BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

AFRICAN SWINE FEVER (ASF)

SURVEY 2022/3

Sciensano/PT VET ASF/1-E

Biological health risks
Quality of laboratories
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A draft version of this report was submitted to the experts on: 15/07/2022.

This report replaces the previous version of the preliminairy report of 20/04/2022.

Authorization of the report: by Ynse Van de Maele, scheme coordinator

Signature of the scheme coordinator.

YnVa2851 Digitally signed by YnVa2851 Date: 2022.07.29 10:04:01 +02'00'

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All the reports are also available on our webpage:
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_nl/rapports_annee.htm
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_fr/rapports_annee.htm

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1 INTRODUCTION

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

2 AIM

This PT was dedicated to detect the agent of African Swine Fever (ASF) (ASF virus) by ELISA (Ab) in serum and by Real Time PCR in serum.

3 MATERIALS AND METHODS

3.1 Serology on serum

3.1.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of ASF serology on serum. The names of the participating laboratories are:

- Sciensano
- ARSIA
- DGZ
- Laboratoire de médecine vétérinaire de l'état (LMVE)
- LSI-Thermofisher Scientific (Lyon)

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Service of Viral reemerging enzootic and BEE diseases, Infectious diseases in animals Directorate, Sciensano.

Information about the **origin** and **preparation** of the samples:

- PT2022ASFSERSERUMPS1, PS2, and PS3 are sera collected on 3 domestic pigs surviving an experimental infection with ASFV Belgium2018/1 strain (genotype II) at day 18 post infection.
- PT2022ASFSERSERUMPS4 is a sera collected on 1 domestic pig surviving an experimental infection with ASFV E70 strain (genotype I) at 21 days post infection.
- PT2022ASFSERSERUMNS1 and NS2 are sera collected on 2 naïve domestic pigs.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (250 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 7 positive and 3 negative samples:

Sample ID	Status
PT2022ASFSERSERUMPS1	POS
PT2022ASFSERSERUMPS2	POS
PT2022ASFSERSERUMPS3	POS
PT2022ASFSERSERUMPS4	POS
PT2022ASFSERSERUMNS1	NEG
PT2022ASFSERSERUMNS2	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

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

3.1.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: ASFSER SERUM	97505	97507	97508	97516	97541
22-1	PS4	PS2	PS1	NS2	PS1
22-2	NS2	PS4	PS4	PS4	NS2
22-3	PS4	NS2	NS2	PS4	PS3
22-4	NS2	PS1	NS2	PS2	PS2
22-5	NS1	PS2	PS3	NS1	NS1
22-6	PS3	NS2	NS1	PS1	PS2
22-7	PS3	NS1	PS2	PS3	PS4
22-8	PS1	PS3	PS2	NS2	PS3
22-9	PS2	PS4	PS3	PS3	PS4
22-10	PS2	PS3	PS4	PS2	NS2

3.2 Virology on serum

3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of ASF virology on serum. The names of the participating laboratories are:

- Sciensano
- ARSIA
- DGZ
- Laboratoire de médecine vétérinaire de l'état (LMVE)
- LSI-Thermofisher Scientific (Lissieu)
- IDEXX (Montpellier)

3.2.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Service of Viral reemerging enzootic and BEE diseases, Infectious diseases in animals Directorate, Sciensano.

Information about the **origin** and **preparation** of the samples:

- PT2022ASFVIRSERUMPS1 is a serum sample obtained from a domestic pig experimentally infected with ASFV Belgium2018/1 stain (genotype II). The serum sample was collected at the euthanasia, 8 days post challenge, as the animal demonstrated clinical signs.
- PT2022ASFVIRSERUMPS3, NS4 and NS5 are, respectively, dilution 1/10, 1/100 and 1/500 of PT2022ASFVIRSERUMPS1 in the serum of one naïve domestic pig.
- PT2022ASFVIRSERUMPS2 is a serum sample obtained from a domestic pig experimentally infected with ASFV Belgium2018/1 stain (genotype II). The serum sample was collected 18 days post challenge on this animal that presented no clinical signs.
- PT2022ASFVIRSERUMNS1 and NS2 are sera collected on 2 naïve domestic pigs.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (500 µl) of each sample using Real Time PCR before the PT. The samples were considered as homogeneous.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 7 positive and 3 negative samples:

