

EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

**Proficiency Testing in Veterinary Diagnosis
African Swine Fever**

SURVEY 2020/1

Sciensano/PT VET ASF/1-E

Expertise and service provision
Quality of laboratories
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Introduction

This survey was dedicated to the detection of ASF virus using molecular methods in swine serum and to the detection of antibodies specific to ASF virus in swine serum using ELISA.

The samples

The samples were prepared by the National Reference Laboratory, Enzootic, vector-borne and bee diseases, Infectious diseases in animals Directorate, Sciensano.

Virology

Homogeneity

3 different samples were used

PT2020ASFVIRPS1, PT2020ASFVIRPS2, PT2020ASFVIRNS3.

The homogeneity of the samples was tested by the NRL on 10 exemplars of each sample.

ID PT 2020	Homogeneity (Ingenasa)										Mean	SD	CV (%)
	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	TEST 6	TEST 7	TEST 8	TEST 9	TEST 10			
PT2020ASFVIRPS1	27,37	26,04	28,35	26,43	27,18	26,13	25,91	25,92	25,87	25,86	26,506	0,849	3,2
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASFVIRPS2	31,56	32,02	32,16	31,27	30,90	34,37	33,53	34,08	33,77	33,54	32,72	1,272	3,9
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASFVIRNS3	>45	>45	>45	>45	>45	>45	>45	>45	>45	>45	>45	NA	NA
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	

200204 D587 D588/RobotExtraction

200204 D588 / RobotExtraction

200205 D591 D583 D589 R418/ManualExtraction

The samples were considered as homogeneous.

Target Values

The target value was determined by the NRL based on the homogeneity tests.

The samples PT2020ASFVIRPS1 and PT2020ASFVIRPS2 were considered as positive. The sample PT2020SFVITNS3 was considered as negative.

Stability

The stability was determined by comparison of the pre-survey results and the results obtained by the NRL during the survey. The samples were considered as stable.

The participants

6 laboratories participated to the ASF Virology survey:

Sciensano ; Arsia (Ciney) , DGZ (Torhout), LMVE (Luxemburg), Biosellal (France), ID-VET (Switzerland).

Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follow:

Laboratory	impar	Par
Sample Order		
V.ASF 2001	PT2020ASFVIRPS1	PT2020ASFVIRPS2
V.ASF 2002	PT2020ASFVIRPS2	PT2020ASFVIRNS3
V.ASF 2003	PT2020ASFVIRPS1	PT2020ASFVIRNS3
V.ASF 2004	PT2020ASFVIRNS3	PT2020ASFVIRPS2
V.ASF 2005	PT2020ASFVIRPS2	PT2020ASFVIRNS3
V.ASF 2006	PT2020ASFVIRNS3	PT2020ASFVIRPS1
V.ASF 2007	PT2020ASFVIRNS3	PT2020ASFVIRPS2
V.ASF 2008	PT2020ASFVIRNS3	PT2020ASFVIRNS3
V.ASF 2009	PT2020ASFVIRPS2	PT2020ASFVIRPS1
V.ASF 2010	PT2020ASFVIRNS3	PT2020ASFVIRNS3

The panel was constituted of 10 samples of 500 ul.

2. Serology

Homogeneity

6 different samples were used:PT2020ASFSEPS1, PT2020ASFSEPS2, PT2020ASFSEPS3, PT2020ASFSENS4, PT2020ASFSENS5, PT2020ASFSENS6

The homogeneity of the samples was tested by the NRL on 10 exemplars of each sample using two different ELISA kits.

ID PT 2020	Homogeneity (Ingenasa)										Mean	SD	CV (%)
	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	TEST 6	TEST 7	TEST 8	TEST 9	TEST 10			
PT2020ASFSEPS1	56.74	58.53	62.27	59.03	61.17	61.66	58.33	58.26	57.46	61.23	59.47	1.944	3.27%
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASFSEPS2	67.40	66.75	71.31	69.56	67.75	68.49	69.53	68.29	67.75	68.15	68.50	1.318	1.92%
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASFSEPS3	79.93	77,4	78.47	82.15	81.16	83.87	80.45	81.62	80.32	82.21	80,79	1.825	2.26%
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASFSENS4	-3.50	-4.64	4,31	-0.62	2.84	-1.93	-1.72	5.68	-6.56	-0.90	-0.70	3.925	556.99%
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
PT2020ASFSENS5	-6.35	-2.85	-1.55	-6.13	-4.98	-6.62	-6.26	-4.72	-9,5	-5.07	-5.37	2.104	-39,0%
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
PT2020ASFSENS6	4.70	6.90	4.70	5.10	7.00	12.60	3.50	19.30	4.90	0.00	6.87	5.399	78.59%
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		

