BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

AFRICAN SWINE FEVER - TYPE II STRAIN (ASF)

PROFICIENCY TEST 2023-1

Sciensano/PT VET ASF/2023-1/E
Biological health risks
Quality of laboratories
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- FR: https://www.sciensano.be/fr/qualite-des-laboratoires
- EN: https://www.sciensano.be/en/quality-laboratories

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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

The aim of the PT was to evaluate the ability of the participating laboratories 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 (serum)

3.1.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of African Swine Fever (ASF) serology on serum. The names of the participating laboratories are:

- Sciensano, department of Viral Re-emerging Enzootic and Bee Diseases
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LNCR / ACSEDIATE
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
- LSI-Thermofisher Scientific (Lyon)

3.1.2 THE SAMPLES

The samples (frozen serum) were prepared by the National Reference Laboratory (NRL), department of Viral Re-emerging Enzootic and Bee Diseases, Sciensano.

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

- PT2023ASFSERPS1, PS2, PS3 and PS5 are sera collected on 4 domestic pigs surviving an experimental infection with ASFV Belgium2018/1 strain (genotype II) at day 18 post infection. PT2023ASFSERPS1 has an blocking % value close to the cut-off and can be considered as Positive or doubtful.
- PT2022ASFSERSERUMPS4 is a sera collected on 1 domestic pig surviving an experimental infection with ASFV E70 strain (genotype I) at 21 days post infection.
- PT2023ASFSERNS1, NS2 and NS3 are sera collected on 3 naïve domestic pigs.

The ASF positive samples have been inactivated 30 min at 70°C.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (250 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of different of samples: 6 positive and 4 negative samples.

Sample ID	Repetition	Status
PT2023ASFSERPS1	1	POS or NI*
PT2023ASFSERPS2	2	POS
PT2023ASFSERPS3	1	POS
PT2023ASFSERPS4	1	POS
PT2023ASFSERPS5	1	POS
PT2023ASFSERNS1	2	NEG
PT2023ASFSERNS2	1	NEG
PT2023ASFSERNS3	1	NEG

(POS = positive; NEG = negative; NI = not interpretable)

* = This low positive sample has been collected on an infected animal at the early moment of seroconversion, implying that the result can be doubtful. Therefore, for this sample, POS or NI are accepted as correct results.

3.1.5 STABILITY

The criteria for stability is that the status of the sample in Post-PT remains the status assigned in pre-PT test. The stability check was conform.

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	97505	97507	97508	97510	97516	97541
23-1	NS3	NS2	NS1	PS3	PS3	PS2
23-2	PS3	PS2	PS2	NS1	NS3	PS5
23-3	NS1	PS5	PS1	PS5	PS1	NS2
23-4	PS1	NS3	NS2	PS2	NS1	NS1
23-5	NS1	PS4	PS3	NS3	PS2	PS3
23-6	PS5	PS1	NS1	PS1	PS4	PS2
23-7	PS2	PS3	NS3	PS4	PS2	NS1
23-8	NS2	NS1	PS5	PS2	NS1	PS4
23-9	PS2	PS2	PS2	NS2	PS5	NS3
23-10	PS4	NS1	PS4	NS1	NS2	PS1

3.2 Virology (serum)

3.2.1 THE PARTICIPANTS

Eight laboratories participated in the proficiency test of African Swine Fever (ASF) virology on serum. The names of the participating laboratories are:

- Sciensano, department of Viral Re-emerging Enzootic and Bee Diseases)
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LNCR / ACSEDIATE
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
- LSI-Thermofisher Scientific (Lissieu)
- IDEXX (Montpellier)
- BIO CHENE VERT - Laboratoire d’Analyse de Biologie Vétérinaire

3.2.2 THE SAMPLES

The samples (frozen serum) were prepared by the National Reference Laboratory (NRL), department of Viral Re-emerging Enzootic and Bee Diseases, Sciensano.

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

- PT2023ASFVIRPS1 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.
- PT2023ASFVIRPS2 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, 7 days post challenge, as the animal demonstrated clinical signs.
- PT2023ASFVIRPS4 and PS5 are, respectively, dilution 1/10 and 1/100 of the serum collected at 8 days post challenge from another domestic pig experimentally infected with ASFV Belgium2018/1 stain (genotype II) and presenting severe clinical signs.
- PT2023ASFVIRPS3 is a 1/10 dilution of the serum collected from the same animal as PT2023ASFVIRPS4 and PS5 but in RNA-later.
- PT2023ASFVIRNS1 and NS2 are sera collected on 2 naïve domestic pigs.

