BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

PROFICIENCY TEST 2023-2

Sciensano/PT VET PRRS/2023-2/E
Biological health risks
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- NL: https://www.sciensano.be/nl/kwaliteit-van-laboratoria
- FR: https://www.sciensano.be/fr/qualite-des-laboratoires
- EN: https://www.sciensano.be/en/quality-laboratories

TABLE OF CONTENTS

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 Porcine Reproductive and Respiratory Syndrome (PRRS) (PRRS 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 Porcine Reproductive and Respiratory Syndrome (PRRS) serology on serum. The names of the participating laboratories are:

- Sciensano, department of Viral Re-emerging Enzootic and Bee Diseases
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- ANSES-Ploufragan-Unité Virologie Immunologie Porcines
- IDVET (France)
- INDICAL BIOSCIENCE GmbH

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:

- **PT2023PRRSSERNS1** and **PT2023PRRSSERNS2** are two serum samples collected from two domestic pigs originated from a PRRS-free herd located in Flanders.
- **PT2023PRRSSERPS1** is an undiluted serum collected from one domestic pig originated from a conventional herd and collected 23 days after experimental challenge with PRRSV (Flanders/13 strain).
- **PT2023PRRSSERPS2** is an undiluted serum collected from one domestic pig originated from a PRRSV-free herd and collected at 14 days after experimental vaccination with Porcilis.
- **PT2023PRRSSERPS3** is an undiluted serum collected from one domestic pig originated from a PRRSV-free herd and collected at 14 days after experimental vaccination with Ingelvac.
- **PT2023PRRSSERPS4** is an undiluted serum collected from one domestic pig originated from a PRRSV-free herd and collected at 21 days after experimental vaccination with Unistrain.

- **PT2023PRRSSERPS5** is a serum sample collected from one domestic pig from conventional herd vaccinated two times with PRRSFLEX (21 days apart) and experimentally challenge with PRRSV (Flanders/13 strain) 3 weeks after the second vaccination. The serum sample was collected 23 days after challenge.
- **PT2023PRRSSERPS6** is an undiluted serum collected from one domestic pig originated from a PRRSV-free herd and collected at 21 days after experimental vaccination with Suvayn.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (100 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criterion to consider that the homogeneity are 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
PT2023PRRSSERPS1	1	POS
PT2023PRRSSERPS2	1	POS
PT2023PRRSSERPS3	1	POS
PT2023PRRSSERPS4	1	POS
PT2023PRRSSERPS5	1	POS
PT2023PRRSSERPS6	1	POS
PT2023PRRSSERNS1	2	NEG
PT2023PRRSSERNS2	2	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The criterion 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: PRRSSER	97505	97507	97508	97515	97522	97532
23-1	PS5	NS1	PS4	PS3	NS2	NS1
23-2	PS3	PS6	NS1	PS5	PS4	NS2
23-3	NS1	PS4	NS2	PS4	NS1	PS6
23-4	NS1	PS3	PS1	NS1	NS1	NS1
23-5	PS2	NS2	PS5	PS6	PS2	PS4
23-6	NS2	PS5	PS6	NS2	PS3	PS2
23-7	PS4	PS2	NS1	NS2	PS5	PS5
23-8	PS6	NS2	NS2	NS1	PS6	NS2
23-9	PS1	NS1	PS2	PS1	NS2	PS1
23-10	NS2	PS1	PS3	PS2	PS1	PS3

3.2 Virology (serum)

3.2.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Porcine Reproductive and Respiratory Syndrome (PRRS) virology on serum. The names of the participating laboratories are:

- Sciensano, department of Viral Re-emerging Enzootic and Bee Diseases)
- Dierengezondheidszorg Vlaanderen (DGZ)
- ANSES-Ploufragan-Unité Virologie Immunologie Porcines
- INDICAL BIOSCIENCE GmbH

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:

- **PT2023PRRSVIRNS1** is a serum sample collected from one domestic pig originated from a PRRS-free herd located in Flanders
- **PT2023PRRSVIRPS1 and PT2023PRRSVIRPS2** are two serum samples collected from two domestic pigs originated from a PRRSV-free herd and collected at 17 days after experimental infection with PRRSV (Flanders/13 strain). **PT2023PRRSVIRPS1** is stored in the preservative reagent Primestore (0,5 ml serum + 1,5 ml Primestore). **PT2023PRRSVIRPS2** is undiluted (no preservative).
- **PT2023PRRSVIRPS3** and **PT2023PRRSVIRPS5** are two dilutions of a virus culture of Flanders13 strain on Porcine Alveolar Macrophages (10E6,7 TCID50/ ml). **PT2023PRRSVIRPS3** is the dilution at 10E-3 of the virus culture and the estimated virus concentration is 5000 TCID50/ml. **PT2023PRRSVIRPS5** is the dilution at 10E-5 of the virus culture and the estimated virus concentration is 50 TCID50/ml.

