BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

Q-FEVER (QF)

SURVEY 2022/2

Sciensano/PT VET QF/1-E

Biological health risks
Quality of laboratories
J. Wytsmanstreet, 14
1050 Brussels | Belgium

www.sciensano.be

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COMMITTEE OF EXPERTS

Sciensano					
Secretariat		PHONE:	02/642.55.22	FAX:	02/642.56.45
Ynse Van de Maele	Scheme coordinator	PHONE:	02/642 55 24		
		e-mail:	Ynse.vandemaele@sciensano.be		
Bernard China	Alternate coordinator	PHONE:	02/642 53 85		
		e-mail:	Bernard.china@sciensano.be		
Experts	**Institute**				
Marcella Mori	Sciensano				

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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 10:02:23 +02'00'

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TABLE OF CONTENTS

1 INTRODUCTION 4
2 AIM 4
3 MATERIALS AND METHODS 4
3.1 Serology on serum 4
3.1.1 The participants 4
3.1.2 The samples 4
3.1.3 Homogeneity 5
3.1.4 Target values 5
3.1.5 Stability 5
3.1.6 Randomisation and panel composition 6
3.2 Serology on milk 6
3.2.1 The participants 6
3.2.2 The samples 7
3.2.3 Homogeneity 7
3.2.4 Target values 7
3.2.5 Stability 7
3.2.6 Randomisation and panel composition 8
4 SURVEY TIMELINE 8
5 RESULTS 9
5.1 Serology on serum 9
5.1.1 Results per sample 9
5.1.2 Used method 9
5.1.3 Conclusion 9
5.2 Serology on milk 10
5.2.1 Results per sample 10
5.2.2 Used method 10
5.2.3 Conclusion 10
6 ANNEXES (NOT UNDER ACCREDITATION) 11
6.1 Annex 1: Quantitative results 11
6.1.1 Serology on serum 11
6.1.2 Serology on milk 16
6.2 Annex 2: Additional information 19

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 assess the ability of the participating laboratories to identify the absence or presence of Q-Fever (*Coxiella burnetii*)-specific antibodies by ELISA in serum and/or milk samples .

3 MATERIALS AND METHODS

3.1 Serology on serum

3.1.1 THE PARTICIPANTS

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

- Sciensano
- ARSIA
- DGZ
- Anses-SOPHIA ANTIPOLIS- LNR Q FEVER
- LSI-Thermofisher Scientific

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology, Scientific Directorate infectious diseases in animals, Sciensano. The sample set consisted of sera from cattle (n=4 field samples) and goat (n=2 experimental-challenge samples) origin. Samples were either free from detectable QFV-specific antibodies (n=1, coded 'PT2022QFSERSERUMNS1') or containing detectable QFV-specific antibodies (n=5, coded 'PT2022QFSERSERUMPS1', 'PT2022QFSERSERUMPS2', 'PT2022QFSERSERUMPS3', 'PT2022QFSERSERUMPS4', and 'PT2022QFSERSERUMPS5'). For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on the results obtained using the PrioCHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification.

Sample ID	Origine	Background
PT2022QFSERSERUMPS1	Bovine	Infected
PT2022QFSERSERUMPS2	Bovine	Infected
PT2022QFSERSERUMPS3	Bovine	Infected
PT2022QFSERSERUMPS4	Caprine	Vaccinated/challenged
PT2022QFSERSERUMPS5	Caprine	Vaccinated/challenged
PT2022QFSERSERUMNS1	Bovine	Uninfected/unvaccinated

3.1.3 HOMOGENEITY

A homogeneity check on the aliquoted reference serum samples had been performed as in the context of this PTs under the procedure SOP 2.5/01. Indeed, 10 aliquots of each reference serum sample were analysed using the PrioCHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample (3 aliquots if the samples was formerly characterized on 10 aliquots). Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of the participating laboratories to correctly identify the absence or presence of QFV-specific antibodies in serum.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 15 positive and 5 negative samples:

Sample ID	Status
PT2022QFSERSERUMPS1	POS
PT2022QFSERSERUMPS2	POS
PT2022QFSERSERUMPS3	POS
PT2022QFSERSERUMPS4	POS
PT2022QFSERSERUMPS5	POS
PT2022QFSERSERUMNS1	NEG

POS = positive; NEG = negative

3.1.5 STABILITY

The samples were controlled for stability by the National Reference Laboratory (NRL), Veterinary Bacteriology, Scientific Directorate infectious diseases in animals, Sciensano. All reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) using the PrioCHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific.

