BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

Q FEVER (QF)

PROFICIENCY TEST 2023-11

Sciensano/PT VET QF/2023-11/E
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1 INTRODUCTION

Details relevant to the proficiency test 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 proficiency test was dedicated to detect the agent of Q fever (*Coxiella burnetii*) by realtime qPCR in organ and milk samples.

3 MATERIALS AND METHODS

3.1 Bacteriology (organs)

3.1.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Q fever bacteriology on organ samples. The names of the participating laboratories are:

- Sciensano, service of Veterinary Bacteriology
- ARSIA
- Biosellal
- AZ Sint-Jan Campus Brugge
- Friedrich-Loeffler-Institut - Institut für bakterielle Infektionen und Zoonosen
- Kosovo Food And Veterinary Laboratory

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology Service, Infectious diseases in animals Directorate, Sciensano.

For each reference sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference samples was based on (i) the historical background of the animals/farm and (ii) the results obtained by analytical tests (pre-verification before sending). The pre-verification tests consisted of an *in house* method so as defined by SOP/PRE/03 (extraction protocol) and SOP/BAC/ANA15 (qPCR assay).

Organs were obtained by spiking inactivated animal material free of *C. burnetii*. Samples were spiked with serial dilutions (10^{-1}, 10^{-2}, 10^{-3}) of confluent inactivated axenic cultures of an ovine field-derived *C. burnetii* strain). The estimated concentrations of *C. burnetii* bacteria in the spiked samples were of 3.13E+07, 5.82E+06 and 8.51E+05 genomic equivalents (GE)/ml, respectively.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on ten aliquots (newly produced organ samples) of each sample using qPCR before and after the PT. The NRL obtained each time the same qualitative result. Therefore, 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 four different samples. However, positive sample OR3 was replicated two times. Therefore, the panel included five samples in total.

Sample content	Repetition	Expected result
PT2023QF_OR1	1	POS
PT2023QF_OR2	1	POS
PT2023QF_OR3	2	POS
PT2023QF_OR4	1	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The stability was determined by comparison of the pre-proficiency test results with the results obtained by the NRL during and after the proficiency test. 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 content: PT2023 QF_	97504	97507	97514	97545	97550	97630
OR1	QFORG23-1	QFORG23-1	QFORG23-5	QFORG23-4	QFORG23-1	QFORG23-4
OR2	QFORG23-2	QFORG23-5	QFORG23-3	QFORG23-5	QFORG23-4	QFORG23-1
OR3 (1)	QFORG23-4	QFORG23-2	QFORG23-2	QFORG23-1	QFORG23-3	QFORG23-3
OR3 (2)	QFORG23-5	QFORG23-3	QFORG23-4	QFORG23-2	QFORG23-5	QFORG23-5
OR4	QFORG23-3	QFORG23-4	QFORG23-1	QFORG23-3	QFORG23-2	QFORG23-2

3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the five reference samples is 100%.

3.2 Bacteriology (milk)

3.2.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of Q fever bacteriology on milk samples. The names of the participating laboratories are:

- Sciensano, service of Veterinary Bacteriology
- Biosellal
- Anses-SOPHIA ANTIPOLIS- LNR Q FEVER
- Friedrich-Loeffler-Institut - Institut für bakterielle Infektionen und Zoonosen
- Kosovo Food And Veterinary Laboratory

3.2.2 THE SAMPLES

The samples were prepared by the NRL, Veterinary Bacteriology Service, Infectious diseases in animals Directorate, Sciensano.

For each reference sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference samples was based on (i) the historical background of the animals/farm and (ii) the results obtained by analytical tests (pre-verification before sending). The pre-verification tests consisted of an in house method so as defined by SOP/PRE/03 (extraction protocol) and SOP/BAC/ANA15 (qPCR assay).

The reference milk samples were obtained from bulk tank milk of sheep/goat/cattle farms naturally infected or free of *C. burnetii*. All milks were inactivated (internal procedure) and lyophilized before sending.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on three aliquots (three reference milk samples) of each sample using qPCR before and after the PT. The NRL obtained each time the same qualitative result. Therefore, 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 three different samples. However, repetitions were present in each of the samples. Therefore, the panel included ten samples in total.