The ASF positive samples have been inactivated 30 min at 70°C.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (500 µl) of each sample using RT-qPCR method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of different of samples: 8 positive and 2 negative samples.

Sample ID	Repetition	Status
PT2023ASFVIRPS1	2	POS
PT2023ASFVIRPS2	1	POS
PT2023ASFVIRPS3	1	POS
PT2023ASFVIRPS4	1	POS
PT2023ASFVIRPS5	2	POS
PT2023ASFVIRPS6	1	POS
PT2023ASFVIRNS1	1	NEG
PT2023ASFVIRNS2	1	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

The criteria for stability is that the status of the sample in Post-PT remains the status assigned in pre-PT test. The stability check was conform.

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	97505	97507	97508	97510	97516	97534	97543	97547
23-1	NS1	PS2	PS1	PS1	NS2	PS4	NS1	NS1
23-2	PS5	PS5	PS5	PS6	PS5	NS2	PS5	PS5
23-3	PS4	PS1	PS2	PS1	PS1	PS3	PS4	PS4
23-4	PS5	PS4	PS1	NS2	PS1	PS2	PS5	PS5
23-5	PS1	PS5	NS1	PS5	PS6	PS5	PS1	PS1
23-6	NS2	NS1	PS3	PS5	PS3	PS1	NS2	NS2
23-7	PS1	PS1	PS4	PS3	PS5	PS6	PS1	PS1
23-8	PS3	PS6	NS2	PS2	NS1	PS5	PS3	PS3
23-9	PS6	NS2	PS5	PS4	PS2	PS1	PS6	PS6
23-10	PS2	PS3	PS6	NS1	PS4	NS1	PS2	PS2

4 TIMELINE

Transfer of the samples from NRL to QL: 13/02/2023
Randomization of the samples by QL: 27/02/2023
Sending samples to participants: in the week of 6 March 2023

- Samples serology: frozen at - 20 °C
- Samples virology: frozen at - 20 °C

Deadline for submitting the results: 24/03/2023
Individual report to the participants: 06/04/2023

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of 8 different samples. However, the samples PS2 and NS1 were repeated twice (see table below). Therefore, in total, the panel consisted of 10 samples (6 positive and 4 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.

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	1 (7)	7 POS
PS2	POS	2 (14)	14 POS
PS3	POS	1 (7)	7 POS
PS4	POS	1 (7)	7 POS
PS5	POS	1 (7)	7 POS
NS1	NEG	2 (14)	14 NEG
NS2	NEG	1 (7)	7 NEG
NS3	NEG	1 (7)	7 NEG

(POS = positive; NEG = negative)

5.1.2 USED METHOD

Method	Manufacturer ELISA kit	Name ELISA kit	Short or long incubation protocol	N	NR	NCR	%
ELISA Competition	ID.VET	ID Screen® African Swine Fever Competition	Short	**4**	**40**	**40**	**100**
ELISA Indirect	ID.VET	ID Screen® African Swine Fever Indirect	Short	**1**	**10**	**10**	**100**
ELISA Indirect	Ingenasa	INgezim ASFV-R	Short	**1**	**10**	**10**	**100**
ELISA Indirect	*Unknown**	*Unknown**	Short	**1**	**10**	**10**	**100**
TOTAL				**7**	**70**	**70**	**100**

*(N= number of laboratories; NR = number of results; NCR = number of correct results, * = the laboratory did not specify in the comment section the manufacturer and name of the ELISA kit).*

5.1.3 CONCLUSION

In 2023, six laboratories participated in proficiency test of African Swine Fever serology (serum) organized by Sciensano. 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 serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

5.2 Virology (serum)

RESULTS PER SAMPLE

The panel consisted of 8 different samples. However, samples PS1 and PS5 were repeated twice (see table below). Therefore, in total, the panel consisted of 10 samples (8 positive and 2 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	2 (16)	16 POS
PS2	POS	1 (8)	8 POS
PS3	POS	1 (8)	8 POS
PS4	POS	1 (8)	8 POS
PS5	POS	2 (16)	16 POS
PS6	POS	1 (8)	8 POS
NS1	NEG	1 (8)	8 NEG
NS2	NEG	1 (8)	8 NEG

(POS = positive; NEG = negative)

5.1.2 USED RT-PCR PROTOCOL/KIT

In the table below, the RT-PCR protocols/kits used are listed along with the number of laboratories that have used this protocol/kit with their achieved score.