ID PT 2020	Homogeneity (IDvet)										Mean	SD	CV (%)
	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	TEST 6	TEST 7	TEST 8	TEST 9	TEST 10			
PT2020ASF SERPS1	11.10	10.04	7.55	10.73	9.81	10.06	6.15	6.37	11.72	11.46	9.50	2.064	21,2
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASF SERPS2	8.23	13.36	5.44	7.80	7.97	7.46	4.54	4.10	9.38	17.68	8.60	4.150	48.28
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASF SERPS3	10.87	9.89	8.46	9.90	8.39	8.89	4.91	5.35	12.24	11.98	9.09	2.475	27.24
	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2020ASF SERNS4	98.00	93.47	84.26	108.60	100.80	111.36	85.61	94.84	103.80	99.83	98.06	8.873	9.05
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
PT2020ASF SERNS5	99.43	97.17	85.47	100.55	100.21	103.31	85.10	87.81	103.72	100.52	96.33	7.310	7.59
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
PT2020ASF SERNS6	121.9	120.9	117.01	122.21	127.91	126.71	117.81	121.41	115.91	123.71	121.5	3.932	3.23
	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		

The samples were considered as homogeneous.

The participants

4 laboratories participated to the ASF Serology survey: Sciensano, Arsia (Ciney), DGZ (Torhout), LMVE (Luxemburg).

Target values

The target values were determined by the NRL using the homogeneity results. The samples PT2020ASF SERPS1, PT2020ASF SERPS2 and PT2020ASF SERPS3 were considered as positive and the samples PT2020ASF SERNS4, PT2020ASF SERNS5, PT2020ASF SERNS6 were considered as negative;

Stability

The samples were tested before the survey and during the survey. The results were compared and the samples were considered as stable.

Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follow:

Laboratory	Impar	Par
Sample Order		
S.ASF 2001	PT2020ASF SERPS1	PT2020ASF SERPS3
S.ASF 2002	PT2020ASF SERPS2	PT2020ASF SERPS1
S.ASF2003	PT2020ASF SERPS3	PT2020ASF SERNS5
S.ASF 2004	PT2020ASF SERNS4	PT2020ASF SERNS6
S.ASF 2005	PT2020ASF SERNS5	PT2020ASF SERPS2
S.ASF 2006	PT2020ASF SERNS6	PT2020ASF SERPS3
S.ASF 2007	PT2020ASF SERNS5	PT2020ASF SERNS4
S.ASF 2008	PT2020ASF SERNS4	PT2020ASF SERNS5
S.ASF 2009	PT2020ASF SERPS3	PT2020ASF SERPS1
S.ASF 2010	PT2020ASF SERPS1	PT2020ASF SERNS4

The ASF serology panel consisted of 10 serum samples of 250 ul.

Survey Timeline

Transfer of the samples from NRL to QL: 12/03/2020

Randomization of the samples by QL:

sending samples to participants: 17/03/2020. The samples were sent on dry ice.

Deadline for the results encoding: 31/03/2020

Preliminary report: 15/05/2020

Results

1. Virology

1.1. Results per sample

6 laboratories encoded results giving 6 datasets.

Table RV1. Results per sample

Sample ID	Expected result	Number of repetitions (total results)	Observed result
PT2020ASFVIRPS1	Positive	2 (12)	12 positive results
PT2020ASFVIRPS2	Positive	3 (18)	18 Positive results
PT2020ASFVIRNS3	Negative	5 (30)	28 negative results 2 false positive results

Globally, on 60 encoded results, 58 (96.7%) were correct. Two false positive results were encoded.

1.2. Results per method

Table RV2. Results per method

Method	N	NR	NCR	%	FP	FN	ND
Bio-T kit® ASFV	1	10	10	100	0	0	0
ID Gene African Swine Fever Duplex	4	40	38	95	2	0	0
Home made	1	10	10	100	0	0	0
Total	6	60	58	96.7	2	0	0

NR: number of results, NCR: Number of correct results, FP/ false positive, FN: false negative, ND: not determined.

