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081-PT

PROFICIENCY TESTING 2019

AFRICAN SWINE FEVER (ASF) (TYPE II STRAIN)

Detection of ASF-specific antibodies in serum by

Enzyme Linked Immunosorbent Assay (ELISA)

and/or

Detection of ASF DNA in serum by Polymerase Chain Reaction (PCR)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE START PT: 9 SEPTEMBER 2019

DATE REPORT: 17 JANUARY 2020

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 '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 in porcine serum the absence or presence of ASF-specific antibodies by ELISA and/or ASF DNA by PCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples were tested by means of an ASF antibody ELISA test and/or a PCR. The procedures for the ELISA tests and the PCR assays must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference serum samples for PT2019ASFSER panel

Replicates of 3 reference serum samples of porcine origin, either free from detectable ASF-specific antibodies (n=1; coded 'PT2019ASFSERNS1') or containing detectable ASF-specific antibodies (n=2; coded 'PT2019ASFSERPS1', and 'PT2019ASFSERPS2'), were used. In total, 70 aliquots were distributed to 7 participating laboratories. All participants received 10 aliquots: 4 aliquots of the reference serum samples PT2019ASFSERNS1 and PT2019ASFSERPS1 and 2 aliquots of the reference serum samples PT2019ASFSERPS2. 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 during pre-verification, hereby using the INgezim PPA COMPAC kit from INGENASA.

The reference serum sample PT2019ASFSERNS1 was obtained from an ASF free animal. The reference serum samples PT2019ASFSERPS1 and PT2019ASFSERPS2 were ASF positive and limit serum. For all reference serum samples, the same qualitative result was obtained with the INgezim PPA COMPAC ELISA test from INGENASA. Based on these results, the reference serum sample PT2019ASFSERNS1 was considered as negative serum, the reference serum sample PT2019ASFSERPS1 as positive serum in ASF antibody ELISA. For the reference serum sample PT2019ASFSERPS2 that present limit positive result with INgezim PPA COMPAC ELISA test from INGENASA, all the results (Positive/Doublful/Negative) are acceptable (NI status).

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the INgezim PPA COMPAC ELISA test from INGENASA. For all reference serum samples, the same qualitative result was obtained 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 laboratories to correctly identify the absence or presence of ASF-specific antibodies in serum. In addition, 3 aliquots of each serum sample were tested once after the PT in order to confirm their stability and status (post-verification) using the INgezim PPA COMPAC ELISA test from INGENASA.

III.2.2. Reference serum samples for PT2019ASFVIR panel

Replicates of 7 reference serum samples of porcine origin, either free from detectable ASF DNA (n = 1; coded 'PT2019ASFVIRNS1') or containing detectable ASF DNA (n = 6; coded 'PT2019ASFVIRPS1', 'PT2019ASFVIRPS2', 'PT2019ASFVIRPS3', 'PT2019ASFVIRPS4', 'PT2019ASFVIRPS5' and 'PT2019ASFVIRPS6') were used. In total, 90 aliquots were distributed to 9 participating laboratories. All participants received 10 aliquots: 2 aliquots of the reference serum samples 'PT2019ASFVIRPS2', 'PT2019ASFVIRPS6' and 'PT2019ASFVIRNS1' and 1 aliquot of the reference serum sample 'PT2019ASFVIRPS1', 'PT2019ASFVIRPS3', 'PT2019ASFVIRPS4' and 'PT2019ASFVIRPS5'. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 5).

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 background of the samples and (ii) the results obtained during pre-verification, hereby using a in-house PCR.

