136	7	16	PT2012PTUSERNS3	NEG	NEG	1
137	7	17	PT2012PTUSERPS1	POS	POS	1
138	7	18	PT2012PTUSERNS3	NEG	NEG	1
139	7	19	PT2012PTUSERPS2	POS	POS	1
140	7	20	PT2012PTUSERNS3	NEG	NEG	1
141	8	1	PT2012PTUSERNS3	NEG	NEG	1
142	8	2	PT2012PTUSERPS2	POS	POS	1
143	8	3	PT2012PTUSERPS3	POS/NI	POS	1
144	8	4	PT2012PTUSERPS2	POS	POS	1
145	8	5	PT2012PTUSERPS3	POS/NI	POS	1
146	8	6	PT2012PTUSERPS2	POS	POS	1
147	8	7	PT2012PTUSERPS3	POS/NI	POS	1
148	8	8	PT2012PTUSERNS1	NEG	NEG	1
149	8	9	PT2012PTUSERNS2	NEG	NEG	1
150	8	10	PT2012PTUSERNS1	NEG	NEG	1
151	8	11	PT2012PTUSERNS2	NEG	NEG	1
152	8	12	PT2012PTUSERNS1	NEG	NEG	1
153	8	13	PT2012PTUSERNS2	NEG	NEG	1
154	8	14	PT2012PTUSERPS1	POS	POS	1
155	8	15	PT2012PTUSERNS2	NEG	NEG	1
156	8	16	PT2012PTUSERPS1	POS	POS	1
157	8	17	PT2012PTUSERNS3	NEG	NEG	1
158	8	18	PT2012PTUSERPS1	POS	POS	1
159	8	19	PT2012PTUSERNS3	NEG	NEG	1
160	8	20	PT2012PTUSERPS2	POS	POS	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference milk samples (SAMPLE), the positions of the reference milk samples as placed in the block (LABPOSIT), and the status assigned by the PTU reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012PTUSERNM1	NEG	NEG	1
2	1	2	PT2012PTUSERNM2	NEG	NEG	1
3	1	3	PT2012PTUSERNM1	NEG	NEG	1
4	1	4	PT2012PTUSERNM2	NEG	NEG	1
5	1	5	PT2012PTUSERNM1	NEG	NEG	1
6	1	6	PT2012PTUSERNM2	NEG	NEG	1
7	1	7	PT2012PTUSERNM1	NEG	NEG	1
8	1	8	PT2012PTUSERPM3	POS	POS	1
9	1	9	PT2012PTUSERPM1	POS	POS	1
10	1	10	PT2012PTUSERPM3	POS	POS	1
11	1	11	PT2012PTUSERPM1	POS	POS	1
12	1	12	PT2012PTUSERPM3	POS	POS	1
13	1	13	PT2012PTUSERPM1	POS	POS	1
14	1	14	PT2012PTUSERPM4	POS	POS	1
15	1	15	PT2012PTUSERPM2	POS	POS	1
16	1	16	PT2012PTUSERPM4	POS	POS	1
17	1	17	PT2012PTUSERPM2	POS	POS	1
18	1	18	PT2012PTUSERPM4	POS	POS	1
19	1	19	PT2012PTUSERPM2	POS	POS	1
20	1	20	PT2012PTUSERPM4	POS	POS	1
21	2	1	PT2012PTUSERPM4	POS	POS	1
22	2	2	PT2012PTUSERNM1	NEG	NEG	1
23	2	3	PT2012PTUSERNM2	NEG	NEG	1
24	2	4	PT2012PTUSERNM1	NEG	NEG	1
25	2	5	PT2012PTUSERNM2	NEG	NEG	1
26	2	6	PT2012PTUSERNM1	NEG	NEG	1
27	2	7	PT2012PTUSERNM2	NEG	NEG	1
28	2	8	PT2012PTUSERNM1	NEG	NEG	1
29	2	9	PT2012PTUSERPM3	POS	POS	1
30	2	10	PT2012PTUSERPM1	POS	POS	1
31	2	11	PT2012PTUSERPM3	POS	POS	1
32	2	12	PT2012PTUSERPM1	POS	POS	1
33	2	13	PT2012PTUSERPM3	POS	POS	1
34	2	14	PT2012PTUSERPM1	POS	POS	1
35	2	15	PT2012PTUSERPM4	POS	POS	1
36	2	16	PT2012PTUSERPM2	POS	POS	1
37	2	17	PT2012PTUSERPM4	POS	POS	1
38	2	18	PT2012PTUSERPM2	POS	POS	1
39	2	19	PT2012PTUSERPM4	POS	POS	1
40	2	20	PT2012PTUSERPM2	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012PTUSERPM2	POS	POS	1
42	3	2	PT2012PTUSERPM4	POS	POS	1
43	3	3	PT2012PTUSERNM1	NEG	NEG	1
44	3	4	PT2012PTUSERNM2	NEG	NEG	1
45	3	5	PT2012PTUSERNM1	NEG	NEG	1
46	3	6	PT2012PTUSERNM2	NEG	NEG	1
