Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium
T + 32 2 642 51 11 • F + 32 2 642 50 01
www.sciensano.be

081-PT

PROFICIENCY TESTING 2019

Paratuberculosis (PTU)

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

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE BEGIN PT: 16 DECEMBER 2019

DATE REPORT: 4 FEBRUARY 2020

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 individual bovidae 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 milk samples must be tested by means of a PTU 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 bovidae origin, either free from detectable PTU-specific antibodies (n=2; coded 'PT2019PTUSERNS1' and 'PT2019PTUSERNS2') or containing detectable PTU-specific antibodies (n=3; coded 'PT2019PTUSERPS1', 'PT2019PTUSERPS2' and 'PT2019PTUSERPS3'), were used. In total, 120 aliquots were distributed to 6 participating laboratories. All participants received 3 aliquots of the reference serum samples PT2019PTUSERPS1 and PT2019PTUSERNS1, 4 aliquots of the reference serum sample PT2019PTUSERPS2 and 5 aliquots of the reference serum samples PT2019PTUSERPS3 and PT2019PTUSERNS2, i.e. 20 aliquots in total. The identification numbers of the reference milk 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 (i) the historical background of the animals and (ii) the results obtained by the IDEXX 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 PT2019PTUSERNS1 and PT2019PTUSERNS2 were field sera negative with both ELISA kits. The reference serum samples PT2019PTUSERPS1, PT2019PTUSERPS2 and PT2019PTUSERPS3 were derived from one PTU antibody positive serum from naturaly infected animal non diluted, diluted 1/4 and diluted 1/16 respectively. This positive serum originated from naturally infected animal for which presence of *M. paratuberculosis* was detected by culture in lymph node after slaughter. For each reference serum sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference serum samples PT2019PTUSERNS1 and PT2019PTUSERNS2 were considered as negative sera and the reference serum samples PT2019PTUSERPS1, PT2019PTUSERPS2 and PT2019PTUSERPS3 were considered as positive sera.

After aliquoting the different reference serum samples, a homogeneity check was performed on 5 aliquots of each reference serum sample using the IDEXX 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 5 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, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using ID Screen® Paratuberculosis Indirect Screening antibody ELISA test kit from IDVET.

III.2.2. Reference milk samples

Replicates of 5 reference milk samples, either free from detectable PTU-specific antibodies (n=2; coded 'PT2019PTUSERNM1' and 'PT2019PTUSERNM2') or containing detectable PTU-specific antibodies (n=3; coded 'PT2019PTUSERPM1', 'PT2019PTUSERPM2' and 'PT2019PTUSERPM3'), were used. The reference milk samples PT2019PTUSERNM1 and PT2019PTUSERNM2 were bovine tank milk and commercial milk whereas PT2019PTUSERPM1 was spiked commercial milk with positive serum. PT2019PTUSERPM2 and PT2019PTUSERPM3 were 2 positive field milk samples from 2 different animals of the same positive farm.
In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 3 aliquots of the reference milk sample PT2019PTUSERNM1, 4 aliquots of the reference milk samples PT2019PTUSERPM2, PT2019PTUSERPM3 and PT2019PTUSERNM2 and 5 aliquots of the reference milk sample PT2019PTUSERPM1, i.e. 20 aliquots in total. 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 (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 sample PT2019PTUSERNM1 was bovine tank milk from known PTU free herd and PT2019PTUSERNM2 was a commercial deffatted milk. The reference milk samples PT2019PTUSERPM1 and PT2019PTUSERPM3 derived from a commercial deffated milk spiked with positive serum. PT2019PTUSERPM2 was obtained from an animal that was shown to be shedders by PCR analysis on faeces samples. For each reference milk sample, the same qualitative result was obtained with both ELISA kits used. Taken together, the reference milk samples PT2019PTUSERNM1 and PT2019PTUSERNM2 were considered as negative milk samples, and the reference milk samples PT2019PTUSERPM1, PT2019PTUSERPM2 and PT2019PTUSERPM3 as positive milk samples.

After aliquoting the different reference milk samples, a homogeneity check was performed on 5 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 5 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, 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.