The reference serum sample PT2019ASFVIRNS1 was obtained from an ASF free animal. The reference serum samples PT2019ASFVIRPS1 and PT2019ASFVIRPS2 were serum from an ASF free animal spiked with two dilutions (1/10 and 1/10.000) of a positive culture of ASFV genotype 1 strain. The reference serum samples PT2019ASFVIRPS3, PT2019ASFVIRPS4, PT2019ASFVIRPS5 and PT2019ASFVIRPS6 were serum from an ASF free animal spiked with four dilutions (1/10, 1/100, 1/1.000 and 1/10.000) of a positive culture of ASFV genotype 2 strain (Belgium2018/1). For all reference serum samples, the same qualitative result was obtained with a in-house PCR (Tignon et al. 2011). Based on these results, the reference serum sample PT2019ASFVIRNS1 was considered as ASFV negative serum, the reference serum samples PT2019ASFVIRPS1, PT2019ASFVIRPS2, PT2019ASFVIRPS3, PT2019ASFVIRPS4, PT2019ASFVIRPS5 and PT2019ASFVIRPS6 as ASFV positive sera in PCR.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample. The homogeneity check was performed using the in-house ASF PCR assay. For all reference serum samples, the same qualitative result was obtained 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 laboratories to correctly identify the absence or presence of ASF DNA in serum of porcine origin. In addition, 3 aliquots of each reference serum sample were tested after the PT using the in-house ASF PCR assay in order to confirm the stability and status of the reference serum samples (post-verification).

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

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* 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 each of the 10 aliquots of reference samples used for this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 reference samples is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB9 participated in both the serology and virology component of the PT and received the PT2019ASFSER panel and the PT2019ASFVIR panel.
LAB7 and LAB8 only participated in the virology component of the PT and received the PT2019ASFVIR panel.

The 10 reference serum samples from the PT2019ASFVIR panel were sent frozen (dry ice) to LAB1, LAB2, LAB3, LAB4, LAB5, LAB6, LAB7 and LAB8 by national or international courier on 9th of September 2019. LAB3, LAB4, LAB5 and LAB6 acknowledged receipt of the samples on the same day whereas LAB1 and LAB8 received the samples on 10th of September 2019 and LAB2 and LAB7 on 11th of September 2019 (Table 1).
The 10 reference serum samples from the PT2019ASFSER panel were sent frozen (dry ice) to LAB1, LAB2, LAB3, LAB4, LAB5 and LAB6 by national or international courier on 7th of October 2019. LAB3, LAB4, LAB5 and LAB6 acknowledged receipt of the samples on the same day whereas LAB1 received the samples on 8th of October 2019 and LAB2 on 9th of October 2019 (Table 1).
Concerning LAB9 the 10 reference serum samples from the PT2019ASFVIR and PT2019ASFSER panels were sent frozen (dry ice) by national courier on 7th of October 2019. He acknowledged receipt of the samples on the same.

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 16st of September and 24th of October 2019 (Table 1).

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

Participating laboratory	Reference samples received	Start of analysis ELISA	Start of analysis PCR	Submission of the results (Excel file)
LAB1	VIR 10/09/2019 SER 08/10/2019	11/09/2019	15/10/2019	VIR 20/09/2019 SER 18/10/2019
LAB2	VIR 11/09/2019 SER 09/10/2019	18/09/2019	16/10/2019	VIR 27/09/2019 SER 24/10/2019
LAB3	VIR 09/09/2019 SER 07/10/2019	10/09/2019	09/10/2019	VIR 26/09/2019 SER 10/10/2019
LAB4	VIR 09/09/2019 SER 07/10/2019	10/09/2019	09/10/2019	VIR 26/09/2019 SER 10/10/2019
LAB5	VIR 09/09/2019 SER 07/10/2019	11/09/2019	08/10/2019	VIR 16/09/2019 SER 24/10/2019
LAB6	VIR 09/09/2019 SER 07/10/2019	11&13/09/2019	08/10/2019	VIR 20/09/2019 SER 18/10/2019
LAB7	VIR 11/09/2019	17/09/2019	NA	VIR 27/09/2019
LAB8	VIR 10/09/2019	13/09/2019	NA	VIR 20/09/2019
LAB9	VIR + SER 07/10/2019	08/10/2019	07/10/2019	VIR + SER 14/10/2019

Legend: NA = not applicable

IV.3. Compliance with the procedure

All participating laboratories, except LAB5 and LAB8, 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 ASF-specific antibodies by **ELISA** in serum : all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% of agreement (Table 2).