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: QFSER SERUM	97504	97507	97508	97523	97541
22-1	NS1	PS2	PS5	PS3	PS2
22-2	PS1	PS3	NS1	PS1	PS4
22-3	PS3	PS3	PS2	PS3	PS4
22-4	NS1	NS1	PS3	NS1	PS3
22-5	PS5	PS1	PS4	PS5	PS3
22-6	PS5	PS3	PS1	NS1	PS1
22-7	PS1	PS1	NS1	PS1	NS1
22-8	PS1	PS4	PS1	PS4	PS1
22-9	PS3	PS5	NS1	PS3	PS1
22-10	PS4	NS1	PS3	PS5	PS2
22-11	NS1	PS1	PS2	NS1	NS1
22-12	NS1	PS4	PS5	PS2	NS1
22-13	PS1	PS2	PS3	NS1	PS5
22-14	NS1	NS1	PS1	NS1	NS1
22-15	PS4	PS2	PS2	PS1	PS2
22-16	PS2	NS1	PS3	PS2	PS5
22-17	PS2	NS1	NS1	PS2	NS1
22-18	PS3	PS3	NS1	PS3	PS1
22-19	PS5	PS5	PS4	PS1	PS3
22-20	PS3	PS1	PS1	PS4	PS3

3.2 Serology on milk

3.2.1 THE PARTICIPANTS

Three laboratories participated in the proficiency test of Q-Fever serology on milk. The names of the participating laboratories are:

- Sciensano
- ARSIA
- LSI-Thermofisher Scientific

3.2.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology, Scientific Directorate infectious diseases in animals, Sciensano. The sample set consisted of milk from cattle (n= 4 field samples) and goat (n=1 field sample) origin. Samples were either free from detectable QFV-specific antibodies (n=1, coded 'PT2022QFSERMILKNM1') or containing detectable QFV-specific antibodies (n=4, coded 'PT2022QFSERMILKPS1', 'PT2022QFSERMILKPS2', 'PT2022QFSERMILKPS3', and 'PT2022QFSERMILKPS4'). For each reference milk sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference milk samples was based on the results obtained using the PrioCHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific. The status of the reference milk samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification.

Sample ID	Origine	Background
PT2022QFSERMILKPM1	Bovine	Infected
PT2022QFSERMILKPM2	Caprine	Infected
PT2022QFSERMILKPM3	Bovine	Infected
PT2022QFSERMILKPM4	Bovine	Infected
PT2022QFSERMILKNM1	Bovine	Uninfected/unvaccinated
PT2022QFSERMILKPM1	Bovine	Infected

3.2.3 HOMOGENEITY

A homogeneity check on the aliquoted reference milk samples had been performed as in the context of this PTs under the procedure SOP 2.5/01. Indeed, 10 aliquots of each reference milk sample were analysed using the PrioCHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific, hereby obtaining the same qualitative result for all 10 aliquots of the same reference milk sample (3 aliquots if the samples was formerly characterized on 10 aliquots). Consequently, all reference milk samples were considered as reliable samples in order to evaluate the ability of the participating laboratories to correctly identify the absence or presence of QFV-specific antibodies in milk.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 16 positive and 4 negative samples:

Sample ID	Status
PT2022QFSERMILKPM1	POS
PT2022QFSERMILKPM2	POS
PT2022QFSERMILKPM3	POS
PT2022QFSERMILKPM4	POS
PT2022QFSERMILKNM1	NEG

POS = positive; NEG = negative

3.2.5 STABILITY

The samples were controlled for stability by the National Reference Laboratory (NRL), Veterinary Bacteriology, Scientific Directorate infectious diseases in animals, Sciensano. All reference milk samples were tested once after the PT in order to confirm their stability and status (post-verification) using the Prio CHECK™ Ruminant Q Fever Ab Plate Kit from Thermo Fisher Scientific.

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: QFSERMILK	97504	97507	97541
22-1	NM1	PM4	PM2
22-2	PM2	NM1	PM4
22-3	PM3	PM1	PM1
22-4	PM2	PM3	NM1
22-5	PM4	PM2	PM3
22-6	NM1	PM3	PM4
22-7	PM2	PM1	NM1
22-8	PM4	PM1	PM1
22-9	PM3	NM1	NM1
22-10	PM1	PM4	PM2
22-11	PM2	PM4	NM1
22-12	PM4	PM4	PM2
22-13	PM3	PM3	PM1
22-14	PM4	PM2	PM1
22-15	PM1	PM2	PM3
22-16	PM1	NM1	PM4
22-17	NM1	NM1	PM2
22-18	PM1	PM3	PM3
22-19	NM1	PM1	PM4
22-20	PM3	PM2	PM3

4 SURVEY TIMELINE

Transfer of the samples from NRL to QL: 08/03/2022
Randomization of the samples by QL: 09/03/2022
Sending samples (cooled at 4 °C) to participants: 14/03/2022
Deadline for submitting the results: 08/04/2022
Preliminary report: 27/04/2022

5 RESULTS

5.1 Serology on serum

The panel consisted of 20 different samples, 15 positive and 5 negative samples.