1.3. Results per laboratory, per method and per sample

Table RV3. Results per laboratory

Lab	Method	batch	PT2020ASFVIRPS1		PT2020ASFVIRPS2			PT2020ASFVIRNS3				
			Rep 1	Rep2	Rep1	REP2	Rep3	Rep1	Rep2	Rep3	Resp4	Rep5
97505	Home made	ND	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Neg	Neg	Neg	Neg
97507	ID Gene African Swine Fever Duplex	26	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Neg	Neg	Neg	Neg
97508	ID Gene African Swine Fever Duplex	25	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Neg	Neg	Neg	Neg
97514	Bio-T kit® ASFV	ASFV-01E	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Neg	Neg	Neg	Neg
97516	ID Gene African Swine Fever Duplex	22	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Pos	Neg	Neg	Pos
97522	ID Gene African Swine Fever Duplex	31	Pos.	Pos.	Pos.	Pos.	Pos.	Neg	Neg	Neg	Neg	Neg

Pos: positive; Neg: negative, rep: repetition

For the laboratory encoding 2 false positive results the Ct values were: 34,36 and 32,48 respectively for a cut off of 40.

2. Serology

2.1. Results per sample

6 datasets were encoded. 4 laboratories among them encoded 1 dataset, 2 laboratories encoded 2 datasets

Table RS1. Result per sample

Sample	Expected result	Number of repetition	Observed result
PT2020ASFSEPS1	Positive	2	12 positive
PT2020ASFSEPS2	Positive	1	6 positive
PT2020ASFSEPS3	Positive	2	12 positive
PT2020ASFSENS4	Negative	2	12 Negative
PT2020ASFSENS5	Negative	2	12 Negative
PT2020ASFSENS6	Negative	1	6 Negative

On the 60 encoded results, 100% were correct.

2.2. Used methods

Table RS2. Used methods

Method	N
ID Screen African Swine Fever Competition	4
INgezym PPA compac	1
ID Screen African Swine Fever Indirect	1

Table RS3. Used methods per laboratory

Lab	Method
97505	ID Screen African Swine Fever Competition
97505	INgezym PPA compac
97507	ID Screen African Swine Fever Competition
97508	ID Screen African Swine Fever Competition
97508	ID Screen African Swine Fever Indirect
97516	D Screen African Swine Fever Competition

ANNEXES

Annexe 1.

Quantitative data for PCR data

Sample PT2020VIRPS1

Table A1. Quantitative results (Ct)

Lab	REP1	REP2	mean	SD	CV (%)
97505	24.96	25.00	24.98	0.03	0.12
97507	24.23	24.44	24.34	0.15	0.61
97508	21.33	21.08	21.21	0.18	0.83
97514	19.28	19.82	19.55	0.38	1.95
97516	22.07	22.19	22.13	0.08	0.38
97522	23.20	23.10	23.15	0.07	0.31

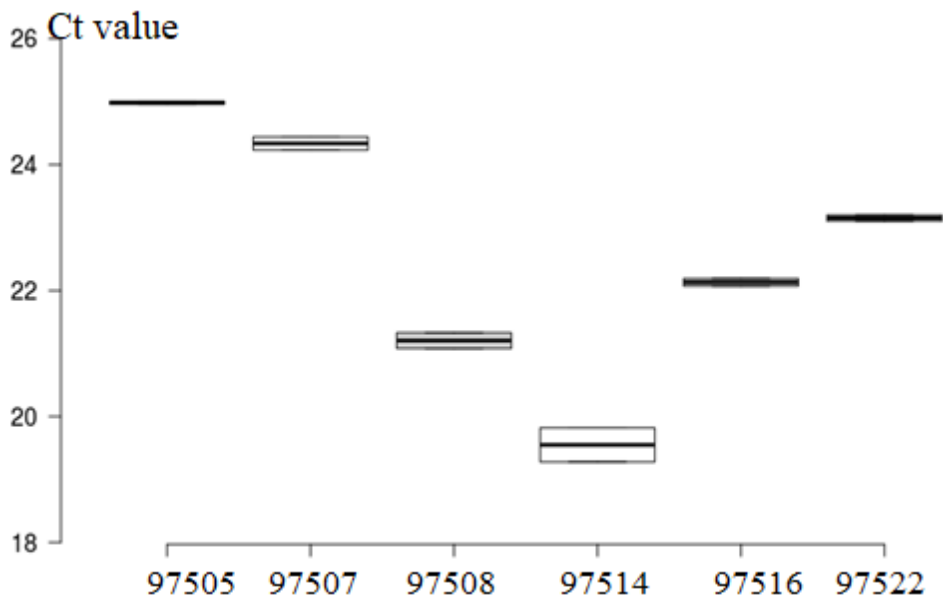


Figure 1. Boxplot dispersion of the results per laboratory for the PS1 sample

Sample PT2020ASFVIRPS2

Table A2. Quantitative results(Ct)