- **PT2023PRRSVIRPS4** is a serum collected from a non-responder piglet ("G9") vaccinated once with PRRSFLEX at 3 weeks of age and experimentally infected with PRRSV (Flanders/13 strain) 6 weeks post vaccination. The serum was collected at 42 days post infection.
- **PT2023PRRSVIRPS6** is an undiluted serum collected from one domestic pig originated from a PRRSV-free herd and collected at 21 days after experimental vaccination with Ingelvac (North American vaccine strain).

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 criterion 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
PT2023PRRSVIRPS1	1	POS EU/ NEG NA
PT2023PRRSVIRPS2	1	POS EU/ NEG NA
PT2023PRRSVIRPS3	1	POS EU/ NEG NA
PT2023PRRSVIRPS4	2	POS EU/ NEG NA
PT2023PRRSVIRPS5	1	POS EU/NEG EU /NEG NA
PT2023PRRSVIRPS6	2	NEG EU/ POS NA
PT2023PRRSVIRNS1	2	NEG EU/ NEG NA

(POS = positive; NEG = negative; EU = European genotype; NA = North American genotype)

3.2.5 STABILITY

The criterion 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 for all samples **except for PT2023PRRSVIRPS3 and PT2023PRRSVIRPS5.** The expected Cp values for PT2023PRRSVIRPS3 were higher than expected but remains over the limit of detection, which was not the case for PT2023PRRSVIRPS5. **PT2023PRRSVIRPS5 gave negative results in the three repeats of the Post-PT**. The expected (weak) positive status has been adapted to Positive or Negative for this sample.

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: PRRSVIR	97505	97508	97515	97532
23-1	NS1	NS1	PS2	NS1
23-2	NS1	PS1	PS6	PS5
23-3	PS4	PS6	NS1	PS4
23-4	PS6	PS4	NS1	PS4
23-5	PS2	NS1	PS3	PS3
23-6	PS6	PS6	PS1	PS2
23-7	PS3	PS4	PS4	PS1
23-8	PS4	PS3	PS6	NS1
23-9	PS1	PS2	PS4	PS6
23-10	PS5	PS5	PS5	PS6

4 TIMELINE

Transfer of the samples from NRL to QL: 13/04/2023
Randomization of the samples by QL: 13/04/2023
Sending samples to participants: in the week of the 24th of April 2023

- Samples serology: frozen at - 16 °C
- Samples virology: frozen at - 16 °C

Deadline for submitting the results: 12/05/2023
Individual report to the participants: 07/06/2023

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of 8 different samples. However, the negative samples NS1 and NS2 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	1 (7)	7 POS
PS3	POS	1 (7)	7 POS
PS4	POS	1 (7)	7 POS
PS5	POS	1 (7)	7 POS
PS6	POS	1 (7)	7 POS
NS1	NEG	2 (14)	14 NEG
NS2	NEG	2 (14)	14 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 Indirect	IDEXX	PRRS X3 Ab	Short	**4**	**40**	**40**	**100**
ELISA Indirect	INDICAL (QIAGEN)	Pigtype PRRSV Ab	Short	**2**	**20**	**20**	**100**
ELISA Indirect	ID.VET	ID Screen PRRS Indirect	Short	**1**	**10**	**10**	**10**
TOTAL				**7**	**70**	**70**	**100**