Sample ID	Status
PT2022ASFVIRSERUMPS1	POS
PT2022ASFVIRSERUMPS2	POS
PT2022ASFVIRSERUMPS3	POS
PT2022ASFVIRSERUMPS4	POS
PT2022ASFVIRSERUMPS5	POS
PT2022ASFVIRSERUMNS1	NEG
PT2022ASFVIRSERUMNS2	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

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

3.2.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: ASFVIR SERUM	97505	97507	97508	97516	97534	97543
22-1	PS5	PS5	PS5	NS2	NS1	PS3
22-2	PS1	PS1	PS3	PS1	PS4	PS5
22-3	PS2	PS2	NS1	NS1	NS2	PS4
22-4	PS5	PS4	PS1	PS4	NS1	NS2
22-5	NS2	NS1	PS4	PS4	PS3	PS2
22-6	PS4	NS1	NS1	PS5	PS5	NS1
22-7	PS4	NS2	PS5	PS5	PS2	PS4
22-8	NS1	PS4	PS4	PS2	PS5	PS1
22-9	NS1	PS5	PS2	PS3	PS1	NS1
22-10	PS3	PS3	NS2	NS1	PS4	PS5

4 SURVEY TIMELINE

Transfer of the samples from NRL to QL: 16/03/2022
Randomization of the samples by QL: 17/03/2022
Sending samples (frozen at - 20 °C) to participants: 24/03/2022
Deadline for submitting the results: 15/04/2022
Preliminary report: 20/04/2022

5 RESULTS

5.1 Serology on serum

The panel consisted of 6 different samples, but samples PS2, PS3, PS4 and NS2 were repeated twice. Therefore, in total, the panel consisted of 10 samples (7 positive and 3 negative samples).

One lab had chosen to test two different methods on the same samples, implying that there were two datasets submitted. These additional results are included in the tables below.

5.1.1 RESULTS PER SAMPLE

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	1 (6)	6 POS
PS2	POS	2 (12)	12 POS
PS3	POS	2 (12)	12 POS
PS4	POS	2 (12)	12 POS
NS1	NEG	1 (6)	6 NEG
NS2	NEG	2 (12)	12 NEG

(POS = positive; NEG = negative)

5.1.2 USED METHOD

Method	N	NR	NCR	%
Ingenasa - Ingezym ASF-R	1	10	10	100
ID.VET - ID SCREEN® AFRICAN SWINE FEVER COMPETITION	4	40	40	100
Other	1	10	10	100
TOTAL	**6**	**60**	**60**	**100**

(N= number of laboratories; NR = number of results; NCR = number of correct results)

5.1.3 CONCLUSION

In total, three different methods were used by the laboratories. All these methods achieved 100% correctness, which means that 100 correct results were submitted.

5.2 Virology on serum

The panel consisted of 7 different samples, but samples PS4, PS5 and NS1 were repeated twice. Therefore, in total, the panel consisted of 10 samples (7 positive and 3 negative samples).

5.2.1 RESULTS PER SAMPLE

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	1 (6)	6 POS
PS2	POS	1 (6)	6 POS
PS3	POS	1 (6)	6 POS
PS4	POS	2 (12)	12 POS
PS5	POS	2 (12)	12 POS
NS1	NEG	2 (12)	6 NEG
NS2	NEG	1 (6)	6 NEG

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Method	N	NR	NCR	%
PCR method: Tignon *et al* 2011	1	10	10	100
ID.VET - ID Gene® African Swine Fever Duplex	2	20	20	100
QIAGEN Virotype ASF PCR kit	1	10	10	100
Thermofisher - VetMAX™ African Swine Fever Virus Detection Kit	1	10	10	100
Idexx - RealPCR ASFV DNA mix lot	1	10	10	100
TOTAL	**6**	**60**	**60**	**100**

(N= number of laboratories; NR = number of results; NCR = number of correct results)

5.2.3 EXTRACTION METHOD

Extraction method	N	NR	NCR	%
Indical - IndiMag Pathogen Kit	3	30	30	100
IDVET - ID Gene Mag Universal Extraction kit	1	10	10	100
ThermoFisher Scientific - other	1	10	10	100
QIAGEN - QIAamp DNA Mini kit	1	10	10	100
TOTAL	**6**	**60**	**60**	**100**

5.2.4 CONCLUSION

In total, five different methods were used by the laboratories. All these methods achieved 100% correctness, which means that 100 correct results were submitted.