47	3	7	PT2012PTUSERNM1	NEG	NEG	1
48	3	8	PT2012PTUSERNM2	NEG	NEG	1
49	3	9	PT2012PTUSERNM1	NEG	NEG	1
50	3	10	PT2012PTUSERPM3	POS	POS	1
51	3	11	PT2012PTUSERPM1	POS	POS	1
52	3	12	PT2012PTUSERPM3	POS	POS	1
53	3	13	PT2012PTUSERPM1	POS	POS	1
54	3	14	PT2012PTUSERPM3	POS	POS	1
55	3	15	PT2012PTUSERPM1	POS	POS	1
56	3	16	PT2012PTUSERPM4	POS	POS	1
57	3	17	PT2012PTUSERPM2	POS	POS	1
58	3	18	PT2012PTUSERPM4	POS	POS	1
59	3	19	PT2012PTUSERPM2	POS	POS	1
60	3	20	PT2012PTUSERPM4	POS	POS	1
61	4	1	PT2012PTUSERPM4	POS	POS	1
62	4	2	PT2012PTUSERPM2	POS	POS	1
63	4	3	PT2012PTUSERPM4	POS	POS	1
64	4	4	PT2012PTUSERNM1	NEG	NEG	1
65	4	5	PT2012PTUSERNM2	NEG	NEG	1
66	4	6	PT2012PTUSERNM1	NEG	NEG	1
67	4	7	PT2012PTUSERNM2	NEG	NEG	1
68	4	8	PT2012PTUSERNM1	NEG	NEG	1
69	4	9	PT2012PTUSERNM2	NEG	NEG	1
70	4	10	PT2012PTUSERNM1	NEG	NEG	1
71	4	11	PT2012PTUSERPM3	POS	POS	1
72	4	12	PT2012PTUSERPM1	POS	POS	1
73	4	13	PT2012PTUSERPM3	POS	POS	1
74	4	14	PT2012PTUSERPM1	POS	POS	1
75	4	15	PT2012PTUSERPM3	POS	POS	1
76	4	16	PT2012PTUSERPM1	POS	POS	1
77	4	17	PT2012PTUSERPM4	POS	POS	1
78	4	18	PT2012PTUSERPM2	POS	POS	1
79	4	19	PT2012PTUSERPM4	POS	POS	1
80	4	20	PT2012PTUSERPM2	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	9	1	PT2012PTUSERPM2	POS	POS	1
82	9	2	PT2012PTUSERPM4	POS	POS	1
83	9	3	PT2012PTUSERPM2	POS	POS	1
84	9	4	PT2012PTUSERPM4	POS	POS	1
85	9	5	PT2012PTUSERNM1	NEG	NEG	1
86	9	6	PT2012PTUSERNM2	NEG	NEG	1
87	9	7	PT2012PTUSERNM1	NEG	NEG	1
88	9	8	PT2012PTUSERNM2	NEG	NEG	1
89	9	9	PT2012PTUSERNM1	NEG	NEG	1
90	9	10	PT2012PTUSERNM2	NEG	NEG	1
91	9	11	PT2012PTUSERNM1	NEG	NEG	1
92	9	12	PT2012PTUSERPM3	POS	POS	1
93	9	13	PT2012PTUSERPM1	POS	POS	1
94	9	14	PT2012PTUSERPM3	POS	POS	1
95	9	15	PT2012PTUSERPM1	POS	POS	1
96	9	16	PT2012PTUSERPM3	POS	POS	1
97	9	17	PT2012PTUSERPM1	POS	POS	1
98	9	18	PT2012PTUSERPM4	POS	POS	1
99	9	19	PT2012PTUSERPM2	POS	POS	1
100	9	20	PT2012PTUSERPM4	POS	POS	1
101	10	1	PT2012PTUSERPM4	POS	POS	1
102	10	2	PT2012PTUSERPM2	POS	POS	1
103	10	3	PT2012PTUSERPM4	POS	POS	1
104	10	4	PT2012PTUSERPM2	POS	POS	1
105	10	5	PT2012PTUSERPM4	POS	POS	1
106	10	6	PT2012PTUSERNM1	NEG	NEG	1
107	10	7	PT2012PTUSERNM2	NEG	NEG	1
108	10	8	PT2012PTUSERNM1	NEG	NEG	1
109	10	9	PT2012PTUSERNM2	NEG	NEG	1
110	10	10	PT2012PTUSERNM1	NEG	NEG	1
111	10	11	PT2012PTUSERNM2	NEG	NEG	1
112	10	12	PT2012PTUSERNM1	NEG	NEG	1
113	10	13	PT2012PTUSERPM3	POS	POS	1
114	10	14	PT2012PTUSERPM1	POS	POS	1
115	10	15	PT2012PTUSERPM3	POS	POS	1
116	10	16	PT2012PTUSERPM1	POS	POS	1
117	10	17	PT2012PTUSERPM3	POS	POS	1
118	10	18	PT2012PTUSERPM1	POS	POS	1
119	10	19	PT2012PTUSERPM4	POS	POS	1
120	10	20	PT2012PTUSERPM2	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 reference milk samples of bovine origin for the detection of PTU-specific antibodies by ELISA.