5.1.1 RESULTS PER SAMPLE

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	4 (20)	20 POS
PS2	POS	3 (15)	15 POS
PS3	POS	4 (20)	20 POS
PS4	POS	2 (10)	10 POS
PS5	POS	2 (10)	10 POS
NS1	NEG	5 (25)	25 POS

(POS = positive; NEG = negative)

5.1.2 USED METHOD

Method	N	NR	NCR	% Agreement
Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit	4	80	80	100
ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES	1	20	20	100
TOTAL	**5**	**100**	**100**	**100**

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

5.1.3 CONCLUSION

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

5.2 Serology on milk

The panel consisted of 20 different samples, 16 positive and 4 negative samples.

5.2.1 RESULTS PER SAMPLE

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	4 (12)	12 POS
PS2	POS	4 (12)	12 POS
PS3	POS	4 (12)	12 POS
PS4	POS	4 (12)	12 POS
NS1	NEG	4 (12)	12 POS

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Method	N	NR	NCR	% Agreement
Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit	3	60	60	100
TOTAL	**3**	**60**	**60**	**100**

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

5.2.3 CONCLUSION

Only one method was used by the laboratories. This method achieved 100% correctness, which means that 60 correct results were submitted.

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

PT2022QFSERSERUMPS1

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	86.3	110.6	91.1	115.1	121.0
REP2	89.6	72.5	95.4	116.0	124.4
REP3	87.0	93.0	92.6	115.9	133.1
REP4	91.8	100.1	116.3	107.6	124.1
Mean	88.7	94.0	98.8	113.7	125.6
SD	2.5	16.1	11.8	4.1	5.2
CV (%)	2.8	17.1	11.9	3.6	4.1

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

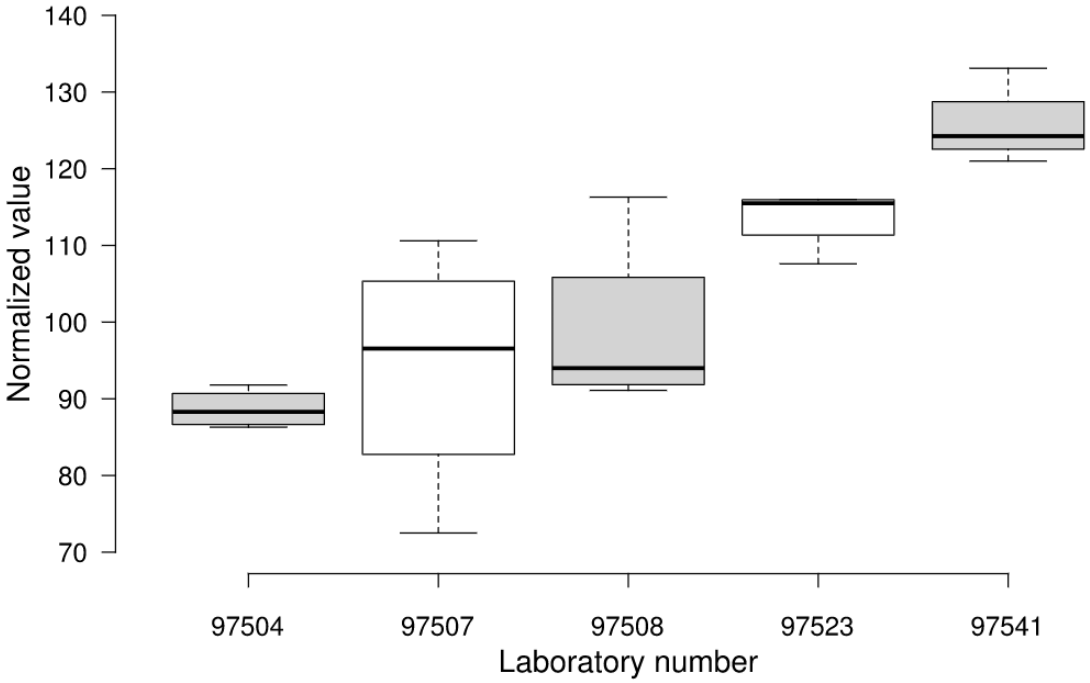


Figure 1. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERSERUMPS2

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	56.7	85.2	58.7	94.8	94.9
REP2	56.0	65.2	67.2	105.7	70.5
REP3	53.9	57.2	59.5	91.3	61.6
Mean	55.5	69.2	61.8	97.3	75.7
SD	1.5	14.4	4.7	7.5	17.3
CV (%)	2.7	20.8	7.6	7.7	22.8