One lab mentioned that their sample 1 and 4 only contained 250 µL each instead of 500 µl, but they reported that it was enough to perform the assay

6 ANNEXES (NOT UNDER ACCREDITATION)

The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/

6.1 Annex 1: Quantitative results

6.1.1 SEROLOGY ON SERUM

PT2022ASFSERSERUMPS2

Lab number	97505-1	97505-2	97507	97508	97516	97541
Method	M_1	M_2	M_2	M_2	M_2	M_3
REP1	74.8	12.1	8.6	11.0	16.8	209
REP2	73.1	9.7	7.3	10.2	14.6	216
Mean	73.9	10.9	7.9	10.6	15.7	212.5
SD	1.2	1.7	0.9	0.5	1.5	4.9
CV (%)	1.7	15.7	11.7	4.8	9.8	2.3

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Ingenasa - Ingezym ASF-R; M_2 = ID.VET - ID SCREEN® AFRICAN SWINE FEVER COMPETITION; M_3 = other)

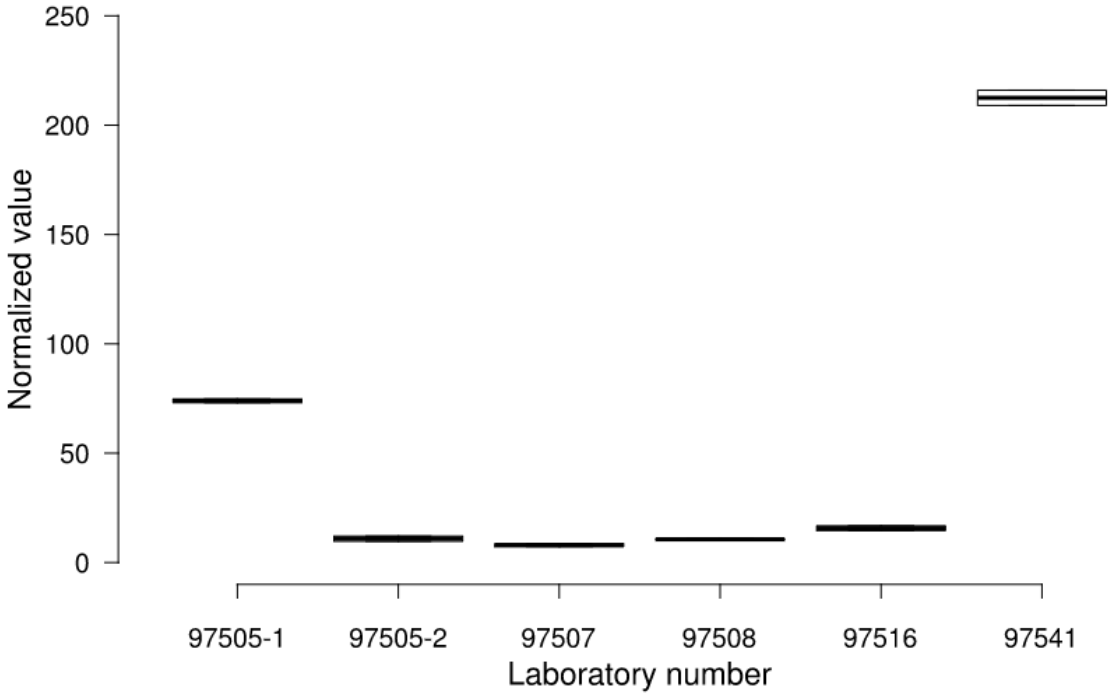


Figure 1. Distribution of the normalized values (box-plots) per laboratory.