Manufacturer RT-qPCR protocol / kit	Name RT-qPCR protocol / kit	N	NR	NCR	%
ID.VET	ID Gene® African Swine Fever Duplex	3	30	30	100
Thermo Fisher	VetMAX™ African Swine Fever Virus Detection Kit	1	10	10	100
IDEXX	RealPCR ASFV DNA mix lot	1	10	10	100
BioX diagnostics	ASV fast time	1	10	10	100
Indical	Virotype ASFV 2.0 PCR Kit	1	10	10	100
Home made	Home made	1	10	10	100
TOTAL		**8**	**80**	**80**	**100**

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

5.1.2 USED EXTRACTION PROTOCOL/KIT

In the table below, the extraction protocols/kits used are listed along with the number of laboratories that have used this protocol/kit with their achieved score.

Manufacturer extraction protocol / kit	Name extraction protocol / kit	N	NR	NCR	%
Indical	IndiMag Pathogen Kit	3	30	30	100
Thermo Fisher Scientific	*Unknown*	2	20	20	100
Thermo Fisher Scientific	MagVet Universal Isolation Kit	1	10	10	100
IDEXX	Real PCR DNA/RNA Magnetic Bead kit	1	10	10	100
BioX diagnostics	Adiamag XL	1	10	10	100
TOTAL		**8**	**80**	**80**	**100**

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

5.1.3 CONCLUSION

In 2023, eight laboratories participated in proficiency test of African Swine Fever virology (serum) organized by Sciensano. 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 serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

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 (SERUM)

PT2023ASFSERPS2

Lab number	97505 (1)	97505 (2)	97507	97508	97510	97516	97541
Method	M_1	M_3	M_1	M_1	M_2	M_1	M_4
OD (REP1)	0,21	0,63	0,25	0,25	1,23	0,26	3,07
OD (REP2)	0,25	0,57	0,30	0,27	1,17	0,31	3,00
Mean	0,23	0,60	0,28	0,26	1,20	0,28	3,04
SD	0,02	0,04	0,04	0,02	0,04	0,03	0,05
CV (%)	10,21	6,82	12,86	7,07	3,29	11,72	1,72

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = ID.VET - ID Screen® African Swine Fever Competition; M_2 = ID.VET - ID Screen® African Swine Fever Indirect; M_3 = Ingenasa - INgezim ASFV-R; M_4 = Unknown).