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

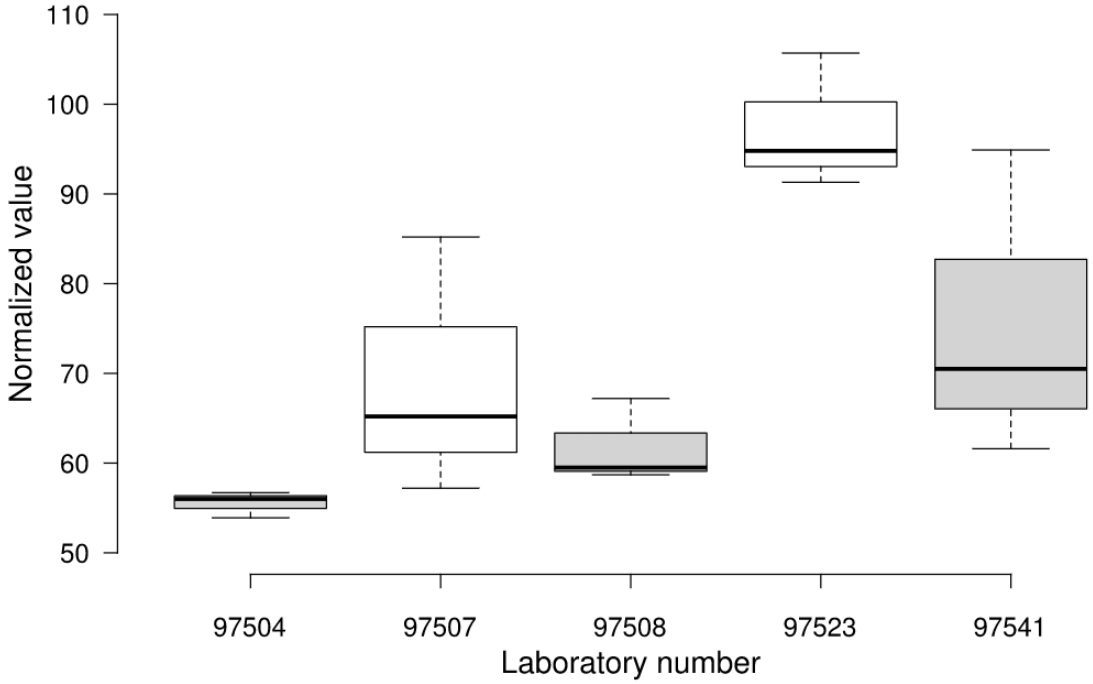


Figure 2. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERSERUMPS3

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	60.2	92.7	67.0	106.2	114.3
REP2	59.9	95.3	66.7	97.7	94.2
REP3	65.7	78.0	64.0	102.9	71.7
REP4	63.3	80.3	69.2	97.5	69.3
Mean	62.3	86.6	67.2	101.1	87.4
SD	2.7	8.7	2.4	4.2	21.2
CV (%)	4.4	10.0	3.6	4.2	24.2

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

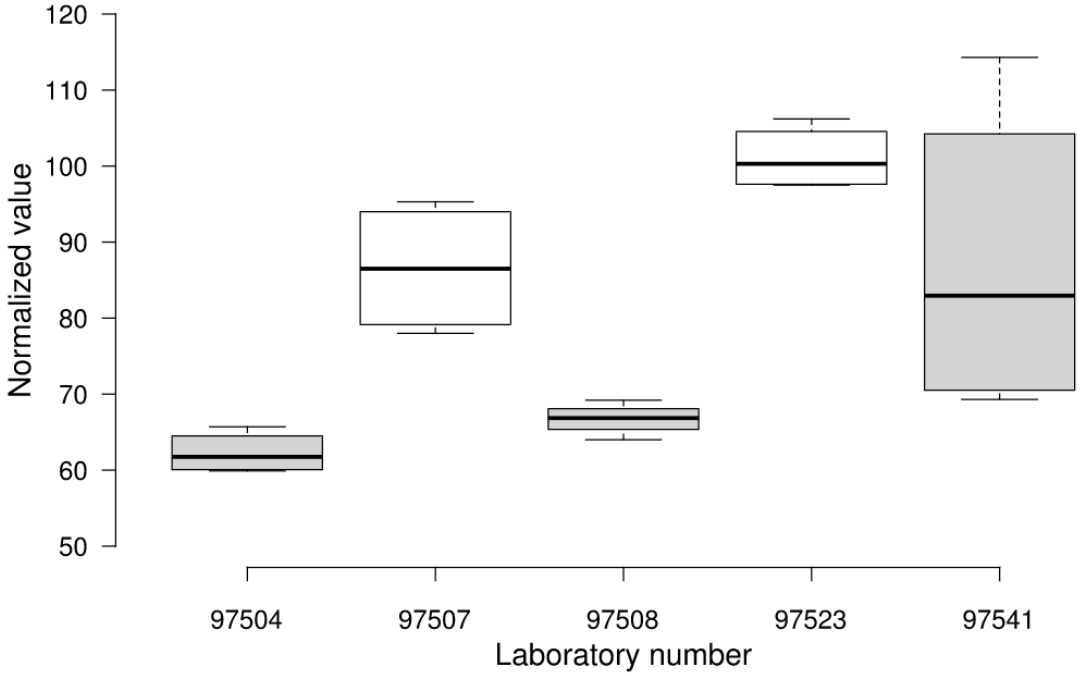


Figure 3. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERSERUMPS4

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	78.6	98.1	80.0	116.4	154.7
REP2	84.6	77.9	94.3	105.0	129.5
Mean	81.6	88.0	87.2	110.7	142.1
SD	4.3	8.7	10.1	8.0	17.9
CV (%)	5.2	10.0	11.6	7.3	12.6