PT2022ASFSERSERUMPS3

Lab number	97505-1	97505-2	97507	97508	97516	97541
Method	M_1	M_2	M_2	M_2	M_2	M_3
REP1	80.8	12.5	9.4	12.4	9.1	219
REP2	79.5	15.7	13.3	12.2	11.6	215
Mean	80.1	14.1	11.4	12.3	10.4	217
SD	0.9	2.3	2.8	0.1	1.8	2.8
CV (%)	1.1	16.2	24.4	0.9	17.0	1.3

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Ingenasa - Ingezym ASF-R; M_2 = ID.VET - ID SCREEN® AFRICAN SWINE FEVER COMPETITION; M_3 = other)

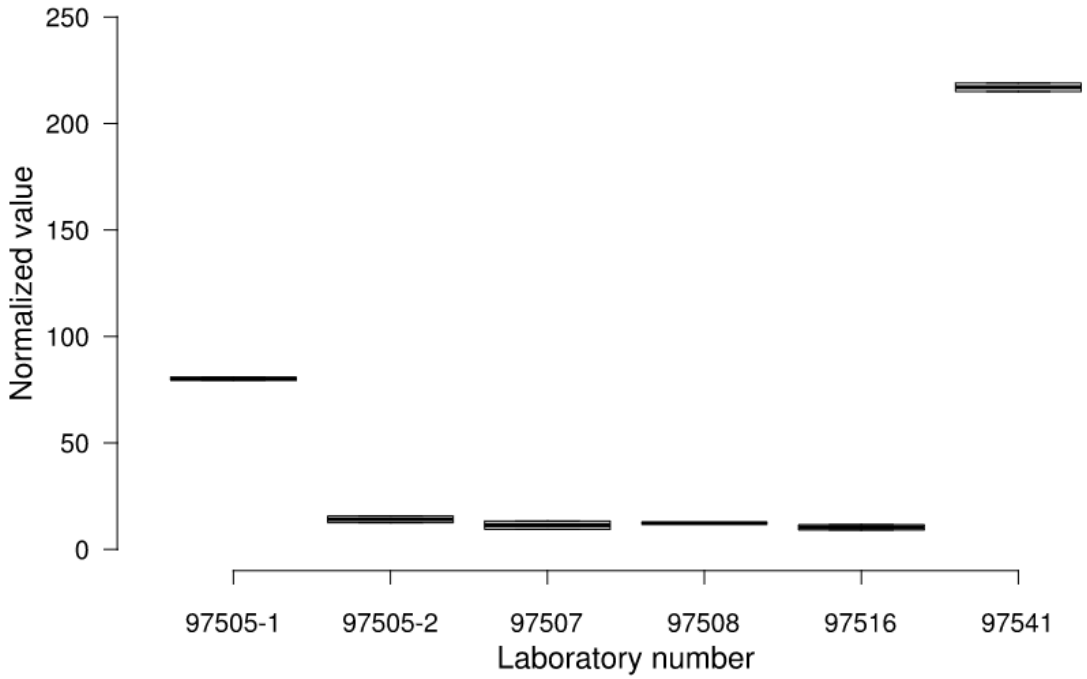


Figure 2. Distribution of the normalized values (box-plots) per laboratory.

PT2022ASFSERSERUMPS4

Lab number	97505-1	97505-2	97507	97508	97516	97541
Method	M_1	M_2	M_2	M_2	M_2	M_3
REP1	61.5	3.7	4.5	3.3	2.9	227
REP2	61.4	3.0	7.9	3.4	3.8	213
Mean	61.4	3.4	6.2	3.4	3.3	220
SD	0.01	3.4	2.4	0.1	0.6	9.9
CV (%)	0.02	100	39.1	3.4	17.2	4.5

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Ingenasa - Ingezym ASF-R; M_2 = ID.VET - ID SCREEN® AFRICAN SWINE FEVER COMPETITION; M_3 = other)