	REP1	REP2	REP3	mean	SD	CV (%)
Lab						
97505	33.92	31.85	34.13	33.30	1.26	3.79
97507	32.69	32.57	32.02	32.43	0.36	1.10
97508	28.72	28.84	28.96	28.84	0.12	0.42
97514	33.03	30.90	30.92	31.62	1.22	3.87
97516	28.96	31.31	30.86	30.38	1.25	4.11
97522	32.36	30.73	30.36	31.15	1.06	3.42

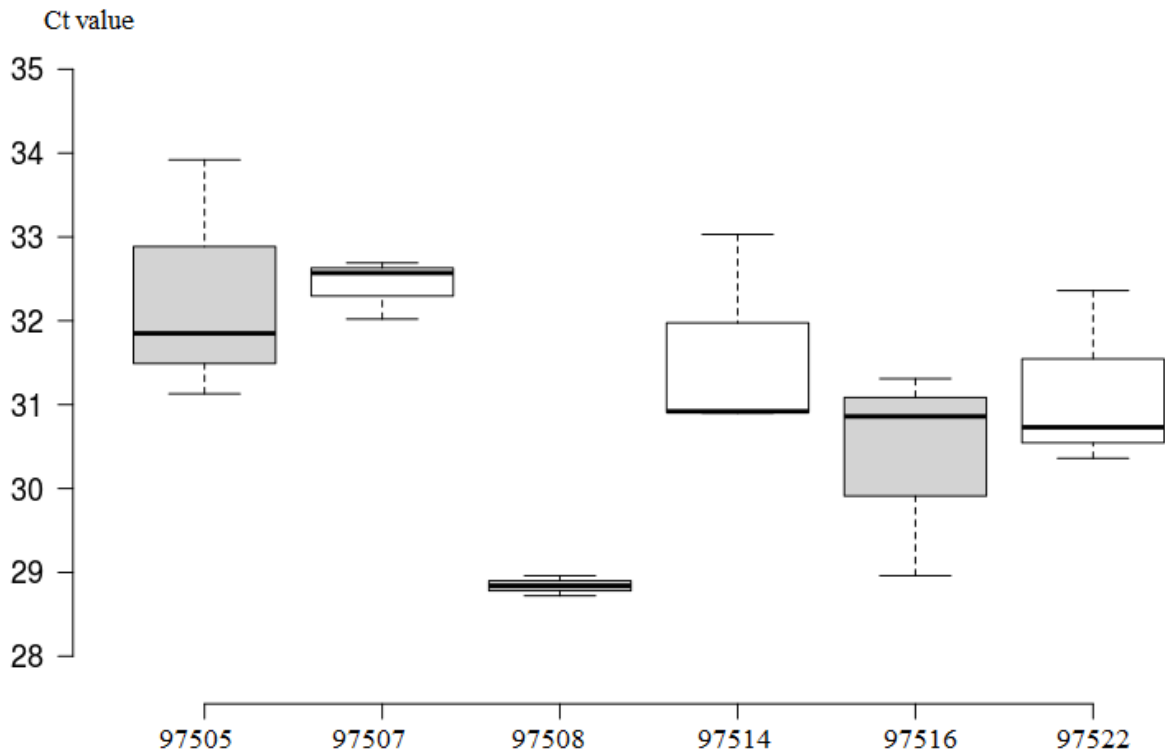


Figure 2.Boxplot of the dispersion of the results per laboratory for the PS2 sample