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

5.1.3 CONCLUSION

In 2023, six laboratories participated in proficiency test of Porcine Reproductive and Respiratory Syndrome 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 7 different samples. However, samples PS4, PS6 and NS1 were repeated twice (see table below). Therefore, in total, the panel consisted of 10 samples (8 positive and 2 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 EU/ NEG NA	1 (5)	5 POS
PS2	POS EU/ NEG NA	1 (5)	5 POS
PS3	POS EU/ NEG NA	1 (5)	5 POS
PS4	POS EU/ NEG NA	2 (10)	10 POS
PS5	POS EU/NEG EU/ NEG NA	1 (5)	4 POS 1 NEG
PS6	NEG EU/ POS NA	2 (10)	10 POS
NS1	NEG EU/ NEG NA	2 (10)	10 NEG

(POS = positive; NEG = negative; EU = European genotype; NA = North American genotype)

* = Laboratories should not be able to distinguish between the two strains (EU-genotype and NA-genotype). It is sufficient to answer 'POS' or 'NEG' as interpretation.

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	%
Thermo Fisher	VTMAX PRRS EU & NA 2.0 Kit	3	30	30	100
Applied Biosystems	VTMAX PRRS EU & NA 2.0 Kit	1	10	10	100
Indical	Virotype PRRSV 2.0 RT-PCR Kit	1	10	10	100
TOTAL		**5**	**50**	**50**	**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	%
Qiagen	QiAmp viral RNA kit	1	10	10	100
Indical Bioscience	IndiMag Pathogen Kit	2	20	20	100
Indical Bioscience	Indispin Pathogen kit	1	10	10	100
Applied Biosystems	MagMAX CORE Nucleic acid purification kit	1	10	10	100
TOTAL		**5**	**50**	**50**	**100**

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

5.1.3 CONCLUSION

In 2023, four laboratories participated in proficiency test of Porcine Reproductive and Respiratory Syndrome 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 VIROLOGY (SERUM)

PT2023PRRSVIRPS4

Lab number	97505 (1)	97505 (2)	97508	97515	97532
Method (RT-qPCR protocol/kit)	M_1	M_1	M_1	M_2	M_3
Ct (REP1)	36.81	36.02	37.09	35.31	37.29
Ct (REP2)	36.74	35.08	36.51	35.53	34.91
Mean	36.78	35.55	36.80	35.42	36.10
SD	0.05	0.66	0.41	0.16	1.68
CV (%)	0.13	1.87	1.11	0.44	4.66

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Thermo Fisher - VTMAX PRRS EU & NA 2.0 Kit, M_2 = Applied Biosystems - VTMAX PRRS EU & NA 2.0 Kit , M_3 = Indical - Virotype PRRSV 2.0 RT-PCR Kit).