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

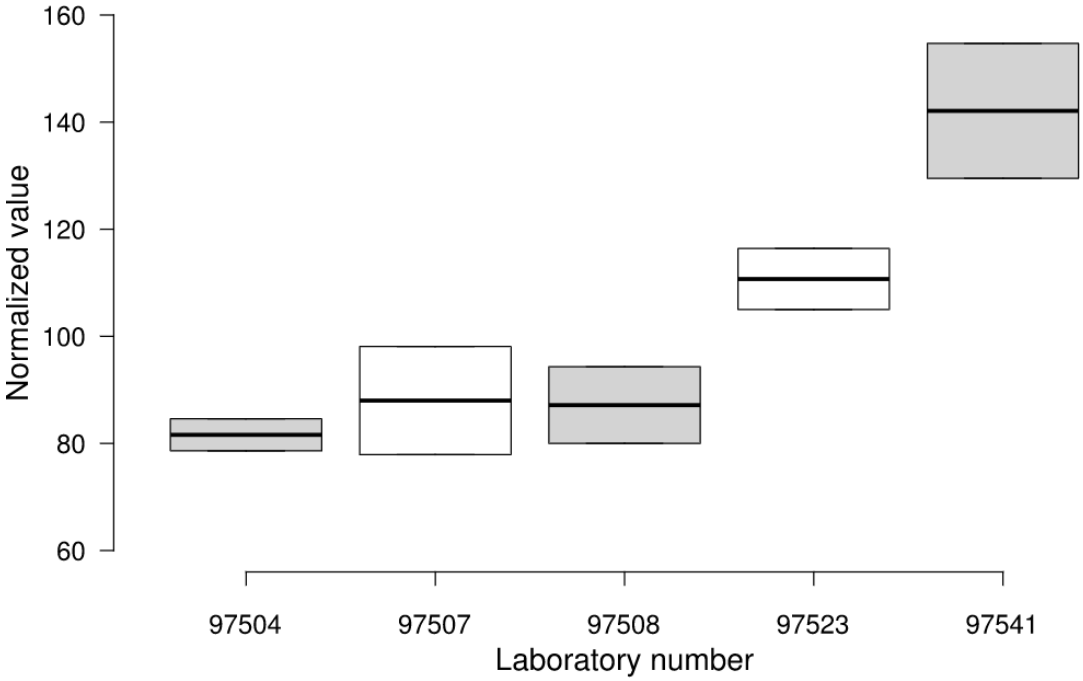


Figure 4. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERSERUMPS5

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	71.5	76.0	71.4	105.6	83.5
REP2	70.9	72.7	84.17	73.1	83.1
Mean	71.2	74.4	77.8	89.3	83.3
SD	0.5	2.3	9.0	23.0	0.2
CV (%)	0.7	3.1	11.6	25.7	0.3