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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

LAB1, LAB2 and LAB3 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, LAB4, LAB5 and LAB6 only participated in the PT serum and hence received 20 aliquots of reference serum samples. LAB7 and LAB8 only participated in the PT milk and hence received 20 aliquots of reference milk samples.
The lyophilized reference serum samples (120 aliquots in total) and reference milk samples (100 aliquots in total) were sent to the 8 participating laboratories by national courier on the 16th of December 2019. LAB1, LAB2, LAB3, LAB5, LAB6, LAB7 and LAB8 acknowledged receipt of the samples on the same day whereas LAB4 received the samples on 17th of December 2019
Analyses were performed between 17th and 31st of December 2019 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 18th of December 2019 and 6th of January 2020 (Table 1). All participating laboratories, except LAB2, respected the deadline of 3th of January 2020 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

Laboratory	Reference samples received	Start of analysis SERUM	Start of analysis MILK	Submission of the results (Excel file)
LAB1	16/12/2019	17/12/2019	18/12/2019	18/12/2019
LAB2	16/12/2019	17/12/2019	17/12/2019	**06/01/2020**
LAB3	16/12/2019	20/12/2019	19/12/2019	02/01/2020
LAB4	17/12/2019	19/12/2019 Idexx + IDVet	NA	02/01/2020
LAB5	16/12/2019	19/12/2019	NA	20/12/2019
LAB6	16/12/2019	23/12/2019 Idexx 24/12/2019 IDVet	NA	02/01/2020
LAB7	16/12/2019	NA	31/12/2019	03/01/2020
LAB8	16/12/2019	NA	17/12/2019	03/01/2020

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories, except LAB2 and LAB8 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**, all 6 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and so achieved 100% of agreement (Table 2).

(ii) For the detection of PTU-specific antibodies in **milk**, the 5 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference milk samples and so achieved 100% of agreement (Table 3).

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

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference **serum** samples assigned by the PTU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference **serum** samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1	**2**	**3**	**4.1**	**4.2**	**5**	**6.1**	**6.2**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

Table 3. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference **milk** samples assigned by the PTU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference **milk** samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR				
	1	**2**	**3**	**7**	**8**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)

IV.4.2. Variability among participating laboratories

(i) For the detection of PTU-specific antibodies in **serum**, no variability between laboratories could be observed since all participants correctly identified all reference serum samples. LAB4 and LAB6 obtained identical qualitative results using ELISA kits from 2 different producers.

(ii) For the detection of PTU-specific antibodies in **milk**, no variability between laboratories could be observed since all participants correctly identified all reference milk samples.

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 the PTU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive;