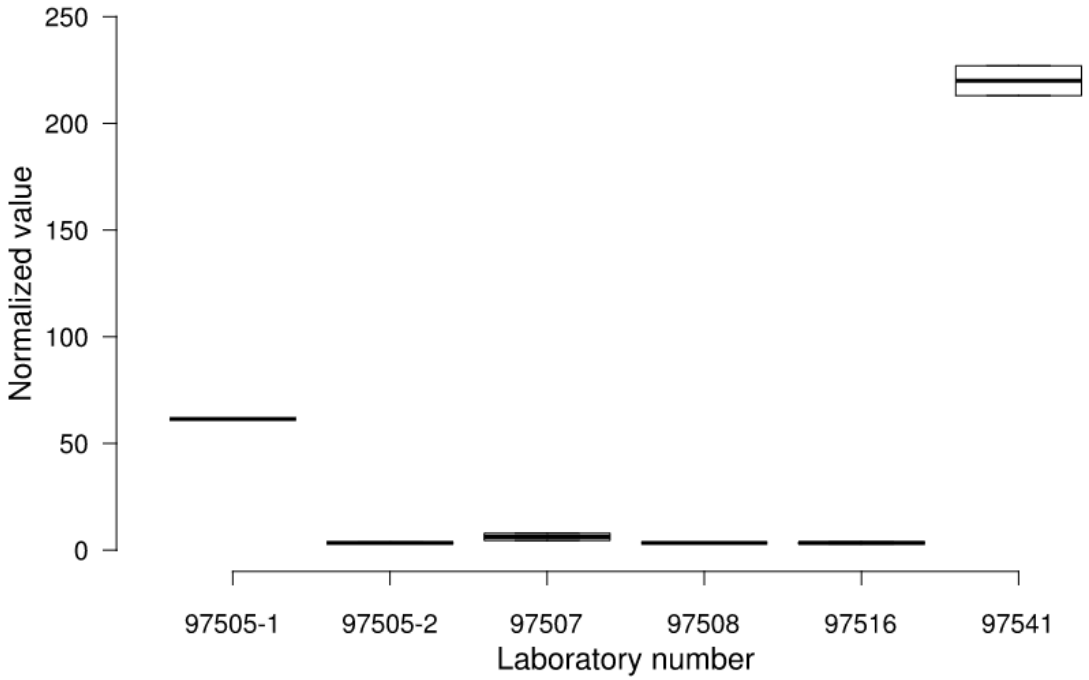


Figure 3. Distribution of the normalized values (box-plots) per laboratory.

PT2022ASFSERSERUMNS2

Lab number	97505-1	97505-2	97507	97508	97516	97541
Method	M_1	M_2	M_2	M_2	M_2	M_3
REP1	-4.5	99.1	102.5	98.9	89.2	0
REP2	2.3	102.0	100.5	96.0	83.4	2
Mean	-1.0	100.5	101.5	97.4	86.3	1
SD	4.8	100.5	1.4	2.0	4.1	1.4
CV (%)	-432.9	100	1.4	2.1	4.8	141.4

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Ingenasa - Ingezym ASF-R; M_2 = ID.VET - ID SCREEN® AFRICAN SWINE FEVER COMPETITION; M_3 = other)