Sample content	Repetition	Expected result
PT2023QF_MI1	4	POS
PT2023QF_MI2	4	POS
PT2023QF_MI3	2	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

The stability was determined by comparison of the pre-proficiency test results with the results obtained by the NRL during and after the proficiency test. 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 content: PT2023QF_	97504	97514	97523	97550	97630
MI1 (1)	QFMILK23-2	QFMILK23-2	QFMILK23-1	QFMILK23-2	QFMILK23-2
MI1 (2)	QFMILK23-5	QFMILK23-4	QFMILK23-3	QFMILK23-6	QFMILK23-3
MI1 (3)	QFMILK23-8	QFMILK23-5	QFMILK23-8	QFMILK23-7	QFMILK23-7
MI1 (4)	QFMILK23-10	QFMILK23-10	QFMILK23-9	QFMILK23-10	QFMILK23-9
MI2 (1)	QFMILK23-3	QFMILK23-3	QFMILK23-4	QFMILK23-1	QFMILK23-4
MI2 (2)	QFMILK23-4	QFMILK23-6	QFMILK23-5	QFMILK23-4	QFMILK23-5
MI2 (3)	QFMILK23-7	QFMILK23-7	QFMILK23-6	QFMILK23-8	QFMILK23-6
MI2 (4)	QFMILK23-9	QFMILK23-8	QFMILK23-7	QFMILK23-9	QFMILK23-10
MI3 (1)	QFMILK23-1	QFMILK23-1	QFMILK23-2	QFMILK23-3	QFMILK23-1
MI3 (2)	QFMILK23-6	QFMILK23-9	QFMILK23-10	QFMILK23-5	QFMILK23-8

3.2.7 THRESHOLD FOR QUALIFICATION

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

4 TIMELINE

Transfer of the samples from NRL to QL: 16/10/2023
Randomization of the samples by QL: 26/10/2023
Sending samples to participants: 30/10/2023

- Storage of the organ samples: frozen (- 20°C)
- Storage of the milk samples: room temperature (20°C)

Deadline for submitting the results: 17/11/2023
Individual report to the participants: 07/12/2023

5 RESULTS

5.1 Bacteriology (organs)

5.1.1 RESULTS PER SAMPLE

The panel consisted of four different samples. However, positive sample OR3 was replicated two times. Therefore, the panel included five samples in total.

Sample content	Status	Number of repetitions (total results)	Observed result
OR1	POS	1 (6)	6 POS
OR2	POS	1 (6)	6 POS
OR3	POS	2 (12)	12 POS
OR4	NEG	1 (6)	6 NEG

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	%
ELITech ELITe InGenius™	ELITe InGenius® SP 200 (protocol)	1	5	5	100
Indical Bioscience	IndiMag Pathogen Kit	1	5	5	100
Biosellal	BioExtract® Column	1	5	5	100
QIAGEN	Qiagen DNAesay Blood and tissue	1	5	5	100
Roche	High Pure PCR template purification Kit	1	5	5	100
Indical Bioscience	IndiSpin Pathogen Kit (50 reactions) Cat. No.: SP54104	1	5	5	100
TOTAL		**6**	**30**	**30**	**100**

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

5.1.3 USED RT-QPCR PROTOCOL/KIT

In the table below, the RT-qPCR 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	%
Homemade	Internal SOP/BAC/ANA/15	1	5	5	100
Thermo Fisher	VetMAX Screening Pack Bovine Abortion (SARP)	1	5	5	100
Biosellal	Bio-T kit® Coxiella burnetii	1	5	5	100
Homemade	Homemade	1	5	5	100
Indical	Bactotype C. burnetii PCR Kit	1	5	5	100
Qiagen	*In house* primers adapted to QuantiTect Probe PCR Kit	1	5	5	100
TOTAL		**6**	**30**	**30**	**100**

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

5.1.4 CONCLUSION

In 2023, six laboratories participated in the proficiency test Q fever bacteriology (organs) organized by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if all the results (100%) provided by the laboratory are in agreement with the status of the reference samples assigned by the NRL of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

5.2 Bacteriology (milk)

5.2.1 RESULTS PER SAMPLE

The panel consisted of three different samples. However, repetitions were present in each of the samples. Therefore, the panel included ten samples in total.