ANNEXE 2

Quantitative Results for ELISA PT2020ASF/SERPS1

Table A3. Normalised Quantitative results

Lab	Method	REP1 (%)	REP2 (%)	mean	SD	CV (%)
97505	ID Screen African Swine Fever Competition	10.7	7.9	9.3	1.98	21.3
97505	INgezym PPA compac	61.1	58.1	59.6	2.12	3.56
97507	ID Screen African Swine Fever Competition	5.725	10.305	8.015	3.24	40.41
97508	ID Screen African Swine Fever Indirect	60.095	72.821	66.458	9.00	13.54
97508	ID Screen African Swine Fever Competition	6.2585	7.6116	6.93505	0.956	13.80
97516	ID Screen African Swine Fever Competition	8.89	9.24	9.065	0.25	2.73

Normalized results (%)

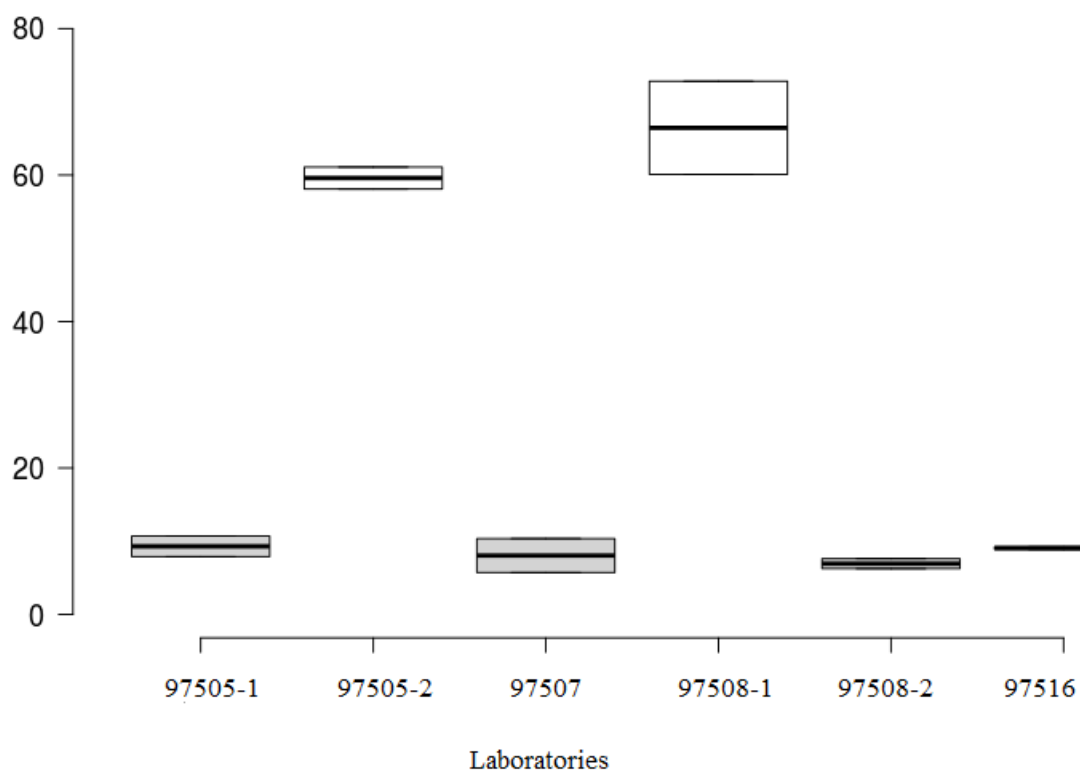


Fig. A3. Boxplot of the dispersion of the results per dataset.

PT2020ASF SERPS3

Tab A4. Normalised quantitative results

LAB	Method	REP1 (%)	REP2 (%)	mean	SD	CV (%)
97505	ID Screen African Swine Fever Competition	8.5	8.1	8.3	0.28	3.4
97505	INgezym PPA compac	79.7	79.3	79.50	0.283	0.36
97507	D Screen African Swine Fever Competition	8.78	14.88	11.83	4.313	36.46
97508	ID Screen African Swine Fever Indirect	70.75	74.44	72.60	2.609	3.59
97508	D Screen African Swine Fever Competition	7.34	5.99	6.66	0.955	14.32
97516	D Screen African Swine Fever Competition	10	9.95	9.97	0.035	0.35