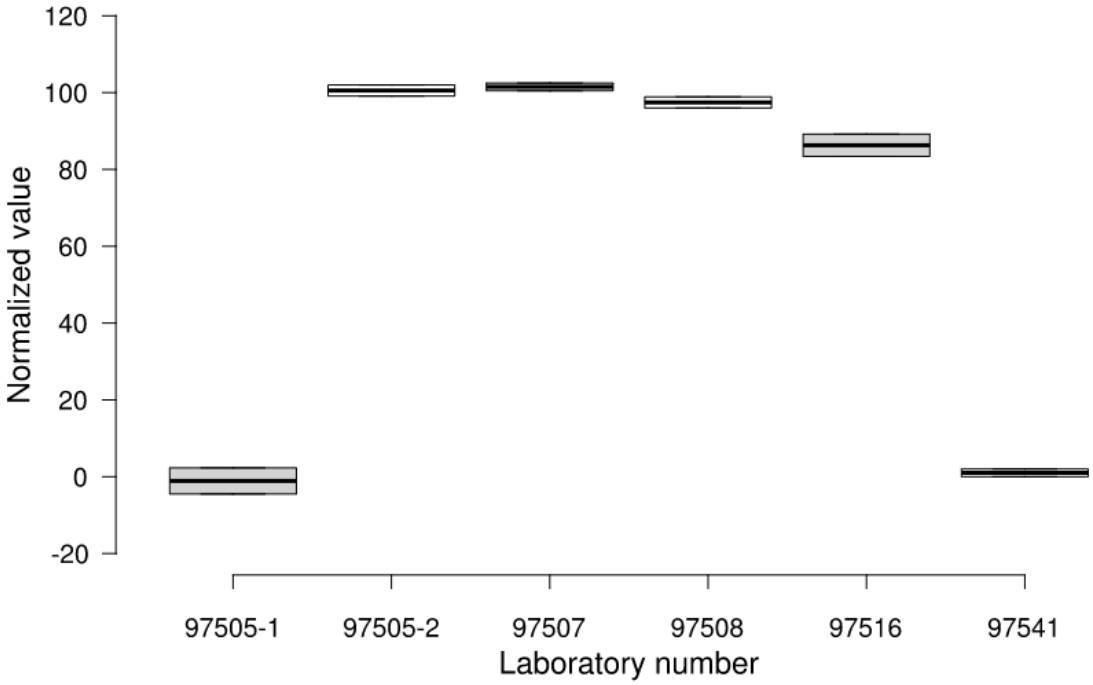


Figure 4. Distribution of the normalized values (box-plots) per laboratory.

6.1.2 VIROLOGY ON SERUM

PT2022ASFVIRSERUMPS4

Lab number	97505	97507	97508	97516	97534	97543
Method	M_1	M_2	M_3	M_2	M_4	M_5
REP1	30.4	29.7	29.9	28.5	30.8	36.5
REP2	30.2	29.3	29.7	28.3	30.9	37.6
Mean	30.3	29.5	29.8	28.4	30.9	37.1
SD	0.1	0.2	0.2	0.1	0.1	0.8
CV (%)	0.4	0.8	0.6	0.3	0.2	2.0

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = PCR method: Tignon et al 2011; M_2 = ID.VET - ID Gene® African Swine Fever Duplex; M_3 = QIAGEN Virotype ASF PCR kit; M_4 = Thermofisher - VetMAX™ African Swine Fever Virus Detection Kit; M_5 = Idexx - RealPCR ASFV DNA mix lot)