For the detection of PTU-specific antibodies in serum, 7 out of 8 participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). LAB1 misclassified 2 aliquots of the positive reference serum sample PT2012PTUSERPS1, 4 aliquots of the positive reference serum sample PT2012PTUSERPS2 and 3 aliquots of the positive reference serum sample PT2012PTUSERPS3 (55% of agreement) (Table 2 and Table 4).
The participating laboratories used PTU antibody ELISA kits from 4 different producers as well as different kits from the same producer and different batches from the same ELISA kit: IDEXX Montpellier SAS (IDEXX Paratuberculosis Screening Ab Test Kit - 3 batches: 1171, 2185 and 2211; IDEXX Paratuberculosis Verification Ab Test Kit - 1 batch: 1044), IDVET (2 batches: 367 and 445), LSI (1 batch: 5-VETPTRS-014) and Synbiotics (1 batch: 12SPTB3N02). LAB3, LAB4, LAB6 and LAB7 on the one hand, and LAB5 and LAB8 on the other hand used a PTU antibody ELISA kit from the same producer (different batches). In addition, all these participants performed the short incubation protocol of the used ELISA kit. Note that LAB6 used another ELISA kit than LAB3, LAB4 and LAB7.

For the detection of PTU-specific antibodies in milk, all 6 participating laboratories provided qualitative results that were in full agreement with the true status of the reference milk samples (100% of agreement) (Table 3 and Table 5).
The participating laboratories used PTU antibody ELISA kits from 2 different producers as well as different batches from the same producer: IDEXX Montpellier SAS (IDEXX Paratuberculosis Screening Ab Test kit - 2 batches: 1171 and 2185), IDVET (1 batch: 367), LSI (1 batch: 5-PTML-003) and Synbiotics (1 batch: 12SPTB3N02). LAB3, LAB4 and LAB10 used a PTU antibody ELISA kit from the same producer. Hereby, LAB3 and LAB10 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 the PTU reference laboratory of CODA-CERVA (see III.3.3.). Consequently, 7 out of 8 participants in the PT serum achieved a satisfactory performance for the detection of PTU-specific antibodies in reference serum samples, whereas all 6 participants in the PT milk achieved a satisfactory performance for the detection of PTU-specific antibodies in reference milk samples. For the PT serum, LAB1 did not reach the required 90% of agreement.

Head CVD-ERA
Yves Van der Stede

Appendix

Names of the participating laboratories

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

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

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

IDEXX Montpellier SAS (Montpellier, France)

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

Laboratoire Service International (LSI) (Lissieu, France)

Melkcontrolecentrum Vlaanderen (MCC) (Lier, Belgium)

Synbiotics Europe (Lyon, France)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs SAS 9.2. (summary statistics) and R (box plots). All quantitative analyses were performed on the normalized data, namely the percentages S/P ratio calculated according to the instructions of the PT provider: [(OD_{Sample} - mean $OD_{Negative\ Kit\ Controls}$) / (mean $OD_{Positive\ Kit\ Controls}$ - mean $OD_{Negative\ Kit\ Controls}$)] *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.