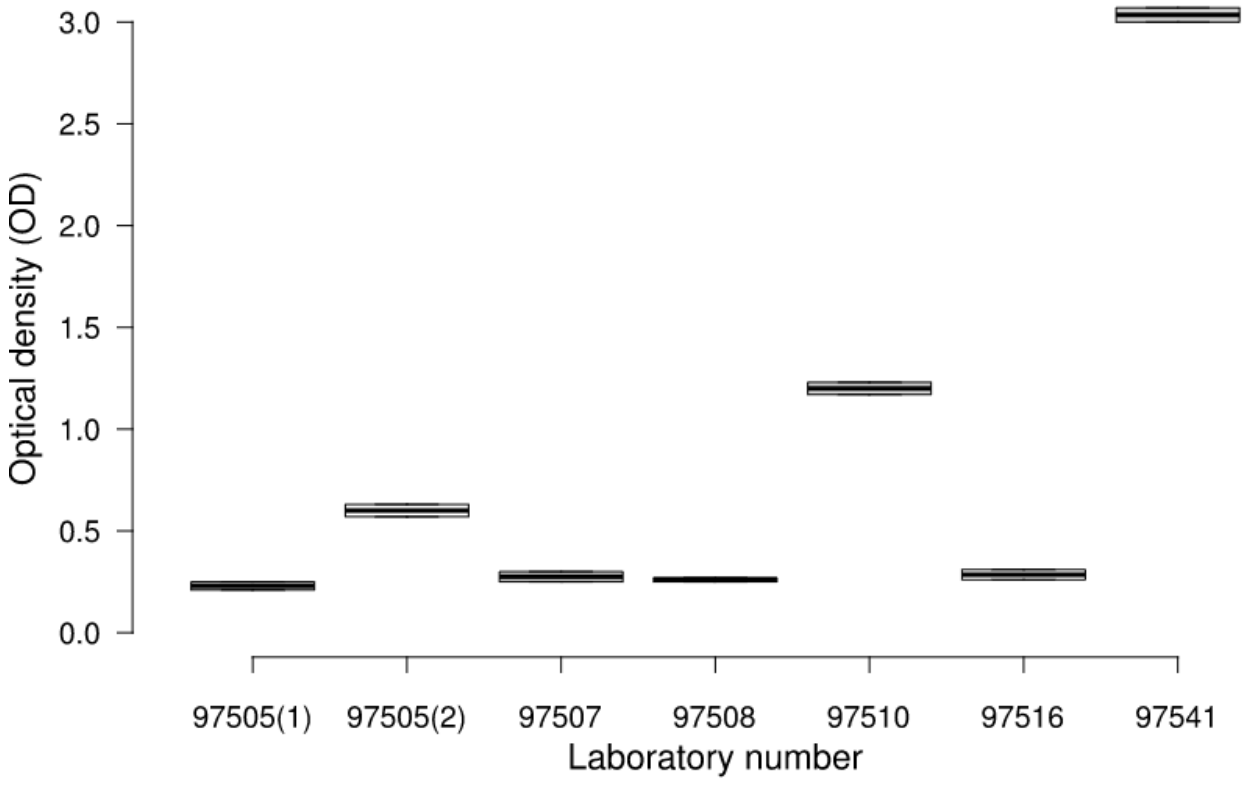


Figure 1. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97505	97507	97508	97510	97516	97534	97543	97547
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_2	M_2	M_4	M_5	M_6
Ct (REP1)	32,94	31,13	31,44	32,60	29,50	35,60	32,35	33,00
Ct (REP2)	33,49	31,12	31,80	32,30	29,48	36,60	32,98	33,00
Mean	33,22	31,13	31,62	32,45	29,49	36,10	32,67	33,00
SD	0,39	0,01	0,25	0,21	0,01	0,71	0,45	0,00
CV (%)	1,17	0,02	0,81	0,65	0,05	1,96	1,36	0,00

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade, M_2 = ID.VET - ID Gene® African Swine Fever Duplex , M_3 = Indical - Virotype ASFV 2.0 PCR Kit, M_4 = Thermofisher - VetMAX™ African Swine Fever Virus Detection Kit, M_5 = IDEXX - RealPCR ASFV DNA mix lot ,M_6 = BioX diagnostics - ASV fast time).