Sample content	Status	Number of repetitions (total results)	Observed result
MI1	POS	4 (20)	20 POS
MI2	POS	4 (20)	20 POS
MI3	NEG	2 (10)	10 NEG

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	%
ELITech ELITe InGenius™	ELITe InGenius® SP 200	1	10	10	100
Biosellal	BioExtract® Column	1	10	10	100
QIAGEN	Qiagen DNAesay Blood and tissue	1	10	10	100
Roche	High Pure PCR template preparation Kit	1	10	10	100
Indical Bioscience	IndiSpin Pathogen Kit (50 reactions) Cat. No.: SP54104	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 USED RT-QPCR PROTOCOL/KIT

In the table below, the RT-qPCR 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	%
Homemade	Internal SOP/BAC/ANA/15	1	10	10	100
Biosellal	Bio-T kit® Coxiella burnetii	1	10	10	100
Homemade	Home Made	1	10	10	100
Indical	Bactotype C burnetii PCR Kit	1	10	10	100
Qiagen	*In house* primers adapted to QuantiTect Probe PCR Kit	1	10	10	100
TOTAL		**5**	**50**	**50**	**100**

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

5.1.4 CONCLUSION

In 2023, five laboratories participated in the proficiency test Q fever bacteriology (milk) 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 the laboratory are in agreement with the status of the reference samples assigned by the NRL 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)

This quantitative data is not under BELAC-accreditation and is solely for the information of the laboratories.

6.1 Annex 1: Quantitative results

Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel.

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

6.1.1 BACTERIOLOGY (ORGANS)

PT2023QF-OR3

Lab number	97504	97507	97514	97545	97550	97630
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_4	M_5	M_6
Ct (REP1)	32,87	32,14	30,35	31,67	34,00	36,81
Ct (REP2)	36,39	34,00	30,54	32,92	34,00	39,66
Mean	34,63	33,07	30,45	32,30	34,00	38,24
SD	2,49	1,32	0,13	0,88	0,00	2,02
CV (%)	7,19	3,98	0,44	2,74	0,00	5,27

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Homemade - Internal SOP/BAC/ANA/15; M_2 = Thermo Fisher - VetMAX Screening Pack Bovine Abortion (SARP); M_3 = Biosellal - Bio-T kit® Coxiella burnetii; M_4 = Homemade; M_5 = Indical - Bactotype C. burnetii PCR Kit; M_6 = Qiagen - QuantiTect Probe PCR Kit).

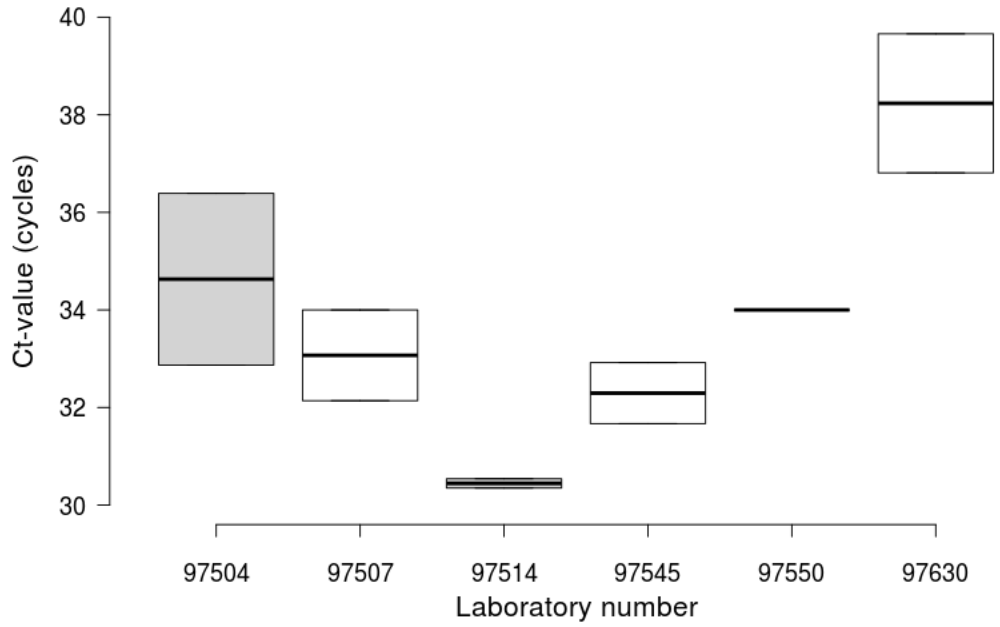


Figure 1. Distribution of the Ct-values (box-plots) per laboratory.

6.1.2 BACTERIOLOGY (MILK)

PT2023QF_MI1

Lab number	97504	97514	97523	97550	97630
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_4	M_5
Ct (REP1)	25,88	24,57	26,30	28,00	31,03
Ct (REP2)	25,96	23,71	26,10	28,00	30,76
Ct (REP3)	26,01	24,22	26,30	29,00	30,90
Ct (REP4)	25,81	24,75	26,40	28,00	31,22
Mean	25,92	24,31	26,28	28,250	30,978
SD	0,088	0,46	0,13	0,500	0,195
CV (%)	0,34	1,88	0,48	1,770	0,628

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade - Internal SOP/BAC/ANA/15; M_2 = Biosellal - Bio-T kit® Coxiella burnetii; M_3 = Homemade; M_4 = Indical - Bactotype C burnetii PCR Kit; M_5 = Qiagen - QuantiTect Probe PCR Kit).