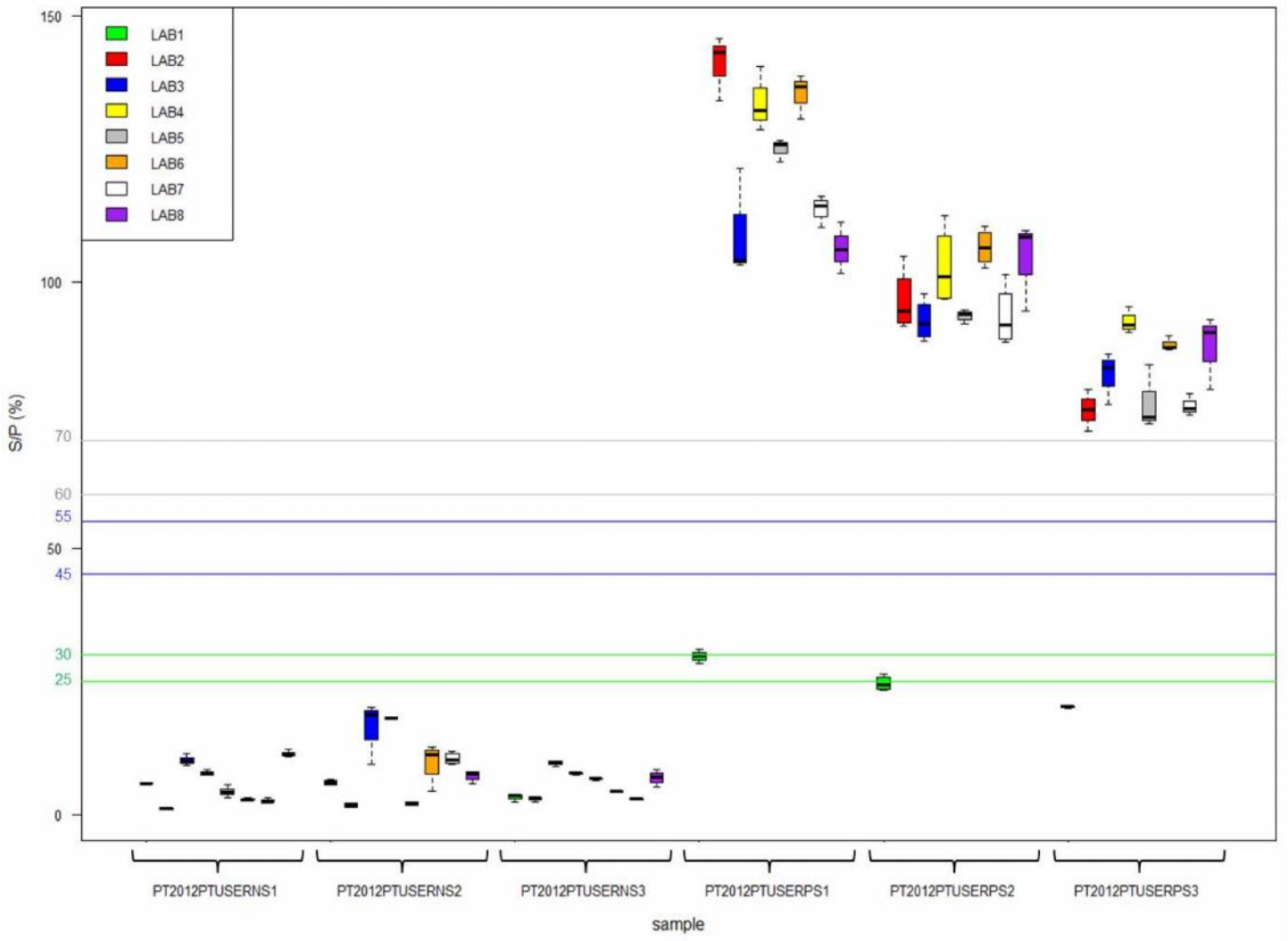

Figure 1. Box plots showing the percentages S/P ratio per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in green (25-30%; LAB1), blue (45-55%; LAB3, LAB4, LAB6, LAB7), grey (60-70%; LAB5, LAB8) and grey - lower line - (60%; LAB2), respectively. LAB3, LAB4, LAB6 and LAB7 on the one hand, and LAB5 and LAB8 on the other hand used a PTU antibody ELISA kit from the same producer (LAB6 used another ELISA kit than LAB3, LAB4 and LAB7). In addition, all these participants performed the short incubation protocol of the used ELISA kit.

Box plots of the percentages S/P ratio per reference milk sample and per participating laboratory were also made using the statistical software R and are shown in Figure 2A and Figure 2B. Because LAB2 calculated percentages S/P ratio using a weak positive kit control, it obtained percentages S/P ratio that were much higher compared to the other partipating laboratories, which used a strong positive kit control (Figure 2A). Therefore, box plots are also shown without LAB2 (Figure 2B).

Remark: To calculate the percentages S/P ratio, the PT provider used the formula [(OD_{Sample} - mean $OD_{Negative\ Kit\ Controls}$) / (mean $OD_{Positive\ Kit\ Controls}$ - mean $OD_{Negative\ Kit\ Controls}$)] *100. Because LAB2 calculated the percentages S/P ratio using the formula [OD_{Sample} / mean $OD_{Positive\ Kit\ Controls}$] * 100, the cut-offs for the PTU antibody ELISA kit used by LAB2 were adapted accordingly (41-56% instead of 60-70%).