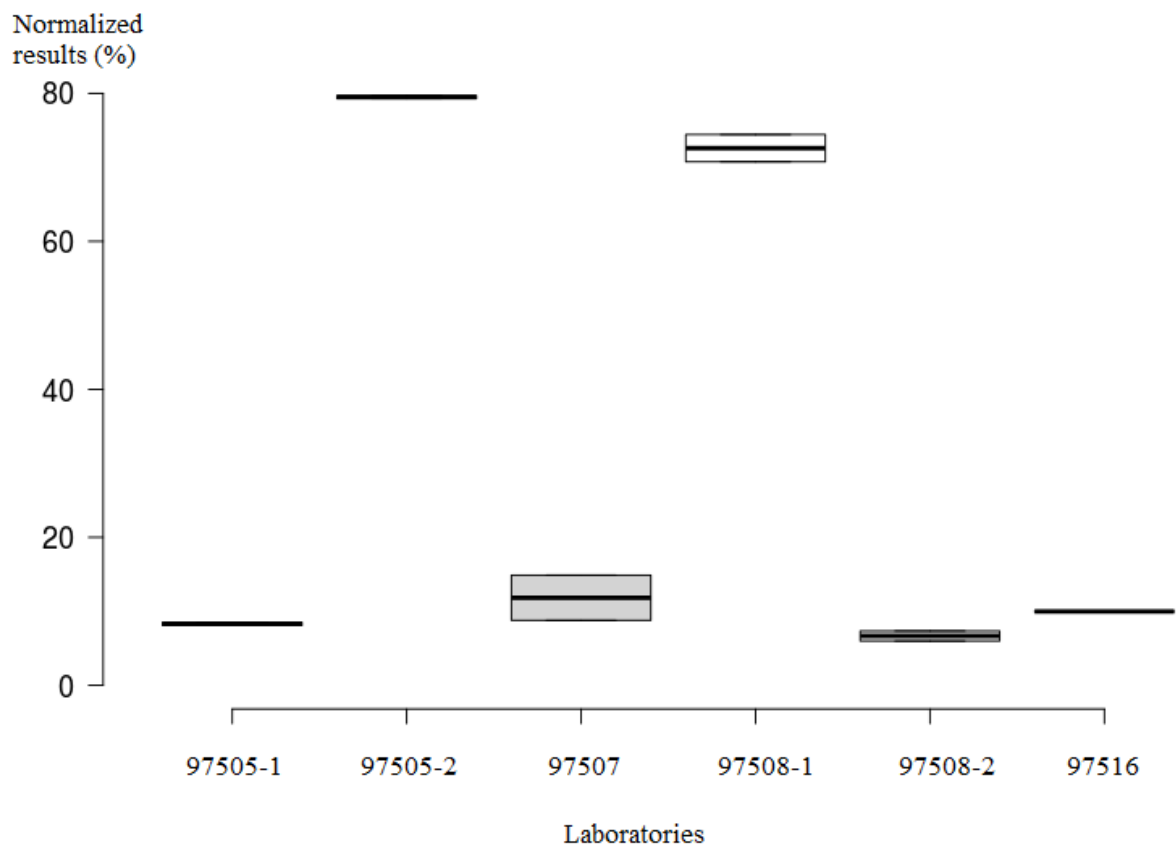


Figure A4. Boxplot distribution of the results per dataset.

PT2020ASFSEERNS4

Table A5. Normalized quantitative results

LAB	Method	REP1(%)	REP2 (%)	mean	SD	CV (%)
97505	ID Screen African Swine Fever Competition	103.5	87.6	95.55	11.24	11.8
97505	INgezym PPA compac	16.8	11.8	14.30	3.54	24.72
97507	D Screen African Swine Fever Competition	85.878	92.748	89.31	4.86	5.44
97508	ID Screen African Swine Fever Indirect	3.54	3.99	3.76	0.32	8.45
97508	D Screen African Swine Fever Competition	89.41	93.2	91.31	2.68	2.94
97516	D Screen African Swine Fever Competition	91.5	91.9	91.70	0.28	0.31