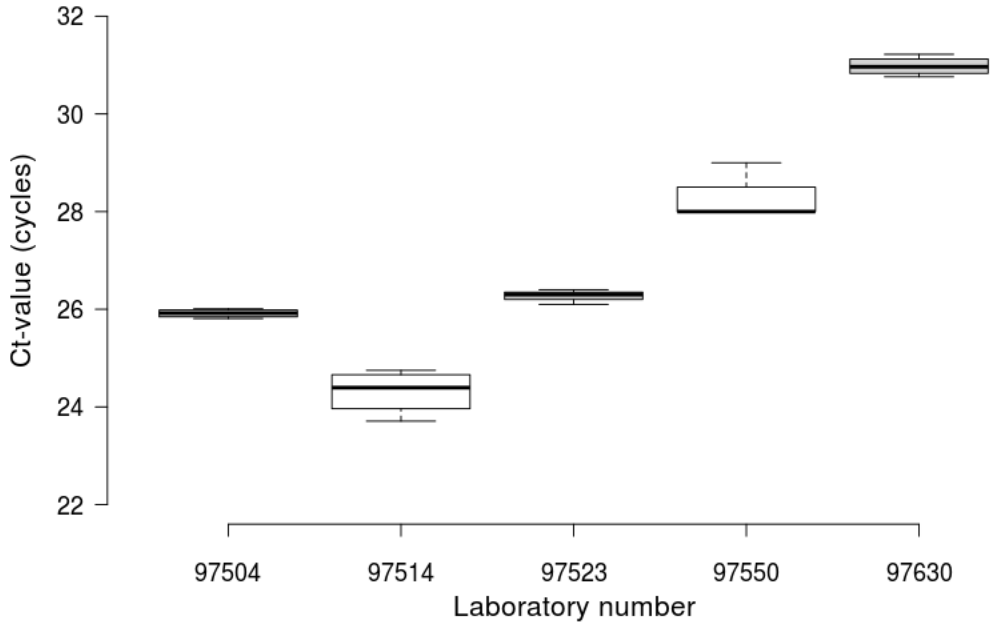


Figure 2. Distribution of the Ct-values (box-plots) per laboratory.

Lab number	97504	97514	97523	97550	97630
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_4	M_5
Ct (REP1)	26,18	26,27	27,80	29,00	31,44
Ct (REP2)	26,90	26,56	28,40	28,00	34,77
Ct (REP3)	27,03	26,64	28,60	28,00	32,43
Ct (REP4)	26,99	26,75	28,60	29,00	31,79
Mean	26,78	26,56	28,35	28,50	32,61
SD	0,40	0,21	0,38	0,58	1,50
CV (%)	1,50	0,773-	1,34	2,03	4,60

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade - Internal SOP/BAC/ANA/15; M_2 = Biosellal - Bio-T kit® Coxiella burnetii; M_3 = Homemade; M_4 = Indical - Bactotype C burnetii PCR Kit; M_5 = Qiagen - QuantiTect Probe PCR Kit).