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019PTUSERPS1	POS	POS	1
2	1	2	PT2019PTUSERPS3	POS	POS	1
3	1	3	PT2019PTUSERNS2	NEG	NEG	1
4	1	4	PT2019PTUSERPS3	POS	POS	1
5	1	5	PT2019PTUSERNS1	NEG	NEG	1
6	1	6	PT2019PTUSERNS1	NEG	NEG	1
7	1	7	PT2019PTUSERPS1	POS	POS	1
8	1	8	PT2019PTUSERPS3	POS	POS	1
9	1	9	PT2019PTUSERNS2	NEG	NEG	1
10	1	10	PT2019PTUSERPS2	POS	POS	1
11	1	11	PT2019PTUSERNS2	NEG	NEG	1
12	1	12	PT2019PTUSERPS3	POS	POS	1
13	1	13	PT2019PTUSERNS2	NEG	NEG	1
14	1	14	PT2019PTUSERPS2	POS	POS	1
15	1	15	PT2019PTUSERNS2	NEG	NEG	1
16	1	16	PT2019PTUSERPS2	POS	POS	1
17	1	17	PT2019PTUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
18	1	18	PT2019PTUSERPS1	POS	POS	1
19	1	19	PT2019PTUSERPS3	POS	POS	1
20	1	20	PT2019PTUSERPS2	POS	POS	1
21	2	1	PT2019PTUSERPS2	POS	POS	1
22	2	2	PT2019PTUSERPS1	POS	POS	1
23	2	3	PT2019PTUSERPS3	POS	POS	1
24	2	4	PT2019PTUSERNS1	NEG	NEG	1
25	2	5	PT2019PTUSERPS2	POS	POS	1
26	2	6	PT2019PTUSERNS2	NEG	NEG	1
27	2	7	PT2019PTUSERPS2	POS	POS	1
28	2	8	PT2019PTUSERNS2	NEG	NEG	1
29	2	9	PT2019PTUSERPS1	POS	POS	1
30	2	10	PT2019PTUSERPS3	POS	POS	1
31	2	11	PT2019PTUSERNS1	NEG	NEG	1
32	2	12	PT2019PTUSERPS2	POS	POS	1
33	2	13	PT2019PTUSERNS2	NEG	NEG	1
34	2	14	PT2019PTUSERPS1	POS	POS	1
35	2	15	PT2019PTUSERNS1	NEG	NEG	1
36	2	16	PT2019PTUSERPS3	POS	POS	1
37	2	17	PT2019PTUSERNS2	NEG	NEG	1
38	2	18	PT2019PTUSERPS3	POS	POS	1
39	2	19	PT2019PTUSERNS2	NEG	NEG	1
40	2	20	PT2019PTUSERPS3	POS	POS	1
41	3	1	PT2019PTUSERPS1	POS	POS	1
42	3	2	PT2019PTUSERPS3	POS	POS	1
43	3	3	PT2019PTUSERNS2	NEG	NEG	1
44	3	4	PT2019PTUSERPS3	POS	POS	1
45	3	5	PT2019PTUSERNS1	NEG	NEG	1
46	3	6	PT2019PTUSERNS1	NEG	NEG	1
47	3	7	PT2019PTUSERPS1	POS	POS	1
48	3	8	PT2019PTUSERPS3	POS	POS	1
49	3	9	PT2019PTUSERNS2	NEG	NEG	1
50	3	10	PT2019PTUSERPS2	POS	POS	1
51	3	11	PT2019PTUSERNS2	NEG	NEG	1
52	3	12	PT2019PTUSERPS3	POS	POS	1
53	3	13	PT2019PTUSERNS2	NEG	NEG	1
54	3	14	PT2019PTUSERPS2	POS	POS	1
55	3	15	PT2019PTUSERNS2	NEG	NEG	1
56	3	16	PT2019PTUSERPS2	POS	POS	1
57	3	17	PT2019PTUSERNS1	NEG	NEG	1
58	3	18	PT2019PTUSERPS1	POS	POS	1
59	3	19	PT2019PTUSERPS3	POS	POS	1
60	3	20	PT2019PTUSERPS2	POS	POS	1
61	4.1	1	PT2019PTUSERPS2	POS	POS	1
62	4.1	2	PT2019PTUSERPS1	POS	POS	1
63	4.1	3	PT2019PTUSERPS3	POS	POS	1
64	4.1	4	PT2019PTUSERNS1	NEG	NEG	1
65	4.1	5	PT2019PTUSERPS2	POS	POS	1
66	4.1	6	PT2019PTUSERNS2	NEG	NEG	1
67	4.1	7	PT2019PTUSERPS2	POS	POS	1