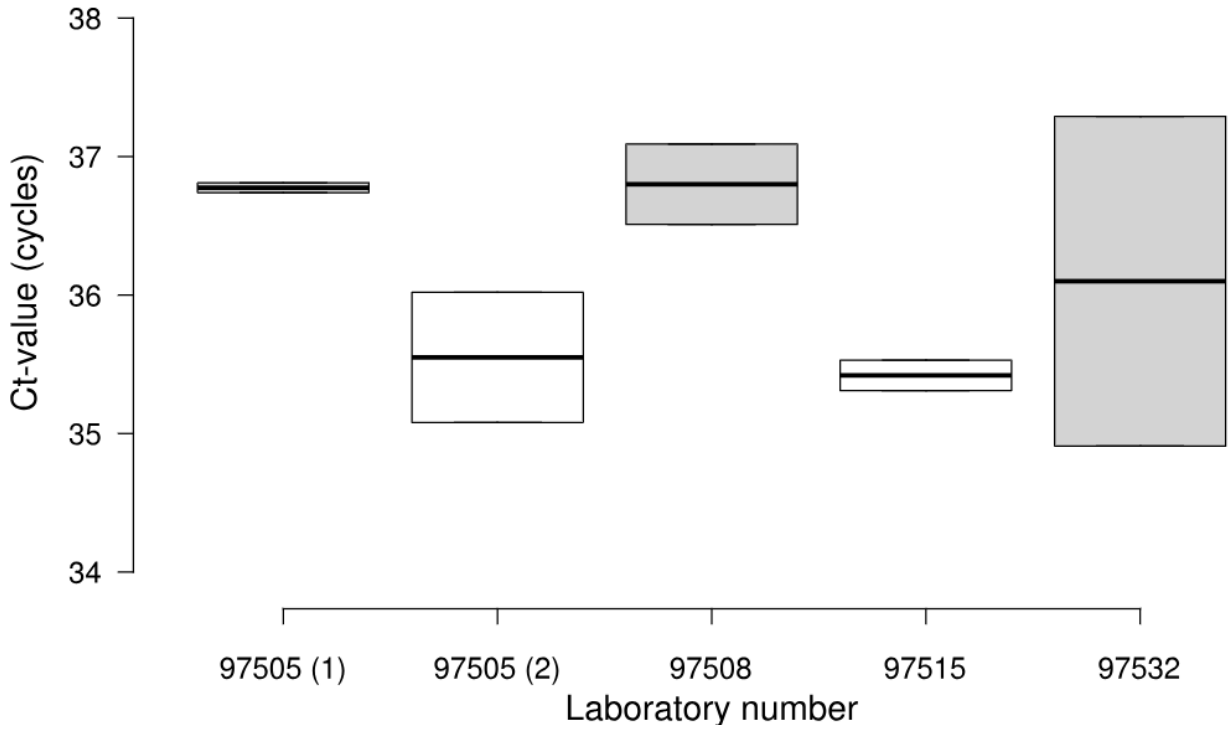


Figure 1. Distribution of the Ct-values (box-plots) per laboratory.

Lab number	97505 (1)	97505 (2)	97508	97515	97532
Method (RT-qPCR protocol/kit)	M_1	M_1	M_1	M_2	M_3
Ct (REP1)	34.16	31.67	33.78	32.3	32.55
Ct (REP2)	34.11	33.03	33.02	32.47	31.27
Mean	34.14	32.35	33.40	32.39	31.91
SD	0.04	0.96	0.54	0.12	0.91
CV (%)	0.10	2.97	1.61	0.37	2.84

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Thermo Fisher - VTMAX PRRS EU & NA 2.0 Kit, M_2 = Applied Biosystems - VTMAX PRRS EU & NA 2.0 Kit , M_3 = Indical - Virotype PRRSV 2.0 RT-PCR Kit).