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

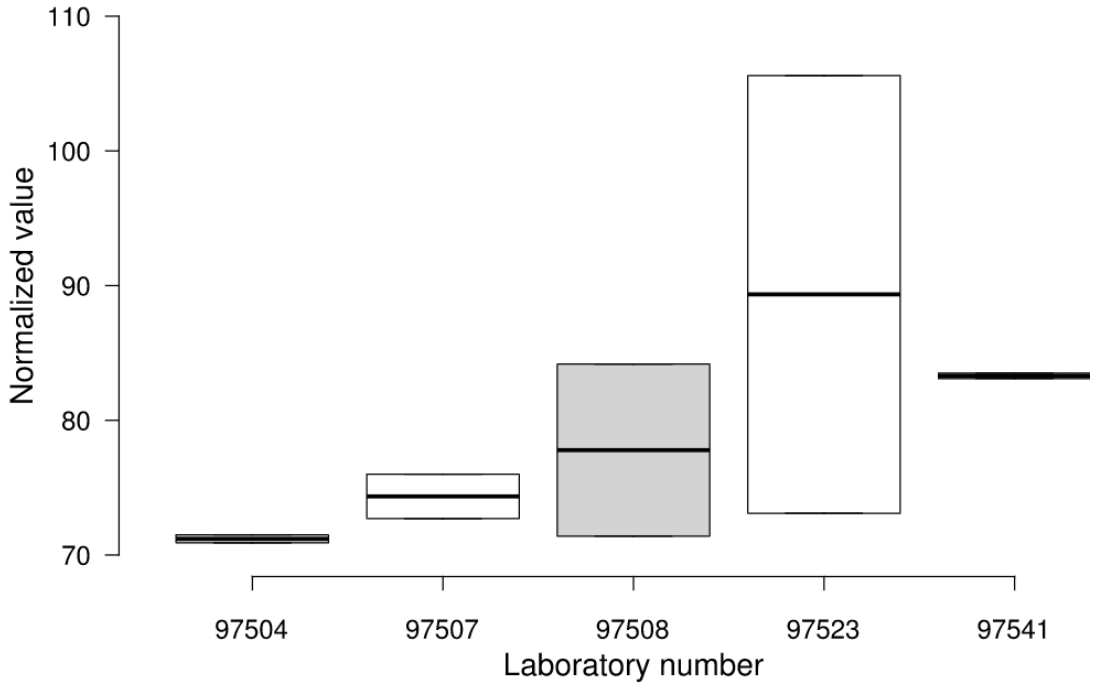


Figure 5. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERSERUMNS1

Lab number	97504	97507	97508	97523	97541
Method	M_1	M_1	M_1	M_2	M_1
REP1	-4.0	-6.0	-11.6	10.9	-8.2
REP2	-3.8	-5.6	-10.7	9.0	-7.7
REP3	-3.5	-6.1	-10.8	9.8	-8.41
REP4	-3.6	-6.7	-10.8	9.8	-8.0
REP5	-3.4	-5.6	-10.6	9.6	-8.8
Mean	-3.6	-6.0	-10.9	9.8	-8.2
SD	0.3	0.5	0.4	0.7	0.4
CV (%)	-7.2	-7.8	-3.6	6.9	-4.9

(REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit ; M_2 = ID.VET - ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES)

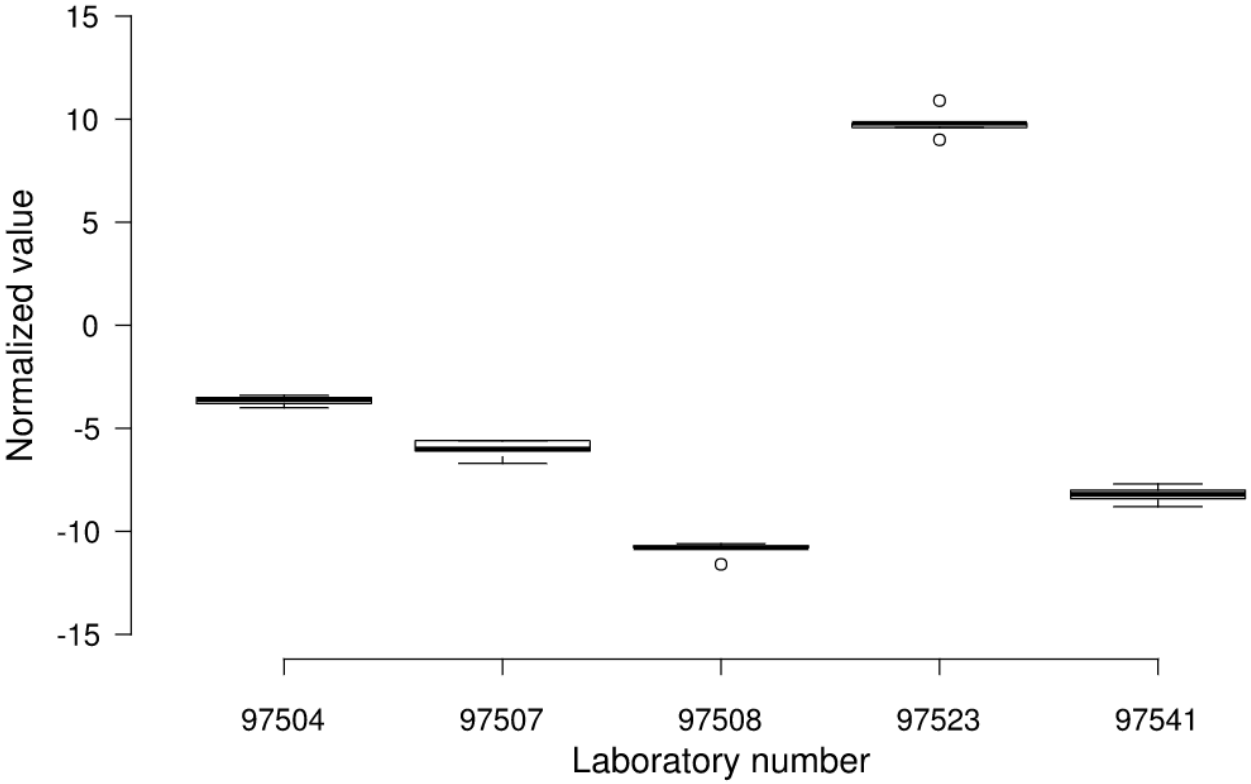


Figure 6. Distribution of the normalized values (box-plots) per laboratory.