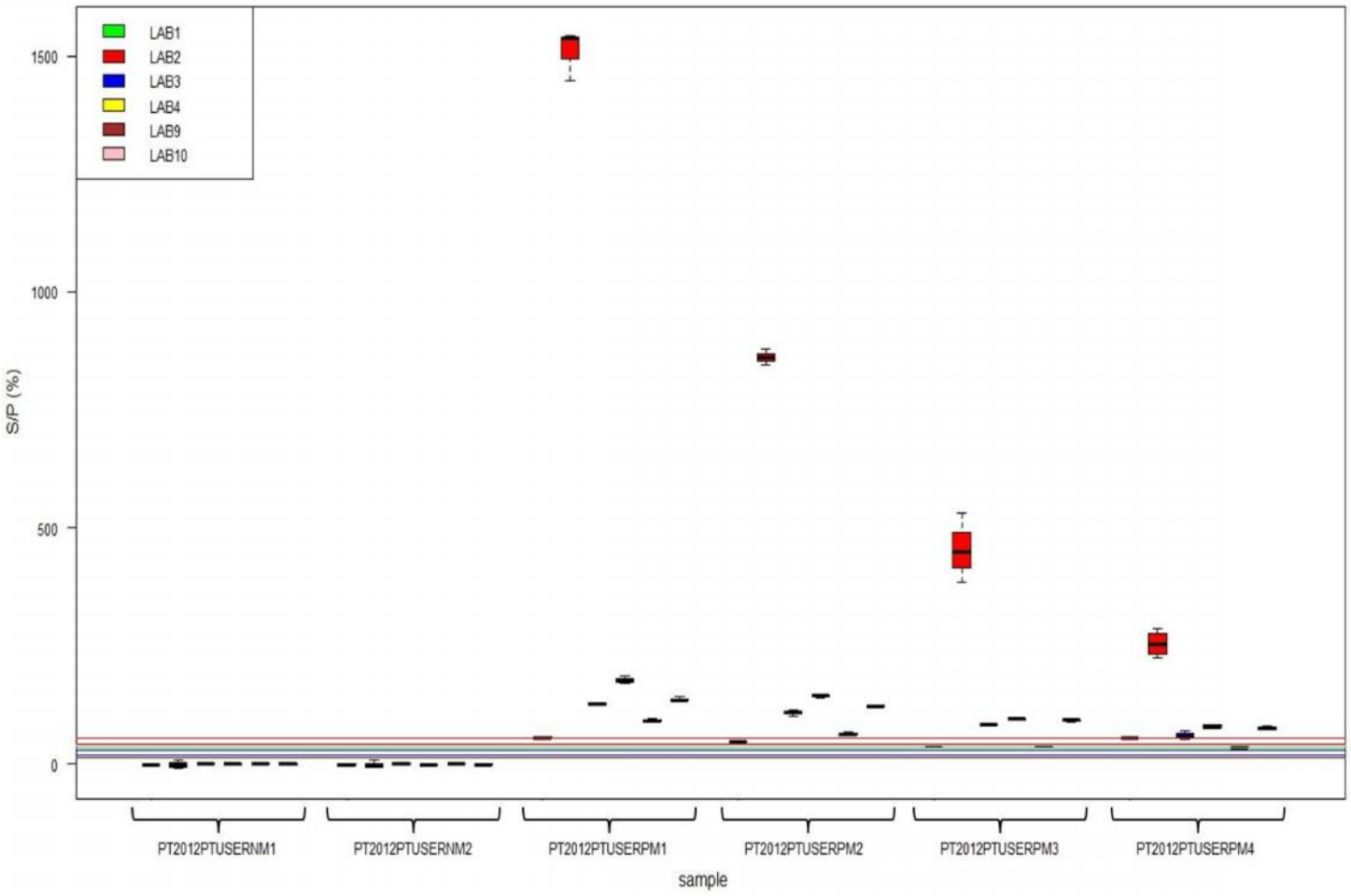

Figure 2A. Box plots showing the percentages S/P ratio per reference milk sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are shown in brown (15%; LAB9), blue (20-30%; LAB3, LAB4), green (35%; LAB1), blue - upper line - and pink (30-40%; LAB10), red (41-56%; LAB2) respectively. LAB3, LAB4 and LAB10 used a PTU antibody ELISA kit from the same producer (LAB10 applied alternative cut-off values). Hereby, LAB3 and LAB10 used the same batch.

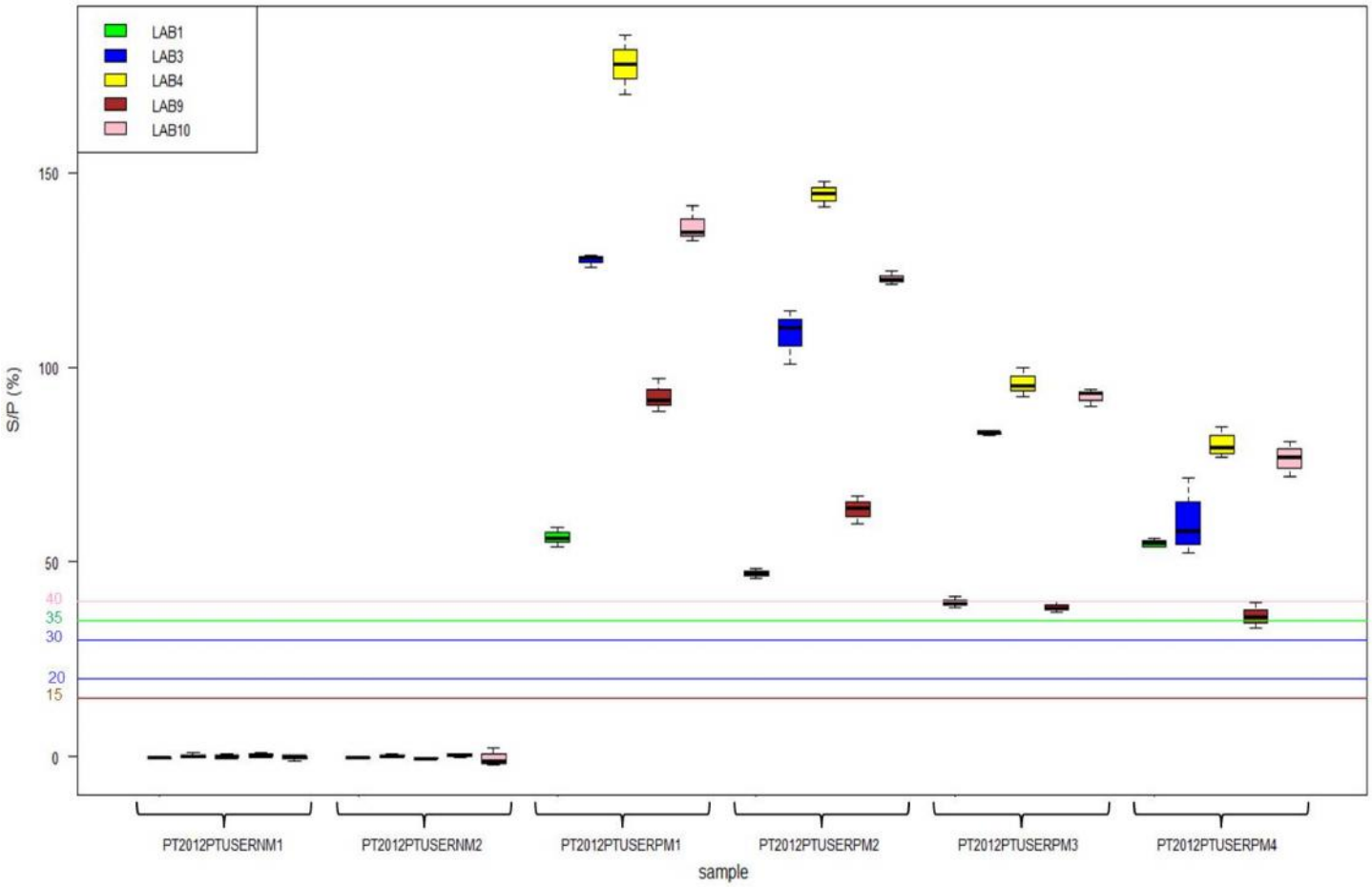

Figure 2B. Box plots showing the percentages S/P ratio per reference milk sample and per participating laboratory (without LAB2). Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. Cut-off values applied by the participating laboratories are shown in brown (15%; LAB9), blue (20-30%; LAB3, LAB4), green (35%; LAB1), blue - upper line - and pink (30-40%; LAB10), respectively. LAB3, LAB4 and LAB10 used a PTU antibody ELISA kit from the same producer (LAB10 applied alternative cut-off values). Hereby, LAB3 and LAB10 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 the percentages S/P ratio per reference serum/milk sample and per participating laboratory.