(ii) For the detection of ASF DNA by **PCR** in serum : LAB2, LAB3, LAB4, LAB5, LAB6, LAB7 and LAB8 provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB1 misclassified 2 aliquots (80% of agreement) and LAB9 misclassified 1 aliquots (90% of agreement) (Table 3).

A quantitative data analysis (box plots) is shown for educational purposes in Annex1.

Table 2. ELISA: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the ASF reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR								
	1	**2.1**	**2.2**	**3**	**4.1**	**4.2**	**5**	**6**	**9**
failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
success	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

Table 3. PCR : Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the ASF reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 10 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
failure	**2 (20)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**1 (10)**
success	**8 (80)**	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	**9 (90)**

IV.4.2. Variability among participating laboratories

(i) For detection of ASF-specific antibodies by **ELISA** in serum, no variability between the participants could be observed since all participants correctly identified all reference serum samples.

(ii) For the detection of ASF DNA by **PCR** in serum, no variability between LAB2, LAB3, LAB4, LAB5, LAB6, LAB7 and LAB8 could be observed since these participants correctly identified all reference serum samples. LAB1 misclassified 1 out of the 2 aliquots of the reference serum samples PT2019ASFVIRPS2 and PT2019ASFVIRNS1 (respectively NEG instead of POS and POS instead of NEG). LAB9 misclassified 1 out of the 2 aliquots of the reference serum samples PT2019ASFVIRNS1 (NI instead of NEG).

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 4 (ELISA) and Table 5 (PCR).