68	4.1	8	PT2019PTUSERNS2	NEG	NEG	1
69	4.1	9	PT2019PTUSERPS1	POS	POS	1
70	4.1	10	PT2019PTUSERPS3	POS	POS	1
71	4.1	11	PT2019PTUSERNS1	NEG	NEG	1
72	4.1	12	PT2019PTUSERPS2	POS	POS	1
73	4.1	13	PT2019PTUSERNS2	NEG	NEG	1
74	4.1	14	PT2019PTUSERPS1	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
75	4.1	15	PT2019PTUSERNS1	NEG	NEG	1
76	4.1	16	PT2019PTUSERPS3	POS	POS	1
77	4.1	17	PT2019PTUSERNS2	NEG	NEG	1
78	4.1	18	PT2019PTUSERPS3	POS	POS	1
79	4.1	19	PT2019PTUSERNS2	NEG	NEG	1
80	4.1	20	PT2019PTUSERPS3	POS	POS	1
81	4.2	1	PT2019PTUSERPS2	POS	POS	1
82	4.2	2	PT2019PTUSERPS1	POS	POS	1
83	4.2	3	PT2019PTUSERPS3	POS	POS	1
84	4.2	4	PT2019PTUSERNS1	NEG	NEG	1
85	4.2	5	PT2019PTUSERPS2	POS	POS	1
86	4.2	6	PT2019PTUSERNS2	NEG	NEG	1
87	4.2	7	PT2019PTUSERPS2	POS	POS	1
88	4.2	8	PT2019PTUSERNS2	NEG	NEG	1
89	4.2	9	PT2019PTUSERPS1	POS	POS	1
90	4.2	10	PT2019PTUSERPS3	POS	POS	1
91	4.2	11	PT2019PTUSERNS1	NEG	NEG	1
92	4.2	12	PT2019PTUSERPS2	POS	POS	1
93	4.2	13	PT2019PTUSERNS2	NEG	NEG	1
94	4.2	14	PT2019PTUSERPS1	POS	POS	1
95	4.2	15	PT2019PTUSERNS1	NEG	NEG	1
96	4.2	16	PT2019PTUSERPS3	POS	POS	1
97	4.2	17	PT2019PTUSERNS2	NEG	NEG	1
98	4.2	18	PT2019PTUSERPS3	POS	POS	1
99	4.2	19	PT2019PTUSERNS2	NEG	NEG	1
100	4.2	20	PT2019PTUSERPS3	POS	POS	1
101	5	1	PT2019PTUSERPS1	POS	POS	1
102	5	2	PT2019PTUSERPS3	POS	POS	1
103	5	3	PT2019PTUSERNS2	NEG	NEG	1
104	5	4	PT2019PTUSERPS3	POS	POS	1
105	5	5	PT2019PTUSERNS1	NEG	NEG	1
106	5	6	PT2019PTUSERNS1	NEG	NEG	1
107	5	7	PT2019PTUSERPS1	POS	POS	1
108	5	8	PT2019PTUSERPS3	POS	POS	1
109	5	9	PT2019PTUSERNS2	NEG	NEG	1
110	5	10	PT2019PTUSERPS2	POS	POS	1
111	5	11	PT2019PTUSERNS2	NEG	NEG	1
112	5	12	PT2019PTUSERPS3	POS	POS	1
113	5	13	PT2019PTUSERNS2	NEG	NEG	1
114	5	14	PT2019PTUSERPS2	POS	POS	1
115	5	15	PT2019PTUSERNS2	NEG	NEG	1
116	5	16	PT2019PTUSERPS2	POS	POS	1
117	5	17	PT2019PTUSERNS1	NEG	NEG	1
118	5	18	PT2019PTUSERPS1	POS	POS	1
119	5	19	PT2019PTUSERPS3	POS	POS	1
120	5	20	PT2019PTUSERPS2	POS	POS	1
121	6.1	1	PT2019PTUSERPS2	POS	POS	1
122	6.1	2	PT2019PTUSERPS1	POS	POS	1
123	6.1	3	PT2019PTUSERPS3	POS	POS	1
124	6.1	4	PT2019PTUSERNS1	NEG	NEG	1
125	6.1	5	PT2019PTUSERPS2	POS	POS	1
126	6.1	6	PT2019PTUSERNS2	NEG	NEG	1
127	6.1	7	PT2019PTUSERPS2	POS	POS	1
128	6.1	8	PT2019PTUSERNS2	NEG	NEG	1
129	6.1	9	PT2019PTUSERPS1	POS	POS	1
130	6.1	10	PT2019PTUSERPS3	POS	POS	1
131	6.1	11	PT2019PTUSERNS1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