Remark: For the PT milk, LAB2 calculated percentages S/P ratio using a weak positive kit control whereas the other partipating laboratories used a strong positive kit control. Consequently, LAB2 obtained percentages S/P ratio that were much higher compared to the other participants. In order to avoid bias of the comparative quantitative data analysis, data from LAB2 were not included in the calculation of Mandel's h- and k-statistics for the PT milk.

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 1,75 for the PT serum (p=8) and 1,57 for the PT milk (p=5). For the PT serum, the maximum value for Mandel's k-statistic is 1,67 for the reference serum samples PT2012PTUSERNS1, PT2012PTUSERNS3, PT2012PTUSERPS1 and PT2012PTUSERPS3 (p=8 and n=3), and 1,56 for the reference serum samples PT2012PTUSERNS2 and PT2012PTUSERPS2 (p=8 and n=4). For the PT milk, the maximum value for Mandel's k-statistic is 1,62 for the reference milk samples PT2012PTUSERNM2, PT2012PTUSERPM1, PT2012PTUSERPM2 and PT2012PTUSERPM3 (p=5 and n=3) and 1,53 for the reference milk samples PT2012PTUSERNM1 and PT2012PTUSERPM4 (p=5 and n=4).

For the detection of PTU-specific antibodies in serum, 7 out of 8 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples: LAB2 until LAB8. In contrast, LAB1 showed an increased value for Mandel's h-statistic for the positive reference serum samples PT2012PTUSERPS1 (h=-2,31), PT2012PTUSERPS2 (h=-2,42) and PT2012PTUSERPS3 (h=-2,39). LAB3, LAB4, LAB6 and LAB7 on the one hand, and LAB5 and LAB8 on the other hand used different batches of a PTU antibody ELISA kit from the same producer (LAB6 used another ELISA kit than LAB3, LAB4 and LAB7). In addition, all these participants performed the short incubation protocol of the used ELISA kit.
Furthermore, 4 out of 8 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples: LAB1, LAB2, LAB4 and LAB7. The other participants showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB3 for the negative reference serum sample PT2012PTUSERNS2 (k=2,17) and the positive reference serum sample PT2012PTUSERPS1 (k=1,89), LAB5 for the negative reference serum sample PT2012PTUSERNS1 (k=1,68), LAB6 for the negative reference serum sample PT2012PTUSERNS2 (k=1,66) and LAB8 for the negative reference serum sample PT2012PTUSERNS3 (k=2,38) and the positive reference serum sample PT2012PTUSERPS3 (k=1,68).

For the detection of PTU-specific antibodies in milk, all 5 participating laboratories obtained a satisfactory between-laboratory consistency for all reference milk samples. LAB3, LAB4 and LAB10 used a PTU antibody ELISA kit from the same producer. Hereby, LAB3 and LAB10 used the same batch.
Only 2 out of 5 participating laboratories obtained a satisfactory within-laboratory consistency for all reference milk samples: LAB1 and LAB9. The other participants showed an increased value for Mandel's k-statistic for at least 1 reference milk sample: LAB3 for the positive reference milk samples PT2012PTUSERPM2 (k=1,76) and PT2012PTUSERPM4 (k=1,83), LAB4 for the positive reference milk samples PT2012PTUSERPM1 (k=1,65) and PT2012PTUSERPM3 (k=1,71), and LAB10 for the negative reference milk sample PT2012PTUSERNM2 (k=2,15).

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 samples were analysed together), status level (all reference serum samples with the same status were analysed together) and sample level (all reference serum 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.

Remark: Because LAB2 calculated percentages S/P ratio using a weak positive kit control, it obtained percentages S/P ratio that were much higher compared to the other partipating laboratories, which used a strong positive kit control. In order to avoid bias of the comparative quantitative data analysis, data from LAB2 were not included in the ANOVA for the PT milk.

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 were observed for both the negative and the positive (incl. positive/non-interpretable) reference serum samples. For the negative reference serum samples, LAB3 and LAB4 reported percentages S/P ratio that were significantly higher than those reported by LAB1, LAB2, LAB5, LAB6 and LAB7, while LAB3, LAB4 and LAB8 reported percentages S/P ratio that were significantly higher than those reported by LAB2. For the positive (incl. positive/non-interpretable) reference serum samples, LAB2 reported percentages S/P ratio that were significantly lower than those reported by the other participants.

For the PT milk, statistically significant differences were observed between laboratories at a global level, sample level and status level. At a global level, LAB4 reported percentages S/P ratio that were significantly higher than those reported by LAB1. At the status level, significant differences between laboratories were observed for the positive reference milk samples but not for the negative reference milk samples. For the positive reference milk samples, LAB3, LAB4 and LAB10 reported percentages S/P ratio that were significantly higher than those reported by LAB1 and LAB9. Noteworthy, LAB3, LAB4 and LAB10 used a PTU antibody ELISA kit from the same producer (LAB3 and LAB10 used the same batch).