Table 4 ELISA: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the ASF reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; NI (non-interpretable / doubtful); POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019ASFSERNS1	NEG	NEG	1
2	1	2	PT2019ASFSERPS1	POS	POS	1
3	1	3	PT2019ASFSERNS1	NEG	NEG	1
4	1	4	PT2019ASFSERPS1	POS	POS	1
5	1	5	PT2019ASFSERNS1	NEG	NEG	1
6	1	6	PT2019ASFSERPS2	NI	NEG	1
7	1	7	PT2019ASFSERPS1	POS	POS	1
8	1	8	PT2019ASFSERNS1	NEG	NEG	1
9	1	9	PT2019ASFSERPS1	POS	POS	1
10	1	10	PT2019ASFSERPS2	NI	NEG	1
11	2.1	1	PT2019ASFSERPS1	POS	POS	1
12	2.1	2	PT2019ASFSERPS2	NI	NEG	1
13	2.1	3	PT2019ASFSERNS1	NEG	NEG	1
14	2.1	4	PT2019ASFSERPS1	POS	POS	1
15	2.1	5	PT2019ASFSERNS1	NEG	NEG	1
16	2.1	6	PT2019ASFSERPS1	POS	POS	1
17	2.1	7	PT2019ASFSERNS1	NEG	NEG	1
18	2.1	8	PT2019ASFSERPS1	POS	POS	1
19	2.1	9	PT2019ASFSERPS2	NI	NEG	1
20	2.1	10	PT2019ASFSERNS1	NEG	NEG	1
21	2.2	1	PT2019ASFSERPS1	POS	POS	1
22	2.2	2	PT2019ASFSERPS2	NI	NEG	1
23	2.2	3	PT2019ASFSERNS1	NEG	NEG	1
24	2.2	4	PT2019ASFSERPS1	POS	POS	1
25	2.2	5	PT2019ASFSERNS1	NEG	NEG	1
26	2.2	6	PT2019ASFSERPS1	POS	POS	1
27	2.2	7	PT2019ASFSERNS1	NEG	NEG	1
28	2.2	8	PT2019ASFSERPS1	POS	POS	1
29	2.2	9	PT2019ASFSERPS2	NI	NEG	1
30	2.2	10	PT2019ASFSERNS1	NEG	NEG	1
31	3	1	PT2019ASFSERNS1	NEG	NEG	1
32	3	2	PT2019ASFSERPS1	POS	POS	1
33	3	3	PT2019ASFSERNS1	NEG	NEG	1
34	3	4	PT2019ASFSERPS1	POS	POS	1
35	3	5	PT2019ASFSERNS1	NEG	NEG	1
36	3	6	PT2019ASFSERPS2	NI	NEG	1
37	3	7	PT2019ASFSERPS1	POS	POS	1
38	3	8	PT2019ASFSERNS1	NEG	NEG	1
39	3	9	PT2019ASFSERPS1	POS	POS	1
40	3	10	PT2019ASFSERPS2	NI	NEG	1
41	4.1	1	PT2019ASFSERPS1	POS	POS	1
42	4.1	2	PT2019ASFSERPS2	NI	NEG	1
43	4.1	3	PT2019ASFSERNS1	NEG	NEG	1
44	4.1	4	PT2019ASFSERPS1	POS	POS	1
45	4.1	5	PT2019ASFSERNS1	NEG	NEG	1
46	4.1	6	PT2019ASFSERPS1	POS	POS	1
47	4.1	7	PT2019ASFSERNS1	NEG	NEG	1
48	4.1	8	PT2019ASFSERPS1	POS	POS	1
49	4.1	9	PT2019ASFSERPS2	NI	NEG	1
50	4.1	10	PT2019ASFSERNS1	NEG	NEG	1
51	4.2	1	PT2019ASFSERPS1	POS	POS	1
52	4.2	2	PT2019ASFSERPS2	NI	NEG	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
53	4.2	3	PT2019ASFSERNS1	NEG	NEG	1
54	4.2	4	PT2019ASFSERPS1	POS	POS	1
55	4.2	5	PT2019ASFSERNS1	NEG	NEG	1
56	4.2	6	PT2019ASFSERPS1	POS	POS	1
57	4.2	7	PT2019ASFSERNS1	NEG	NEG	1
58	4.2	8	PT2019ASFSERPS1	POS	POS	1
59	4.2	9	PT2019ASFSERPS2	NI	NEG	1
60	4.2	10	PT2019ASFSERNS1	NEG	NEG	1
61	5	1	PT2019ASFSERNS1	NEG	NEG	1
62	5	2	PT2019ASFSERPS1	POS	POS	1
63	5	3	PT2019ASFSERNS1	NEG	NEG	1
64	5	4	PT2019ASFSERPS1	POS	POS	1
65	5	5	PT2019ASFSERNS1	NEG	NEG	1
66	5	6	PT2019ASFSERPS2	NI	POS	1
67	5	7	PT2019ASFSERPS1	POS	POS	1
68	5	8	PT2019ASFSERNS1	NEG	NEG	1
69	5	9	PT2019ASFSERPS1	POS	POS	1
70	5	10	PT2019ASFSERPS2	NI	POS	1
71	6	1	PT2019ASFSERPS1	POS	POS	1
72	6	2	PT2019ASFSERPS2	NI	NEG	1
73	6	3	PT2019ASFSERNS1	NEG	NEG	1
74	6	4	PT2019ASFSERPS1	POS	POS	1
75	6	5	PT2019ASFSERNS1	NEG	NEG	1
76	6	6	PT2019ASFSERPS1	POS	POS	1
77	6	7	PT2019ASFSERNS1	NEG	NEG	1
78	6	8	PT2019ASFSERPS1	POS	POS	1
79	6	9	PT2019ASFSERPS2	NI	NEG	1
80	6	10	PT2019ASFSERNS1	NEG	NEG	1
81	9	1	PT2019ASFSERNS1	NEG	NEG	1
82	9	2	PT2019ASFSERPS1	POS	POS	1
83	9	3	PT2019ASFSERNS1	NEG	NEG	1
84	9	4	PT2019ASFSERPS1	POS	POS	1
85	9	5	PT2019ASFSERNS1	NEG	NEG	1
86	9	6	PT2019ASFSERPS2	NI	NEG	1
87	9	7	PT2019ASFSERPS1	POS	POS	1
88	9	8	PT2019ASFSERNS1	NEG	NEG	1
89	9	9	PT2019ASFSERPS1	POS	POS	1
90	9	10	PT2019ASFSERPS2	NI	NEG	1