6.1.2 SEROLOGY ON MILK

PT2022QFSERMILKPM1

Lab number	97504	97507	97541
Method	Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit		
REP1	170.5	146.9	152.6
REP2	166.4	138.2	153.3
REP3	165.9	140.4	158.1
REP4	169.0	142.2	161.2
Mean	167.9	141.9	156.3
SD	2.2	3.7	4.1
CV (%)	1.3	2.6	2.6

(REP = repetition; SD = standard deviation; CV = coefficient of variation)

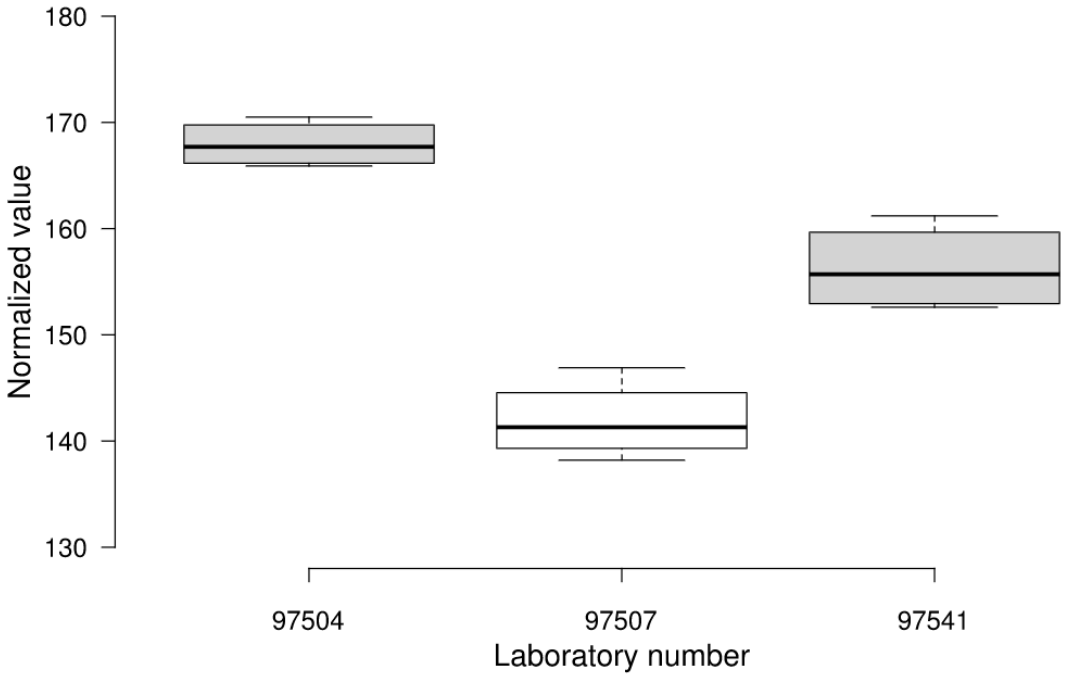


Figure 7. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERMILKPM2

Lab number	97504	97507	97541
Method	Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit		
REP1	200.3	121.5	207.7
REP2	193.6	104.8	181.5
REP3	188.7	132.0	188.8
REP4	205.7	74.3	185.9
Mean	197.1	108.1	191.0
SD	7.5	25.2	11.5
CV (%)	3.8	23.3	6.0

(REP = repetition; SD = standard deviation; CV = coefficient of variation)