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
PT2012PTUSERNS1	1	3	0,01	5,92	5,77	0,81	0,67	1,55	1,40	0,04	0,16	1,79
PT2012PTUSERNS1	2	3	0,06	1,13	5,77	0,81	0,67	1,55	1,40	-1,25	0,38	22,28
PT2012PTUSERNS1	3	3	1,10	10,19	5,77	0,81	0,67	1,55	1,40	1,19	1,57	10,31
PT2012PTUSERNS1	4	3	0,34	7,80	5,77	0,81	0,67	1,55	1,40	0,55	0,87	7,44
PT2012PTUSERNS1	5	3	1,27	4,28	5,77	0,81	0,67	1,55	1,40	-0,40	1,68	26,29
PT2012PTUSERNS1	6	3	0,09	2,84	5,77	0,81	0,67	1,55	1,40	-0,79	0,45	10,66
PT2012PTUSERNS1	7	3	0,26	2,63	5,77	0,81	0,67	1,55	1,40	-0,84	0,77	19,52
PT2012PTUSERNS1	8	3	0,46	11,35	5,77	0,81	0,67	1,55	1,40	1,50	1,01	5,98
PT2012PTUSERNS2	1	4	0,18	5,95	8,99	0,50	2,27	3,20	2,26	-0,50	0,19	7,22
PT2012PTUSERNS2	2	4	0,17	1,73	8,99	0,50	2,27	3,20	2,26	-1,20	0,18	23,79
PT2012PTUSERNS2	3	4	24,19	16,75	8,99	0,50	2,27	3,20	2,26	1,28	2,17	29,37
PT2012PTUSERNS2	4	4	0,04	18,03	8,99	0,50	2,27	3,20	2,26	1,49	0,09	1,11
PT2012PTUSERNS2	5	4	0,10	2,02	8,99	0,50	2,27	3,20	2,26	-1,15	0,14	15,44
PT2012PTUSERNS2	6	4	14,12	9,80	8,99	0,50	2,27	3,20	2,26	0,13	1,66	38,33
PT2012PTUSERNS2	7	4	1,27	10,43	8,99	0,50	2,27	3,20	2,26	0,24	0,50	10,82
PT2012PTUSERNS2	8	4	1,00	7,23	8,99	0,50	2,27	3,20	2,26	-0,29	0,44	13,84
PT2012PTUSERNS3	1	3	0,44	3,14	5,49	0,65	0,69	1,18	0,95	-0,92	0,96	21,07
PT2012PTUSERNS3	2	3	0,17	2,90	5,49	0,65	0,69	1,18	0,95	-1,02	0,60	14,27
PT2012PTUSERNS3	3	3	0,35	9,62	5,49	0,65	0,69	1,18	0,95	1,62	0,85	6,12
PT2012PTUSERNS3	4	3	0,10	7,65	5,49	0,65	0,69	1,18	0,95	0,84	0,45	4,07
PT2012PTUSERNS3	5	3	0,03	6,65	5,49	0,65	0,69	1,18	0,95	0,45	0,25	2,64
PT2012PTUSERNS3	6	3	0,01	4,26	5,49	0,65	0,69	1,18	0,95	-0,49	0,14	2,33
PT2012PTUSERNS3	7	3	0,01	2,89	5,49	0,65	0,69	1,18	0,95	-1,02	0,16	3,84
PT2012PTUSERNS3	8	3	2,71	6,85	5,49	0,65	0,69	1,18	0,95	0,53	2,38	24,05

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012PTUSERPS1	**1**	3	1,77	29,64	111,70	0,86	5,44	14,44	13,38	**-2,31**	0,24	4,48
PT2012PTUSERPS1	2	3	37,14	140,91	111,70	0,86	5,44	14,44	13,38	0,82	1,12	4,33
PT2012PTUSERPS1	**3**	3	105,60	109,47	111,70	0,86	5,44	14,44	13,38	-0,06	**1,89**	9,39
PT2012PTUSERPS1	4	3	37,06	133,76	111,70	0,86	5,44	14,44	13,38	0,62	1,12	4,55
PT2012PTUSERPS1	5	3	4,35	124,94	111,70	0,86	5,44	14,44	13,38	0,37	0,38	1,67
PT2012PTUSERPS1	6	3	17,45	135,18	111,70	0,86	5,44	14,44	13,38	0,66	0,77	3,09
PT2012PTUSERPS1	7	3	9,06	113,53	111,70	0,86	5,44	14,44	13,38	0,05	0,55	2,65
PT2012PTUSERPS1	8	3	23,95	106,20	111,70	0,86	5,44	14,44	13,38	-0,15	0,90	4,61
PT2012PTUSERPS2	**1**	4	1,72	24,59	89,38	0,80	5,07	11,27	10,07	**-2,42**	0,26	5,34
PT2012PTUSERPS2	2	4	35,33	96,36	89,38	0,80	5,07	11,27	10,07	0,26	1,17	6,17
PT2012PTUSERPS2	3	4	15,19	92,71	89,38	0,80	5,07	11,27	10,07	0,12	0,77	4,20
PT2012PTUSERPS2	4	4	55,95	102,76	89,38	0,80	5,07	11,27	10,07	0,50	1,48	7,28
PT2012PTUSERPS2	5	4	1,27	93,63	89,38	0,80	5,07	11,27	10,07	0,16	0,22	1,20
PT2012PTUSERPS2	6	4	11,62	106,39	89,38	0,80	5,07	11,27	10,07	0,64	0,67	3,20
PT2012PTUSERPS2	7	4	33,34	93,44	89,38	0,80	5,07	11,27	10,07	0,15	1,14	6,18
PT2012PTUSERPS2	8	4	50,92	105,17	89,38	0,80	5,07	11,27	10,07	0,59	1,41	6,79
PT2012PTUSERPS3	**1**	3	0,04	20,19	75,10	0,81	4,15	9,60	8,65	**-2,39**	0,05	1,05
PT2012PTUSERPS3	2	3	15,63	75,87	75,10	0,81	4,15	9,60	8,65	0,03	0,95	5,21
PT2012PTUSERPS3	3	3	23,45	82,39	75,10	0,81	4,15	9,60	8,65	0,32	1,17	5,88
PT2012PTUSERPS3	4	3	6,40	92,51	75,10	0,81	4,15	9,60	8,65	0,76	0,61	2,73
PT2012PTUSERPS3	5	3	36,74	77,36	75,10	0,81	4,15	9,60	8,65	0,10	1,46	7,84
PT2012PTUSERPS3	6	3	2,25	88,19	75,10	0,81	4,15	9,60	8,65	0,57	0,36	1,70
PT2012PTUSERPS3	7	3	4,41	76,68	75,10	0,81	4,15	9,60	8,65	0,07	0,51	2,74
PT2012PTUSERPS3	**8**	3	48,77	87,64	75,10	0,81	4,15	9,60	8,65	0,54	**1,68**	7,97