Table 5 PCR: The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the serum samples (SAMPLE), the external identification of the serum samples (LABPOSIT), and the status assigned by the ASF reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; NI (non-interpretable / doubtful); POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019ASFVIRPS3	POS	POS	1
2	1	2	PT2019ASFVIRPS4	POS	POS	1
3	1	3	PT2019ASFVIRPS1	POS	POS	1
4	1	4	PT2019ASFVIRPS6	POS	POS	1
5	1	5	PT2019ASFVIRPS5	POS	POS	1
6	1	6	PT2019ASFVIRPS2	**POS**	**NEG**	**0**
7	1	7	PT2019ASFVIRNS1	**NEG**	**POS**	**0**
8	1	8	PT2019ASFVIRNS1	NEG	NEG	1
9	1	9	PT2019ASFVIRPS2	POS	POS	1
10	1	10	PT2019ASFVIRPS6	POS	POS	1
11	2	1	PT2019ASFVIRPS2	POS	POS	1
12	2	2	PT2019ASFVIRPS3	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
13	2	3	PT2019ASFVIRPS4	POS	POS	1
14	2	4	PT2019ASFVIRNS1	NEG	NEG	1
15	2	5	PT2019ASFVIRPS2	POS	POS	1
16	2	6	PT2019ASFVIRPS1	POS	POS	1
17	2	7	PT2019ASFVIRPS5	POS	POS	1
18	2	8	PT2019ASFVIRPS6	POS	POS	1
19	2	9	PT2019ASFVIRNS1	NEG	NEG	1
20	2	10	PT2019ASFVIRPS6	POS	POS	1
21	3	1	PT2019ASFVIRPS3	POS	POS	1
22	3	2	PT2019ASFVIRPS4	POS	POS	1
23	3	3	PT2019ASFVIRPS1	POS	POS	1
24	3	4	PT2019ASFVIRPS6	POS	POS	1
25	3	5	PT2019ASFVIRPS5	POS	POS	1
26	3	6	PT2019ASFVIRNS1	NEG	NEG	1
27	3	7	PT2019ASFVIRPS2	POS	POS	1
28	3	8	PT2019ASFVIRNS1	NEG	NEG	1
29	3	9	PT2019ASFVIRPS2	POS	POS	1
30	3	10	PT2019ASFVIRPS6	POS	POS	1
31	4	1	PT2019ASFVIRPS2	POS	POS	1
32	4	2	PT2019ASFVIRPS3	POS	POS	1
33	4	3	PT2019ASFVIRPS4	POS	POS	1
34	4	4	PT2019ASFVIRNS1	NEG	NEG	1
35	4	5	PT2019ASFVIRPS2	POS	POS	1
36	4	6	PT2019ASFVIRPS1	POS	POS	1
37	4	7	PT2019ASFVIRPS5	POS	POS	1
38	4	8	PT2019ASFVIRPS6	POS	POS	1
39	4	9	PT2019ASFVIRNS1	NEG	NEG	1
40	4	10	PT2019ASFVIRPS6	POS	POS	1