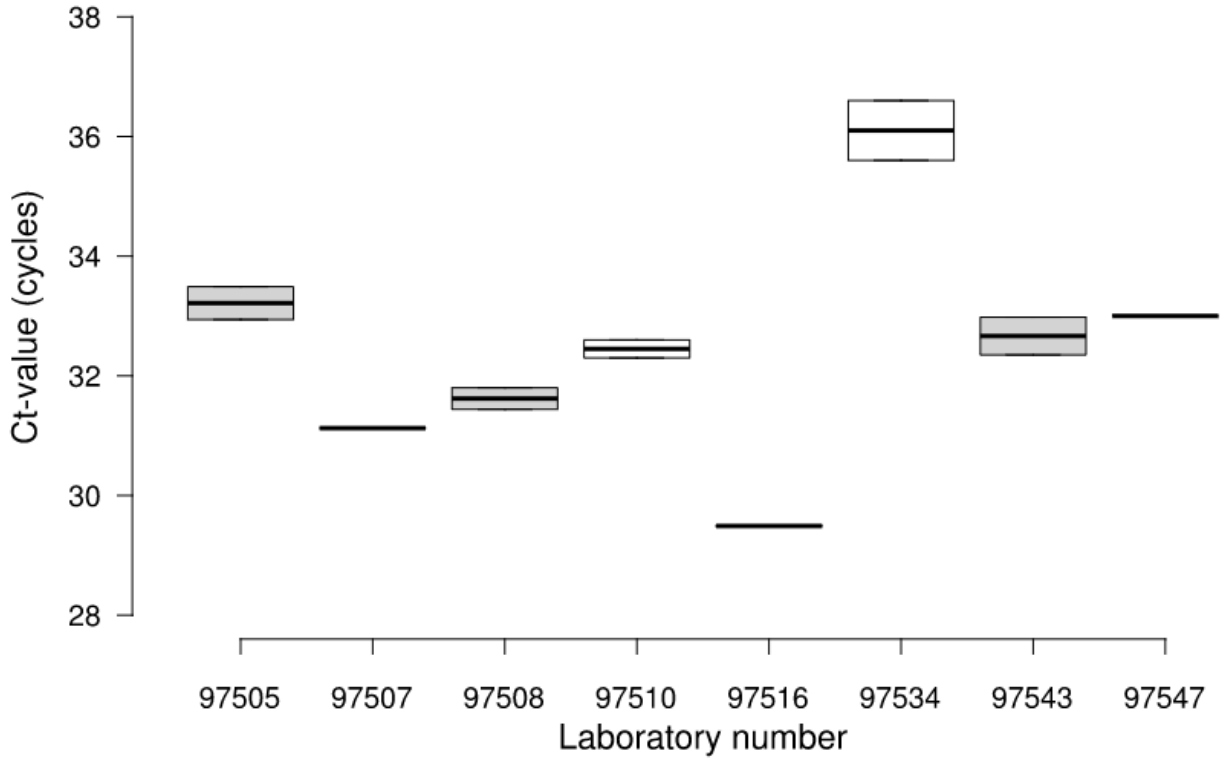


Figure 2. Distribution of the Ct-values (box-plots) per laboratory.

Lab number	97505	97507	97508	97510	97516	97534	97543	97547
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_2	M_2	M_4	M_5	M_6
Ct (REP1)	31,87	28,99	29,28	29,38	27,33	32,80	30,63	30,00
Ct (REP2)	30,96	28,12	29,27	29,72	27,64	33,20	30,31	30,00
Mean	31,42	28,56	29,28	29,55	27,49	33,00	30,47	30,00
SD	0,64	0,62	0,01	0,24	0,22	0,28	0,23	0,00
CV (%)	2,05	2,15	0,02	0,81	0,80	0,86	0,74	0,00

Numbers were rounded to 2 decimal place. (OD = Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade, M_2 = ID.VET - ID Gene® African Swine Fever Duplex , M_3 = Indical - Virotype ASFV 2.0 PCR Kit, M_4 = Thermofisher - VetMAX™ African Swine Fever Virus Detection Kit, M_5 = IDEXX - RealPCR ASFV DNA mix lot ,M_6 = BioX diagnostics - ASV fast time).