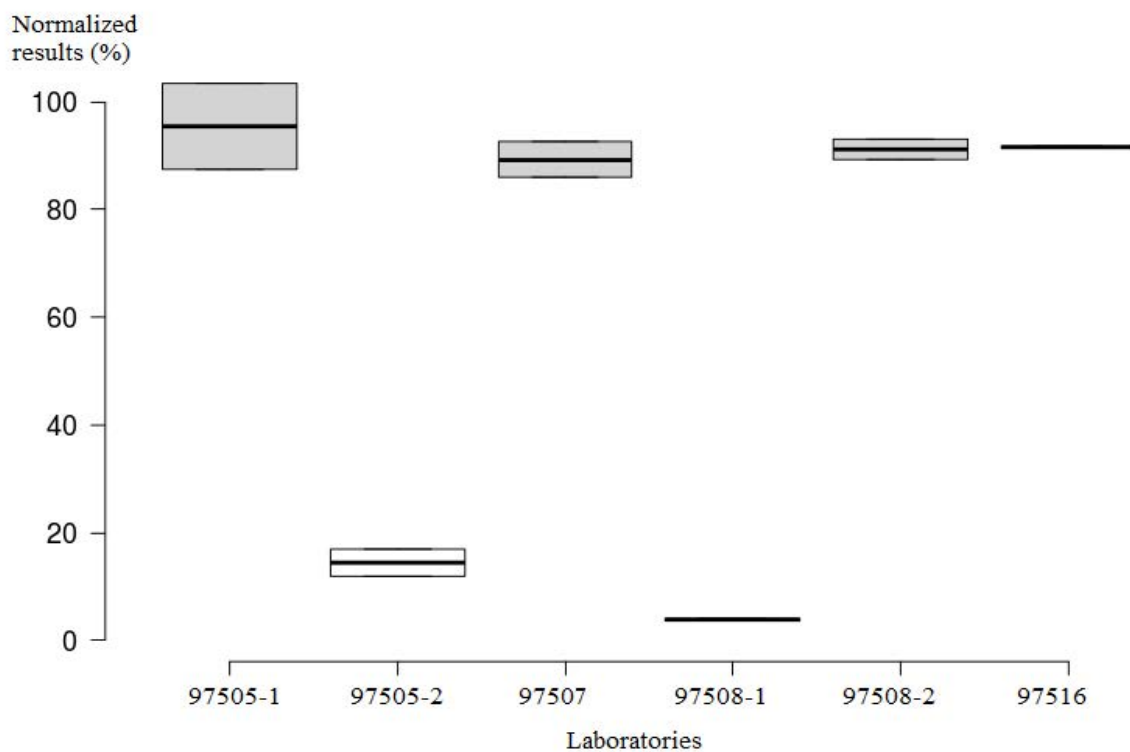


Fig. A5. Boxplot distribution of the results per dataset.

PT2020ASFERNNS5

Table A6. Normalized quantitative results

Lab	Method	REP1(%)	REP2 (%)	mean	SD	CV (%)
97505	ID Screen African Swine Fever Competition	98.4	92.7	95.55	4.03	4.2
97505	INgezym PPA compac	9.2	3.2	6.20	4.24	68.43
97507	D Screen African Swine Fever Competition	85.88	85.59	85.74	0.21	0.24
97508	ID Screen African Swine Fever Indirect	1.33	2.07	1.70	0.52	30.78
97508	ID Screen African Swine Fever Competition	83.12	90.97	87.05	5.55	6.38
97516	ID Screen African Swine Fever Competition	83.5	86.8	85.15	2.33	2.74