41	5	1	PT2019ASFVIRPS3	POS	POS	1
42	5	2	PT2019ASFVIRPS4	POS	POS	1
43	5	3	PT2019ASFVIRPS1	POS	POS	1
44	5	4	PT2019ASFVIRPS6	POS	POS	1
45	5	5	PT2019ASFVIRPS5	POS	POS	1
46	5	6	PT2019ASFVIRNS1	NEG	NEG	1
47	5	7	PT2019ASFVIRPS2	POS	POS	1
48	5	8	PT2019ASFVIRNS1	NEG	NEG	1
49	5	9	PT2019ASFVIRPS2	POS	POS	1
50	5	10	PT2019ASFVIRPS6	POS	POS	1
51	6	1	PT2019ASFVIRPS2	POS	POS	1
52	6	2	PT2019ASFVIRPS3	POS	POS	1
53	6	3	PT2019ASFVIRPS4	POS	POS	1
54	6	4	PT2019ASFVIRNS1	NEG	NEG	1
55	6	5	PT2019ASFVIRPS2	POS	POS	1
56	6	6	PT2019ASFVIRPS1	POS	POS	1
57	6	7	PT2019ASFVIRPS5	POS	POS	1
58	6	8	PT2019ASFVIRPS6	POS	POS	1
59	6	9	PT2019ASFVIRNS1	NEG	NEG	1
60	6	10	PT2019ASFVIRPS6	POS	POS	1
61	7	1	PT2019ASFVIRPS3	POS	POS	1
62	7	2	PT2019ASFVIRPS4	POS	POS	1
63	7	3	PT2019ASFVIRPS1	POS	POS	1
64	7	4	PT2019ASFVIRPS6	POS	POS	1
65	7	5	PT2019ASFVIRPS5	POS	POS	1
66	7	6	PT2019ASFVIRNS1	NEG	NEG	1
67	7	7	PT2019ASFVIRPS2	POS	POS	1
68	7	8	PT2019ASFVIRNS1	NEG	NEG	1
69	7	9	PT2019ASFVIRPS2	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
70	7	10	PT2019ASFVIRPS6	POS	POS	1
71	8	1	PT2019ASFVIRPS2	POS	POS	1
72	8	2	PT2019ASFVIRPS3	POS	POS	1
73	8	3	PT2019ASFVIRPS4	POS	POS	1
74	8	4	PT2019ASFVIRNS1	NEG	NEG	1
75	8	5	PT2019ASFVIRPS2	POS	POS	1
76	8	6	PT2019ASFVIRPS1	POS	POS	1
77	8	7	PT2019ASFVIRPS5	POS	POS	1
78	8	8	PT2019ASFVIRPS6	POS	POS	1
79	8	9	PT2019ASFVIRNS1	NEG	NEG	1
80	8	10	PT2019ASFVIRPS6	POS	POS	1
81	9	1	PT2019ASFVIRPS3	POS	POS	1
82	9	2	PT2019ASFVIRPS4	POS	POS	1
83	9	3	PT2019ASFVIRPS1	POS	POS	1
84	9	4	PT2019ASFVIRPS6	POS	POS	1
85	9	5	PT2019ASFVIRPS5	POS	POS	1
86	9	6	PT2019ASFVIRNS1	**NEG**	**NI**	**0**
87	9	7	PT2019ASFVIRPS2	POS	POS	1
88	9	8	PT2019ASFVIRNS1	NEG	NEG	1
89	9	9	PT2019ASFVIRPS2	POS	POS	1
90	9	10	PT2019ASFVIRPS6	POS	POS	1