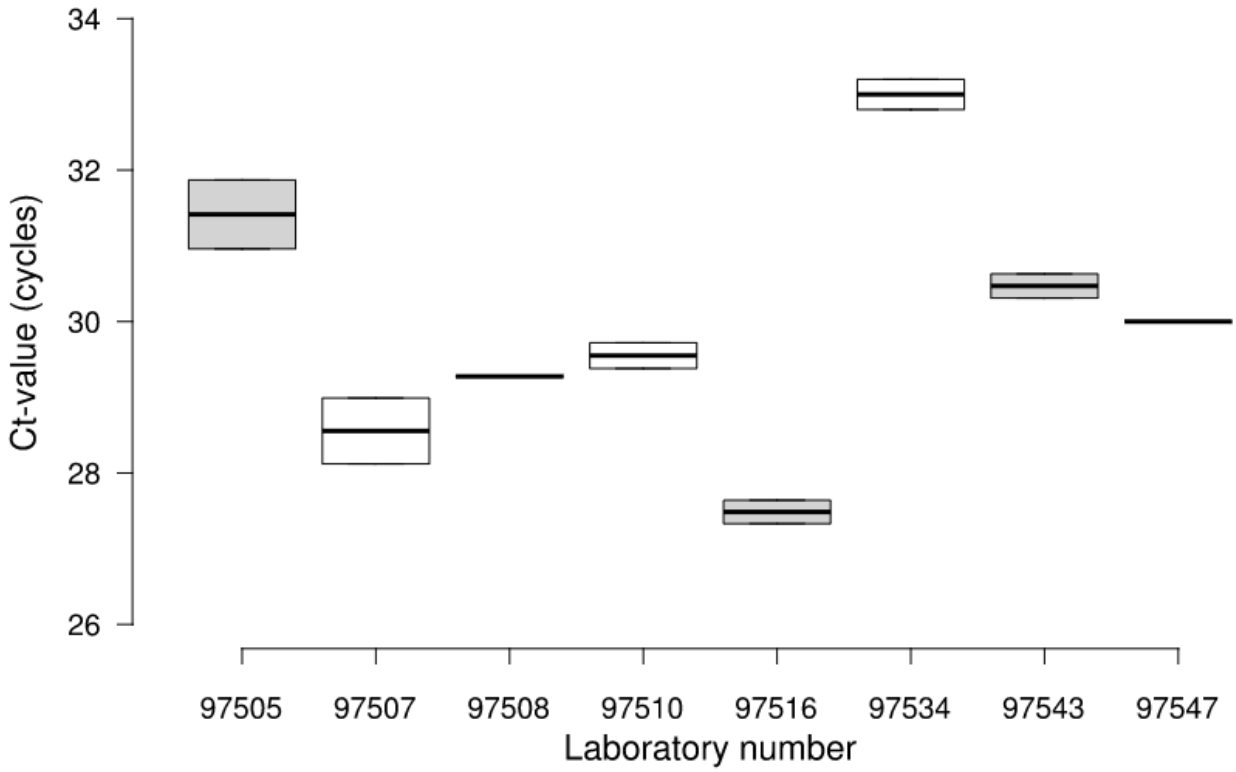


Figure 3. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

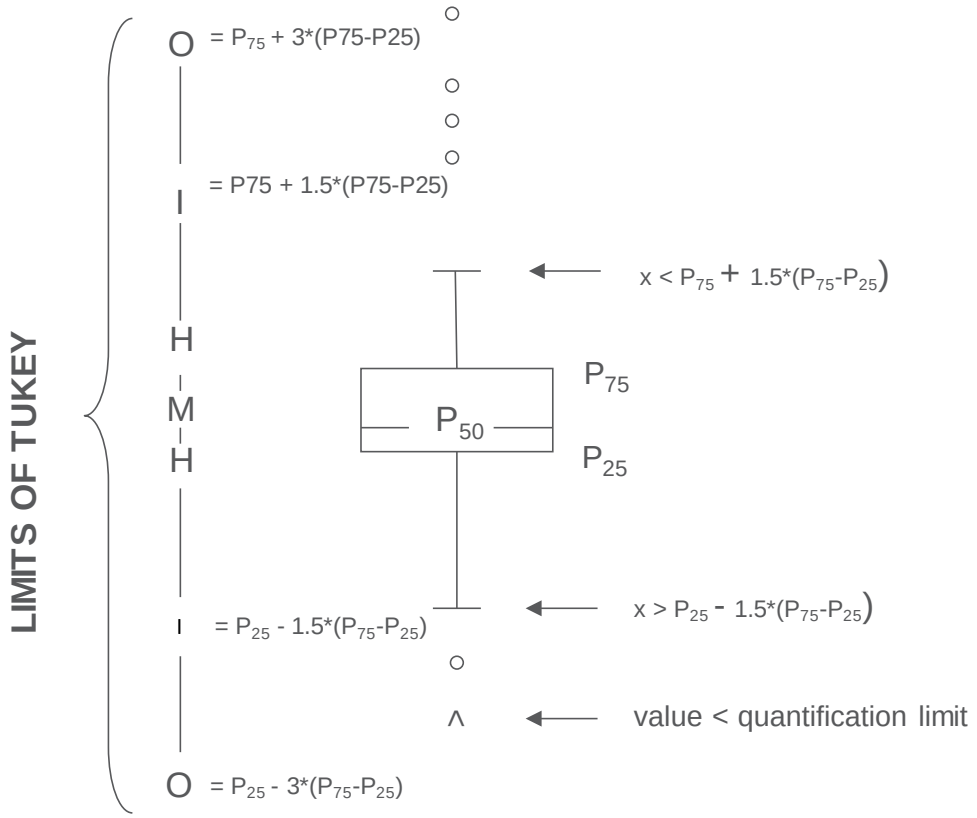
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

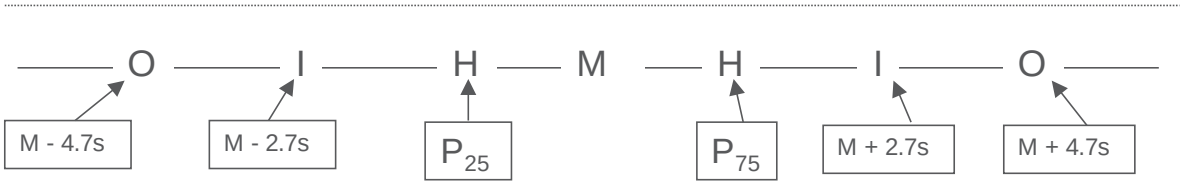
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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

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