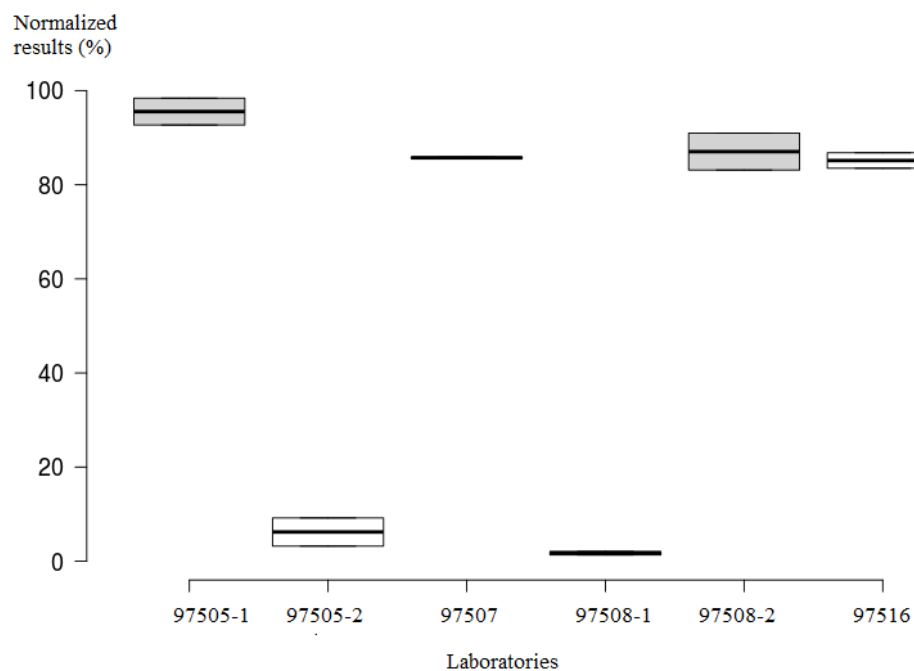


Fig. A6. Boxplot distribution of the results per dataset

Annexe 3: additional information

PRELIMINARY REPORT

The preliminary report of this survey is available on our website via the following link:

[https://www.wiv-isp.be/QML/activities/external_quality/rapports/download/Veterinary/Rapport%20préliminaire/VET%202020-1-VR%20%20\(157\).pdf](https://www.wiv-isp.be/QML/activities/external_quality/rapports/download/Veterinary/Rapport%20préliminaire/VET%202020-1-VR%20%20(157).pdf)

The calendar for Proficiency Testing in Veterinary diagnosis is available on our website:

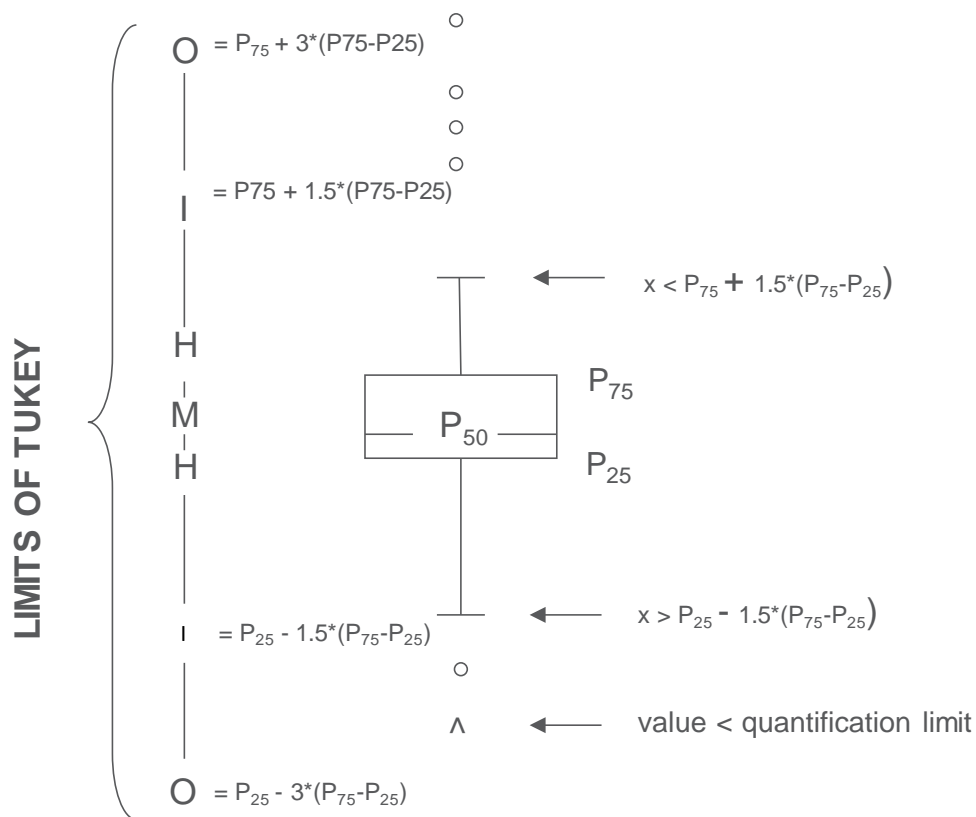
The link is:

https://www.wiv-isp.be/QML/activities/external_quality/calendar/calender_PT%20VET/fr/Calendrier_2020-PT%20VET%202.htm

Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 6 participants:

- a rectangle ranging from percentile 25 (P_{25}) to percentile 75 (P_{75})
- a central line representing the median of the results (P_{50})
- a lower limit showing the smallest value $x > P_{25} - 1.5 * (P_{75} - P_{25})$
- an upper limit representing the largest value $x < P_{75} + 1.5 * (P_{75} - P_{25})$
- all points outside this interval are represented by a dot.



Corresponding limits in case of normal distribution

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