132	6.1	12	PT2019PTUSERPS2	POS	POS	1
133	6.1	13	PT2019PTUSERNS2	NEG	NEG	1
134	6.1	14	PT2019PTUSERPS1	POS	POS	1
135	6.1	15	PT2019PTUSERNS1	NEG	NEG	1
136	6.1	16	PT2019PTUSERPS3	POS	POS	1
137	6.1	17	PT2019PTUSERNS2	NEG	NEG	1
138	6.1	18	PT2019PTUSERPS3	POS	POS	1
139	6.1	19	PT2019PTUSERNS2	NEG	NEG	1
140	6.1	20	PT2019PTUSERPS3	POS	POS	1
141	6.2	1	PT2019PTUSERPS2	POS	POS	1
142	6.2	2	PT2019PTUSERPS1	POS	POS	1
143	6.2	3	PT2019PTUSERPS3	POS	POS	1
144	6.2	4	PT2019PTUSERNS1	NEG	NEG	1
145	6.2	5	PT2019PTUSERPS2	POS	POS	1
146	6.2	6	PT2019PTUSERNS2	NEG	NEG	1
147	6.2	7	PT2019PTUSERPS2	POS	POS	1
148	6.2	8	PT2019PTUSERNS2	NEG	NEG	1
149	6.2	9	PT2019PTUSERPS1	POS	POS	1
150	6.2	10	PT2019PTUSERPS3	POS	POS	1
151	6.2	11	PT2019PTUSERNS1	NEG	NEG	1
152	6.2	12	PT2019PTUSERPS2	POS	POS	1
153	6.2	13	PT2019PTUSERNS2	NEG	NEG	1
154	6.2	14	PT2019PTUSERPS1	POS	POS	1
155	6.2	15	PT2019PTUSERNS1	NEG	NEG	1
156	6.2	16	PT2019PTUSERPS3	POS	POS	1
157	6.2	17	PT2019PTUSERNS2	NEG	NEG	1
158	6.2	18	PT2019PTUSERPS3	POS	POS	1
159	6.2	19	PT2019PTUSERNS2	NEG	NEG	1
160	6.2	20	PT2019PTUSERPS3	POS	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 the PTU reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019PTUSERPM2	POS	POS	1
2	1	2	PT2019PTUSERNM1	NEG	NEG	1
3	1	3	PT2019PTUSERPM1	POS	POS	1
4	1	4	PT2019PTUSERNM2	NEG	NEG	1
5	1	5	PT2019PTUSERPM3	POS	POS	1
6	1	6	PT2019PTUSERNM2	NEG	NEG	1
7	1	7	PT2019PTUSERPM1	POS	POS	1
8	1	8	PT2019PTUSERPM3	POS	POS	1
9	1	9	PT2019PTUSERNM1	NEG	NEG	1
10	1	10	PT2019PTUSERNM1	NEG	NEG	1
11	1	11	PT2019PTUSERPM1	POS	POS	1
12	1	12	PT2019PTUSERPM3	POS	POS	1
13	1	13	PT2019PTUSERNM2	NEG	NEG	1
14	1	14	PT2019PTUSERPM2	POS	POS	1
15	1	15	PT2019PTUSERPM2	POS	POS	1
16	1	16	PT2019PTUSERNM2	NEG	NEG	1
17	1	17	PT2019PTUSERPM1	POS	POS	1
18	1	18	PT2019PTUSERPM1	POS	POS	1
19	1	19	PT2019PTUSERPM3	POS	POS	1
20	1	20	PT2019PTUSERPM2	POS	POS	1
21	2	1	PT2019PTUSERPM1	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
22	2	2	PT2019PTUSERPM2	POS	POS	1
23	2	3	PT2019PTUSERPM3	POS	POS	1
24	2	4	PT2019PTUSERPM1	POS	POS	1
25	2	5	PT2019PTUSERNM1	NEG	NEG	1
26	2	6	PT2019PTUSERPM1	POS	POS	1
27	2	7	PT2019PTUSERPM3	POS	POS	1
28	2	8	PT2019PTUSERNM2	NEG	NEG	1
29	2	9	PT2019PTUSERPM1	POS	POS	1
30	2	10	PT2019PTUSERNM2	NEG	NEG	1
31	2	11	PT2019PTUSERNM2	NEG	NEG	1
32	2	12	PT2019PTUSERPM2	POS	POS	1