V. Discussion

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

For the detection of ASF-specific antibodies in reference serum samples, 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).

ASF antibody ELISA kits from 3 different producers as well as different batches from the same ELISA kit were used: INGENASA (1 batch: 300518), ID.VET (Competition 1 batch: E26 and Indirect 3 batches : E86, D39 and F52) and SVANOVA (1 batch B66682). LAB2 and LAB4 used the two ASF antibody ELISA kit from ID.VET (Competition and Indirect).

For the detection of ASF DNA in reference serum samples, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB7 provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB1 misclassified 1 out of the 2 aliquots of the reference serum samples PT2019ASFVIRPS2 and PT2019ASFVIRNS1 (80% of agreement). LAB9 misclassified 1 out of the 2 aliquots of the reference serum sample PT2019ASFVIRNS1 (90% of agreement) (Table 3 and Table 5).

Five different PCR assays were used by the participating laboratories: LAB1 used the Biosellal Bio-T kit ASFV from Biosellal, LAB2, LAB3, LAB4 and LAB6 used the IDGene ASF Duplex from ID.Vet, LAB7 used the Idexx Real PCR ASF DNA Mix from IDEXX, LAB8 the ADIAVET ASF Fast Time from Bio-X Diagnostic, LAB9 the Biolabs Luna Universal qPCR Master Mix from New Engeland and LAB5 did not communicate the used kit.

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 ASF reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently:

(i) All participants achieved a satisfactory performance for the detection of ASF-specific antibodies in serum samples.

(ii) All participants, except LAB1, achieved a satisfactory performance for the detection of ASF DNA in serum 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)

Biosellal (Dardilly, France)

BIO-X Diagnostics S.A. (Ploufragan, France)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Faculty of Veterinary Medicine, Dept. of Pathology, Veterinary Molecular Diagnostics Platform (Liège, Belgium)

IDEXX (Montpellier, France)

ID.VET (Grabels, France)

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

Sciensano (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 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 only box plots could be performed for the participating laboratories who have used the competition kit from ID.VETand the INGENASA kit. The normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory are shown in Figure 1.

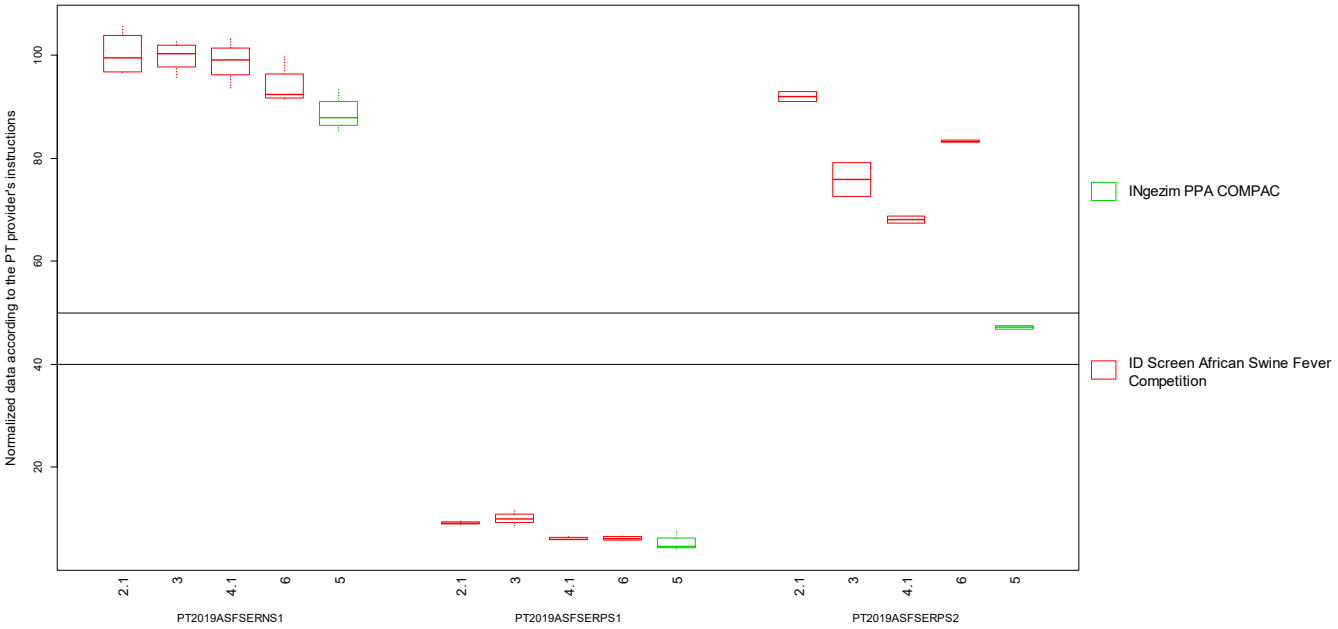

Figure 1. ID.VETCompetition and INGENASA ELISA kit : Box plots showing the normalized data according to the PT provider's instructions per reference serum sample and per participating laboratory.
Cut-off values applied by the participating laboratories are shown by horizontal lines (ID.VET competition 40-50).