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
PT2012PTUSERNM1	1	4	0,01	-0,37	-0,01	0,05	0,48	0,49	0,11	-1,13	0,16	-20,43
PT2012PTUSERNM1	3	4	0,33	0,20	-0,01	0,05	0,48	0,49	0,11	0,63	1,19	291,40
PT2012PTUSERNM1	4	4	0,28	0,03	-0,01	0,05	0,48	0,49	0,11	0,11	1,11	1820,17
PT2012PTUSERNM1	9	4	0,22	0,39	-0,01	0,05	0,48	0,49	0,11	1,25	0,98	119,76
PT2012PTUSERNM1	10	4	0,32	-0,28	-0,01	0,05	0,48	0,49	0,11	-0,86	1,17	-200,00
PT2012PTUSERNM2	1	3	0,01	-0,21	-0,09	0,00	1,09	1,09	0,00	-0,39	0,10	-49,60
PT2012PTUSERNM2	3	3	0,14	0,17	-0,09	0,00	1,09	1,09	0,00	0,86	0,34	213,52
PT2012PTUSERNM2	4	3	0,04	-0,35	-0,09	0,00	1,09	1,09	0,00	-0,83	0,19	-57,85
PT2012PTUSERNM2	9	3	0,23	0,30	-0,09	0,00	1,09	1,09	0,00	1,27	0,44	158,12
PT2012PTUSERNM2	**10**	3	5,47	-0,37	-0,09	0,00	1,09	1,09	0,00	-0,91	**2,15**	-624,24
PT2012PTUSERPM1	1	3	5,91	56,30	118,10	0,96	4,63	23,43	22,97	-1,34	0,52	4,32
PT2012PTUSERPM1	3	3	2,79	127,57	118,10	0,96	4,63	23,43	22,97	0,21	0,36	1,31
PT2012PTUSERPM1	**4**	3	58,20	177,84	118,10	0,96	4,63	23,43	22,97	1,30	**1,65**	4,29
PT2012PTUSERPM1	9	3	18,57	92,48	118,10	0,96	4,63	23,43	22,97	-0,56	0,93	4,66
PT2012PTUSERPM1	10	3	21,88	136,33	118,10	0,96	4,63	23,43	22,97	0,40	1,01	3,43
PT2012PTUSERPM2	1	3	1,76	47,00	97,28	0,96	3,93	20,78	20,40	-1,23	0,34	2,82
PT2012PTUSERPM2	**3**	3	48,13	108,49	97,28	0,96	3,93	20,78	20,40	0,27	**1,76**	6,39
PT2012PTUSERPM2	4	3	11,01	144,55	97,28	0,96	3,93	20,78	20,40	1,16	0,84	2,30
PT2012PTUSERPM2	9	3	13,49	63,53	97,28	0,96	3,93	20,78	20,40	-0,83	0,93	5,78
PT2012PTUSERPM2	10	3	2,95	122,85	97,28	0,96	3,93	20,78	20,40	0,63	0,44	1,40
PT2012PTUSERPM3	1	3	2,21	39,65	69,90	0,98	2,17	14,46	14,30	-1,06	0,69	3,75
PT2012PTUSERPM3	3	3	0,34	83,10	69,90	0,98	2,17	14,46	14,30	0,46	0,27	0,70
PT2012PTUSERPM3	**4**	3	13,70	95,93	69,90	0,98	2,17	14,46	14,30	0,91	**1,71**	3,86
PT2012PTUSERPM3	9	3	1,77	38,31	69,90	0,98	2,17	14,46	14,30	-1,10	0,61	3,48
PT2012PTUSERPM3	10	3	5,47	92,51	69,90	0,98	2,17	14,46	14,30	0,79	1,08	2,53
PT2012PTUSERPM4	1	4	0,93	54,80	61,54	0,80	4,49	9,91	8,84	-0,38	0,22	1,76
PT2012PTUSERPM4	**3**	4	67,48	59,99	61,54	0,80	4,49	9,91	8,84	-0,09	**1,83**	13,69

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012PTUSERPM4	4	4	11,17	80,12	61,54	0,80	4,49	9,91	8,84	1,04	0,75	4,17
PT2012PTUSERPM4	9	4	7,27	36,10	61,54	0,80	4,49	9,91	8,84	-1,43	0,60	7,47
PT2012PTUSERPM4	10	4	13,78	76,69	61,54	0,80	4,49	9,91	8,84	0,85	0,83	4,84

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).