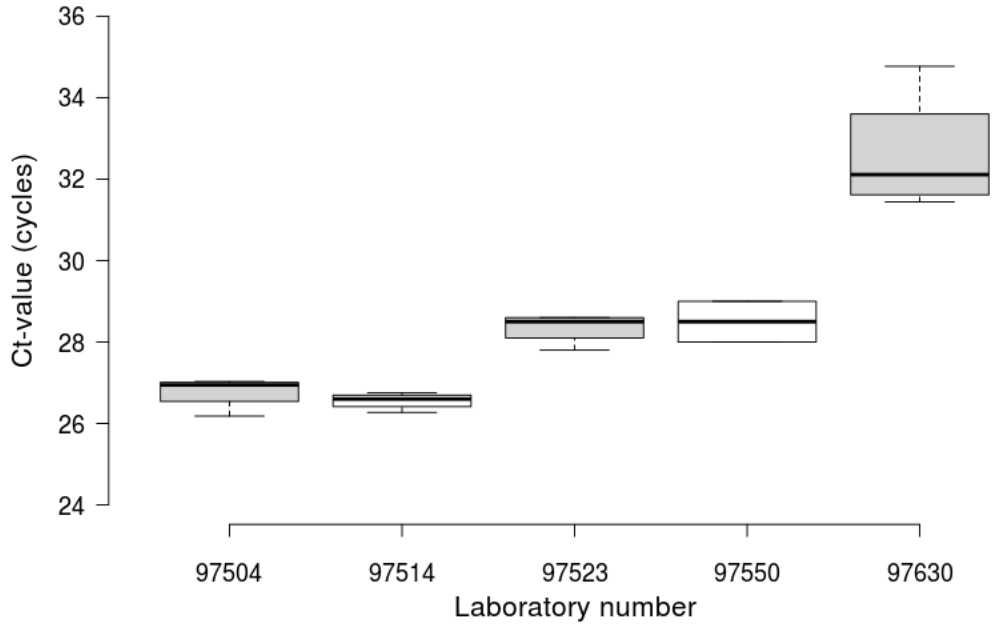


Figure 3. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

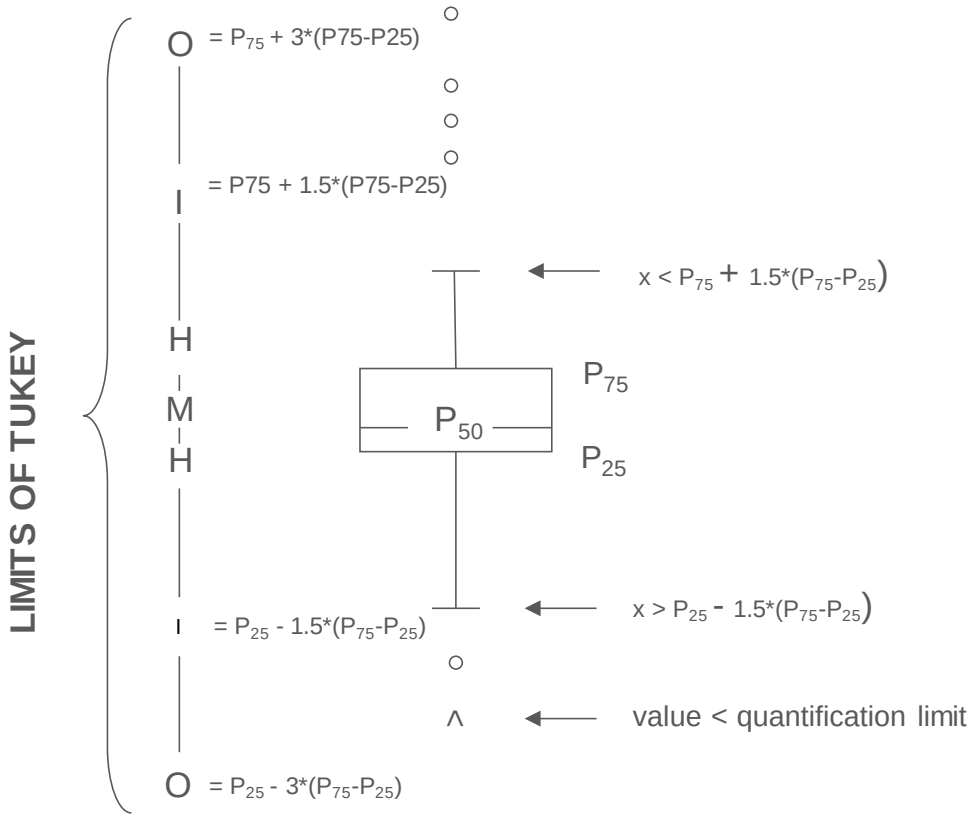
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

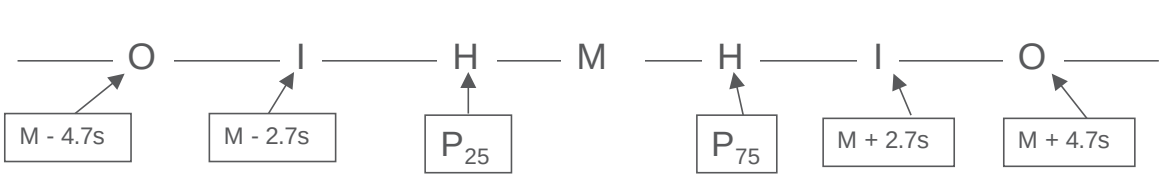
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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





Corresponding limits in case of normal distribution

END

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