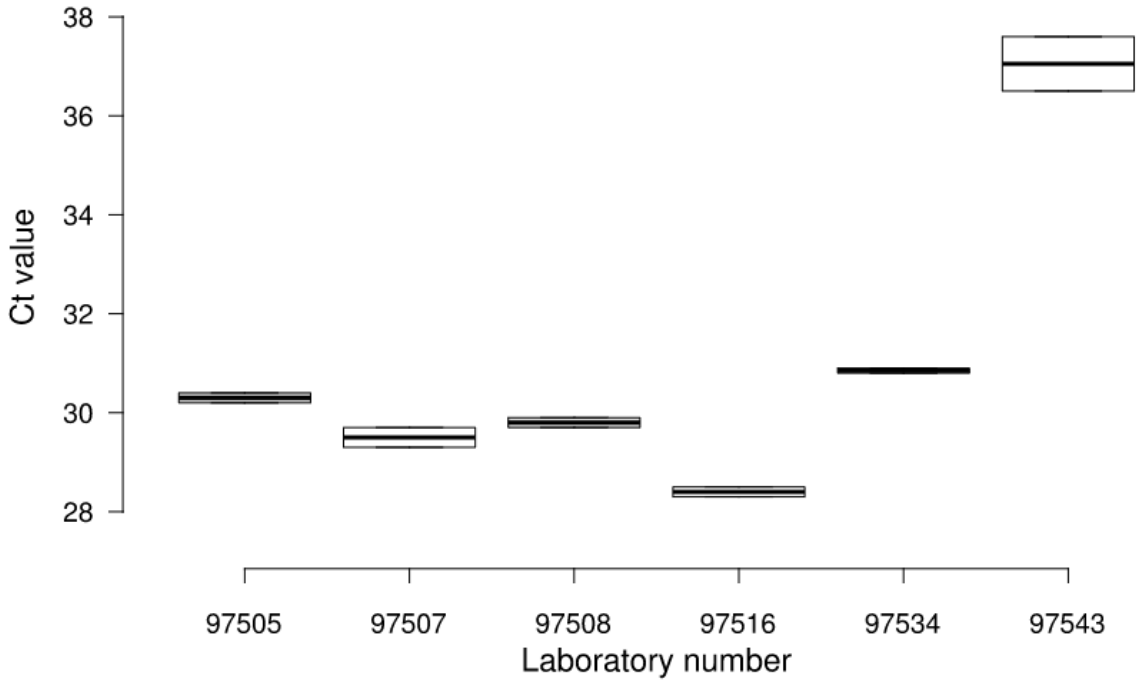


Figure 5. Boxplots of the dispersion of the results (Ct values) per laboratory.

PT2022ASFVIRSERUMPS5

Lab number	97505	97507	97508	97516	97534	97543
Method	M_1	M_2	M_3	M_2	M_4	M_5
REP1	33.0	32.4	32.7	30.7	33.7	37.8
REP2	32.6	32.7	32.1	30.5	33.3	39.6
Mean	32.8	32.5	0.4	30.6	33.5	38.7
SD	0.3	32.5	0.4	0.1	0.3	1.3
CV (%)	0.9	100	100	0.4	0.8	3.2

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = PCR method: Tignon et al 2011; M_2 = ID.VET - ID Gene® African Swine Fever Duplex; M_3 = QIAGEN Virotype ASF PCR kit; M_4 = Thermofisher - VetMAX™ African Swine Fever Virus Detection Kit; M_5 = Idexx - RealPCR ASFV DNA mix lot)

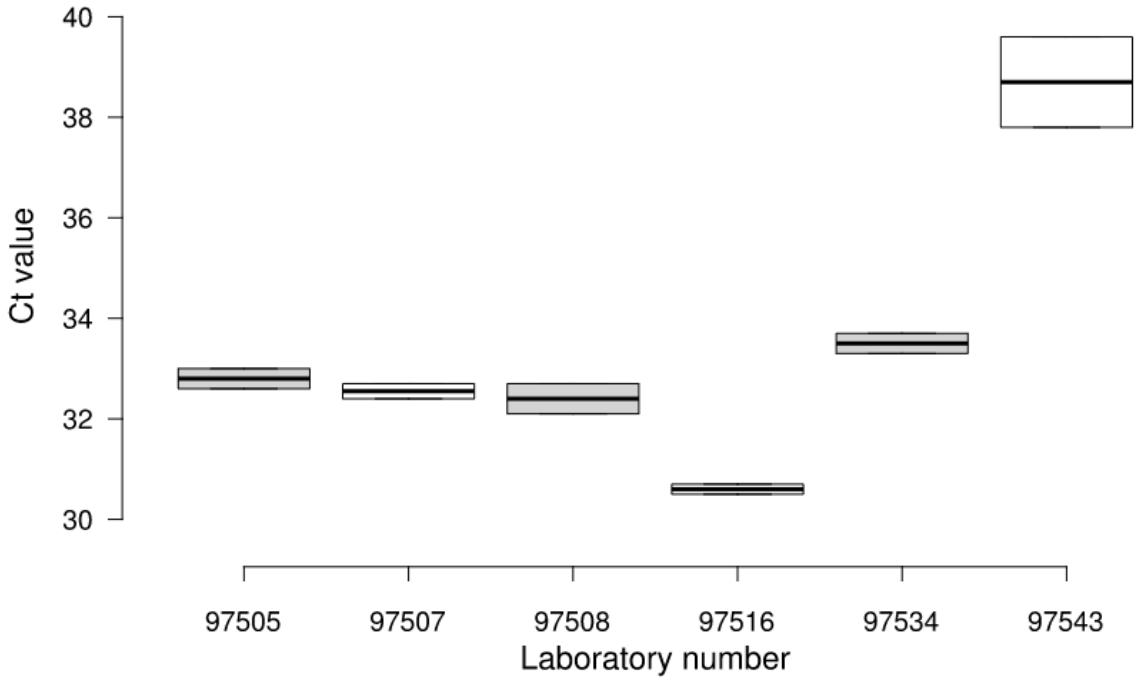


Figure 6. Boxplots of the dispersion of the results (Ct values) per laboratory.