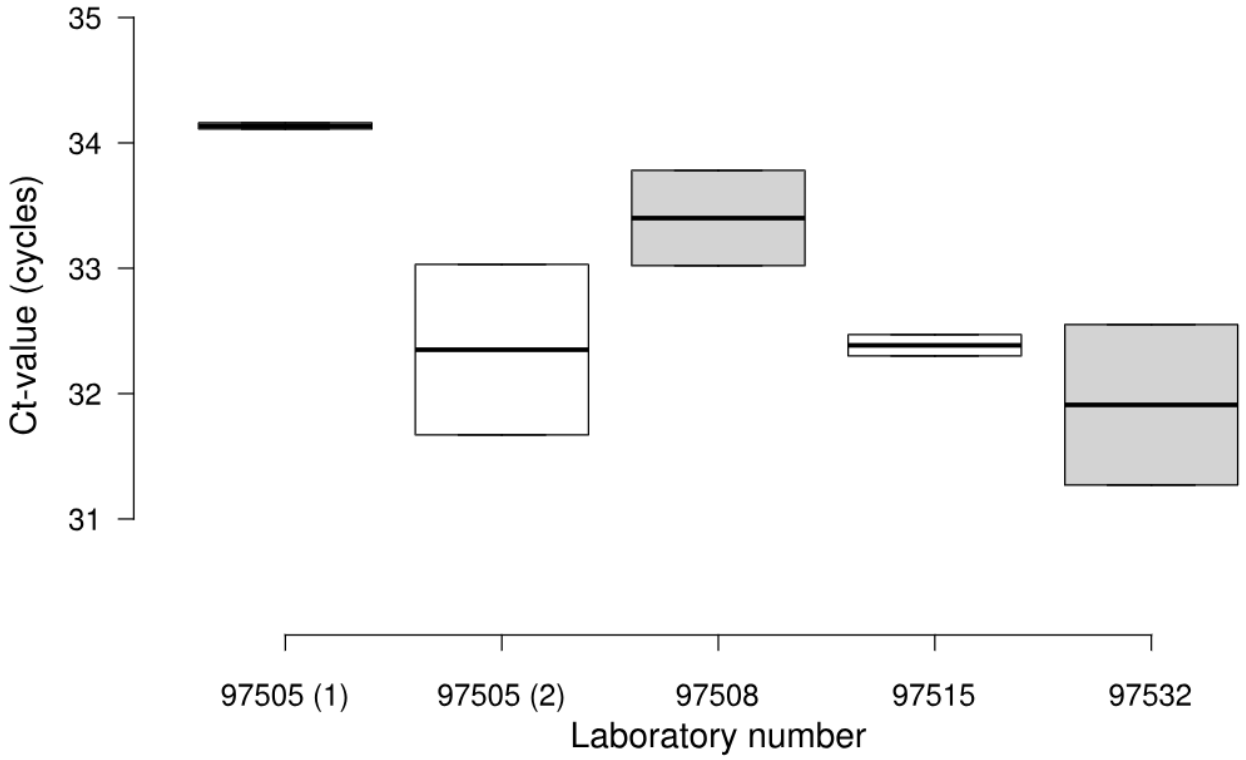


Figure 2. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

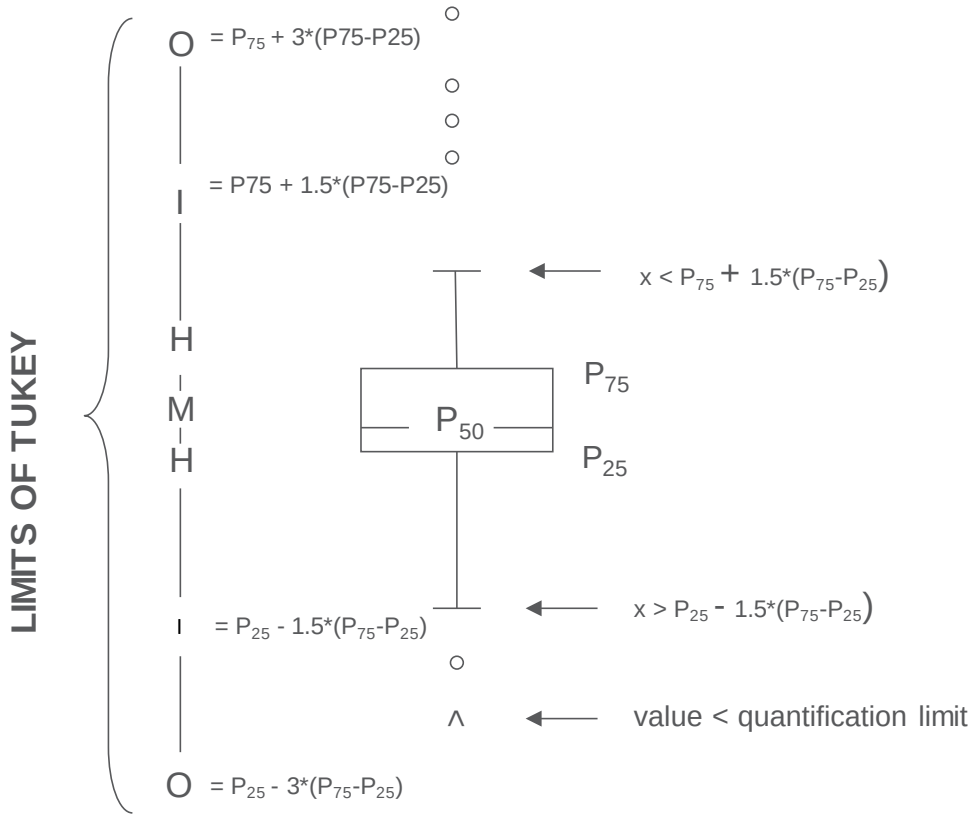
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

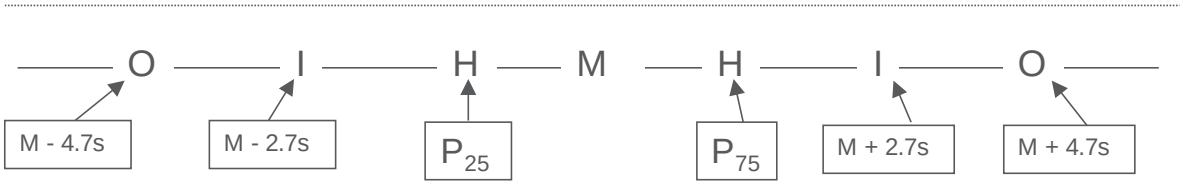
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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