33	2	13	PT2019PTUSERNM2	NEG	NEG	1
34	2	14	PT2019PTUSERPM1	POS	POS	1
35	2	15	PT2019PTUSERPM3	POS	POS	1
36	2	16	PT2019PTUSERPM2	POS	POS	1
37	2	17	PT2019PTUSERNM1	NEG	NEG	1
38	2	18	PT2019PTUSERNM1	NEG	NEG	1
39	2	19	PT2019PTUSERPM2	POS	POS	1
40	2	20	PT2019PTUSERPM3	POS	POS	1
41	3	1	PT2019PTUSERPM2	POS	POS	1
42	3	2	PT2019PTUSERNM1	NEG	NEG	1
43	3	3	PT2019PTUSERPM1	POS	POS	1
44	3	4	PT2019PTUSERNM2	NEG	NEG	1
45	3	5	PT2019PTUSERPM3	POS	POS	1
46	3	6	PT2019PTUSERNM2	NEG	NEG	1
47	3	7	PT2019PTUSERPM1	POS	POS	1
48	3	8	PT2019PTUSERPM3	POS	POS	1
49	3	9	PT2019PTUSERNM1	NEG	NEG	1
50	3	10	PT2019PTUSERNM1	NEG	NEG	1
51	3	11	PT2019PTUSERPM1	POS	POS	1
52	3	12	PT2019PTUSERPM3	POS	POS	1
53	3	13	PT2019PTUSERNM2	NEG	NEG	1
54	3	14	PT2019PTUSERPM2	POS	POS	1
55	3	15	PT2019PTUSERPM2	POS	POS	1
56	3	16	PT2019PTUSERNM2	NEG	NEG	1
57	3	17	PT2019PTUSERPM1	POS	POS	1
58	3	18	PT2019PTUSERPM1	POS	POS	1
59	3	19	PT2019PTUSERPM3	POS	POS	1
60	3	20	PT2019PTUSERPM2	POS	POS	1
61	7	1	PT2019PTUSERPM1	POS	POS	1
62	7	2	PT2019PTUSERPM2	POS	POS	1
63	7	3	PT2019PTUSERPM3	POS	POS	1
64	7	4	PT2019PTUSERPM1	POS	POS	1
65	7	5	PT2019PTUSERNM1	NEG	NEG	1
66	7	6	PT2019PTUSERPM1	POS	POS	1
67	7	7	PT2019PTUSERPM3	POS	POS	1
68	7	8	PT2019PTUSERNM2	NEG	NEG	1
69	7	9	PT2019PTUSERPM1	POS	POS	1
70	7	10	PT2019PTUSERNM2	NEG	NEG	1
71	7	11	PT2019PTUSERNM2	NEG	NEG	1
72	7	12	PT2019PTUSERPM2	POS	POS	1
73	7	13	PT2019PTUSERNM2	NEG	NEG	1
74	7	14	PT2019PTUSERPM1	POS	POS	1
75	7	15	PT2019PTUSERPM3	POS	POS	1
76	7	16	PT2019PTUSERPM2	POS	POS	1
77	7	17	PT2019PTUSERNM1	NEG	NEG	1
78	7	18	PT2019PTUSERNM1	NEG	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
79	7	19	PT2019PTUSERPM2	POS	POS	1
80	7	20	PT2019PTUSERPM3	POS	POS	1
81	8	1	PT2019PTUSERPM2	POS	POS	1
82	8	2	PT2019PTUSERNM1	NEG	NEG	1
83	8	3	PT2019PTUSERPM1	POS	POS	1
84	8	4	PT2019PTUSERNM2	NEG	NEG	1
85	8	5	PT2019PTUSERPM3	POS	POS	1
86	8	6	PT2019PTUSERNM2	NEG	NEG	1
87	8	7	PT2019PTUSERPM1	POS	POS	1
88	8	8	PT2019PTUSERPM3	POS	POS	1
89	8	9	PT2019PTUSERNM1	NEG	NEG	1
90	8	10	PT2019PTUSERNM1	NEG	NEG	1
91	8	11	PT2019PTUSERPM1	POS	POS	1
92	8	12	PT2019PTUSERPM3	POS	POS	1
93	8	13	PT2019PTUSERNM2	NEG	NEG	1
94	8	14	PT2019PTUSERPM2	POS	POS	1
95	8	15	PT2019PTUSERPM2	POS	POS	1
96	8	16	PT2019PTUSERNM2	NEG	NEG	1
97	8	17	PT2019PTUSERPM1	POS	POS	1
98	8	18	PT2019PTUSERPM1	POS	POS	1
99	8	19	PT2019PTUSERPM3	POS	POS	1
100	8	20	PT2019PTUSERPM2	POS	POS	1