For PCR box plots of the Ct or Cp values for the positive reference serum samples PT2019ASFVIRPS2 and PT2019ASFVIRPS6 per participating laboratory are shown in Figure 2.

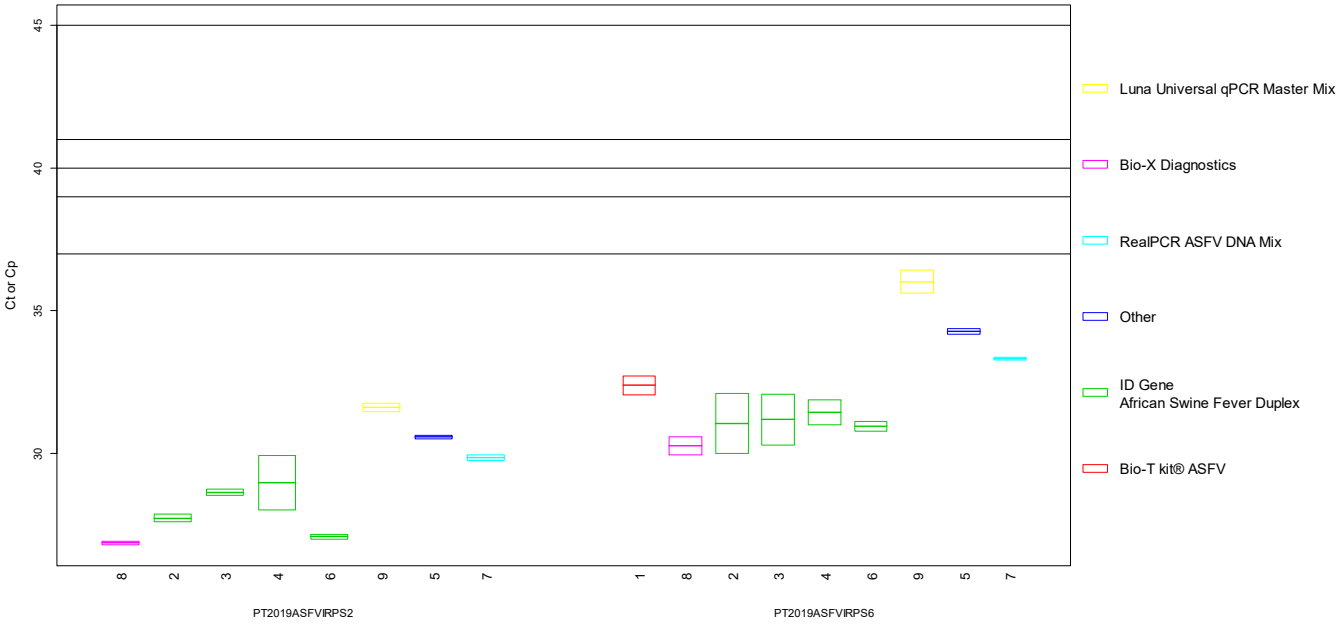

Figure 2. Box plots showing the Ct or Cp values per positive reference serum sample (PT2019ASFVIRPS2 and PT2019ASFVIRPS6) and per participating laboratory.
Cut-off values applied by the participating laboratories are shown by horizontal lines (37-40 for LAB4, 40 for LAB6, 39-41 for LAB9, 40-45 for LAB5 and 45 for LAB3 and LAB8).