6.2 Annex 2: Additional information

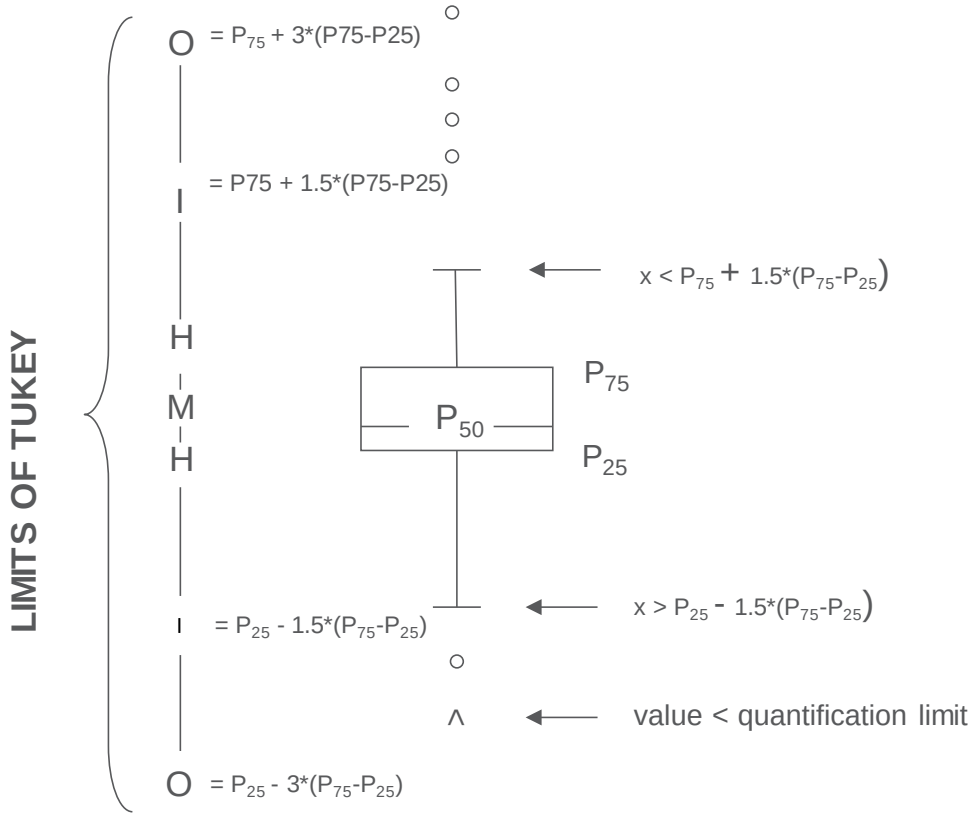
The preliminary report of this survey is available on our website via the following link:
https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

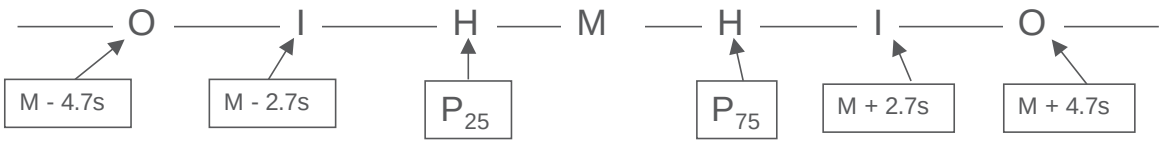
The calendar for Proficiency Testing in Veterinary diagnosis is available on our website:
https://www.wiv-isp.be/QML/activities/external_quality/calendar/kalender.htm

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 3 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

END