V. Discussion

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

For the detection of PTU-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).
PTU antibody ELISA kits from 2 different producers and different batches from the same ELISA kit were used: IDEXX (3 batches: 19120, 8145 and 19083) and IDVet (2 batches: E27 and E59).

For the detection of PTU-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).
PTU antibody ELISA kits from one producer but different batches were used: IDEXX (5 batches: 19120, 19024, 19043, 19097 and 8145).

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 Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.).
Consequently, all participants in the PT serum achieved a satisfactory performance for the detection of PTU-specific antibodies in reference serum samples and all participants in the PT milk achieved a satisfactory performance for the detection of PTU-specific antibodies in reference milk samples.

Coordinator proficiency tests

Katia Knapen and Bernard China

Appendix

Name of the participating laboratories

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

Comité du Lait (Battice, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE (Maisons-Alfort, France)

Lavetan NV (Turnhout, Belgium)

MCC-Vlaanderen (Lier, Belgium)

Sciensano (Uccle, Belgium)

Annex 1: Quantitative data analysis (Box plots)

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R (box plots).

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

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.

For the **antibody ELISA serum reference samples,** box plots of the normalized data according the PT provider per reference sample and per participating laboratory are shown in Figure 1.

Figure 1 (antibody ELISA serum reference samples)

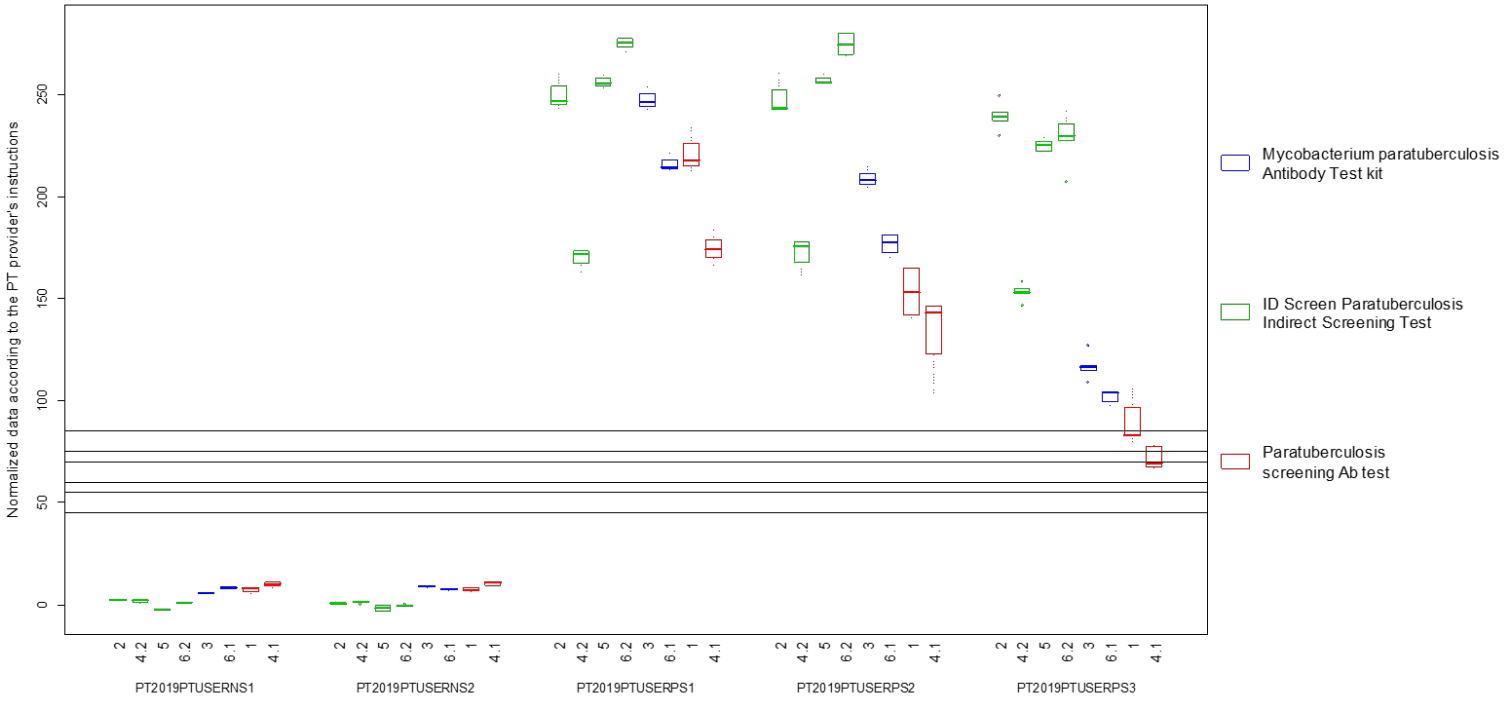

Figure 1. Box plots showing the normalized data according the PT provider per reference serum and per participating laboratory. PTU antibody ELISA kits from 2 different producers were used: IDEXX and IDVet. Cut-off values [IDEXX 45-55 (LAB1, LAB3, LAB4.1, LAB6.1)] and IDVET [60-70 (LAB4.2, LAB5, LAB6.2), or 75-85(LAB2)] are shown by horizontal lines.

For the **antibody ELISA milk reference samples,** box plots of the normalized data according the PT provider per reference sample and per participating laboratory are shown in Figure 2. All the participants used the Paratuberculosis Screening Ab test from IDEXX

Figure 2 (antibody ELISA milk reference samples)

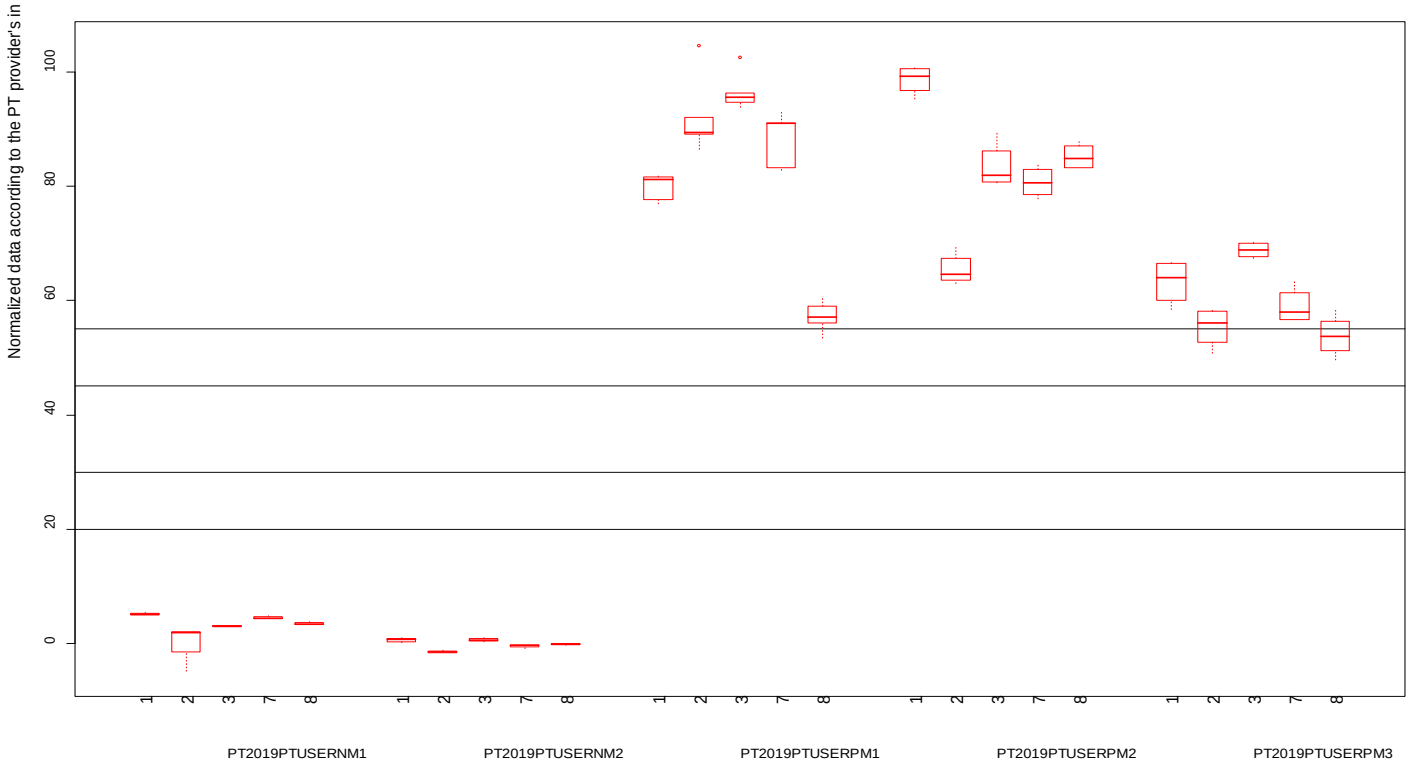

Figure 2. Box plots showing the normalized data according the PT provider per reference milk and per participating laboratory. PTU antibody ELISA kit from one producer were used: IDEXX. Cut-off values [20-30 (LAB2, LAB3, LAB7 and LAB8) or 45-55 (LAB1)] are shown by horizontal lines.