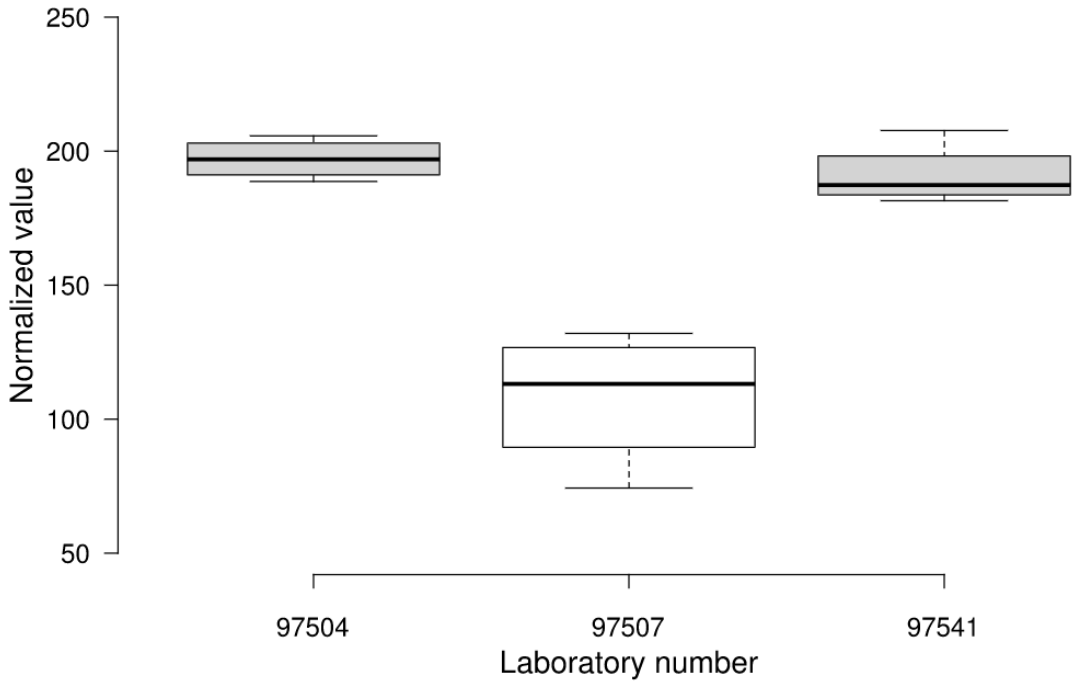


Figure 8. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERMILKPM3

Lab number	97504	97507	97541
Method	Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit		
REP1	154.8	144.3	154.2
REP2	159.4	140.9	145.1
REP3	165.4	139.0	142.3
REP4	154.0	137.1	144.6
Mean	158.4	140.3	146.5
SD	5.2	3.1	5.2
CV (%)	3.3	2.2	3.6

(REP = repetition; SD = standard deviation; CV = coefficient of variation)

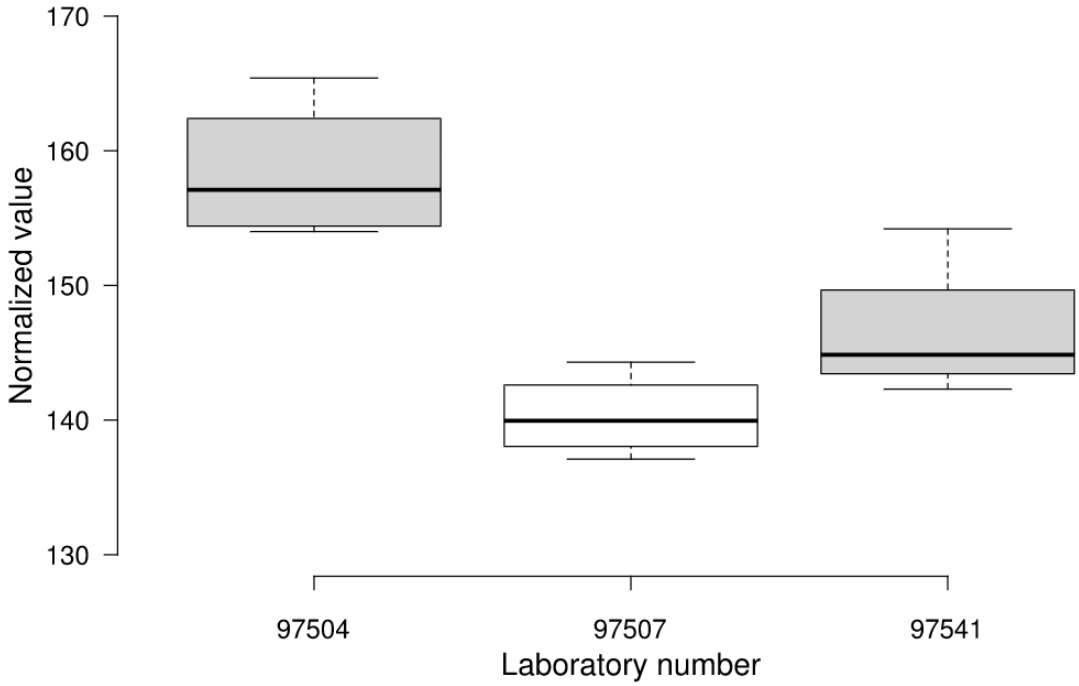


Figure 9. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERMILKPM4

Lab number	97504	97507	97541
Method	Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit		
REP1	119.3	102.1	120.6
REP2	120.1	100.1	125.9
REP3	125.7	108.6	119.0
REP4	128.0	107.0	115.6
Mean	123.3	104.5	120.3
SD	4.3	4.0	4.3
CV (%)	3.4	3.8	3.5

(REP = repetition; SD = standard deviation; CV = coefficient of variation)

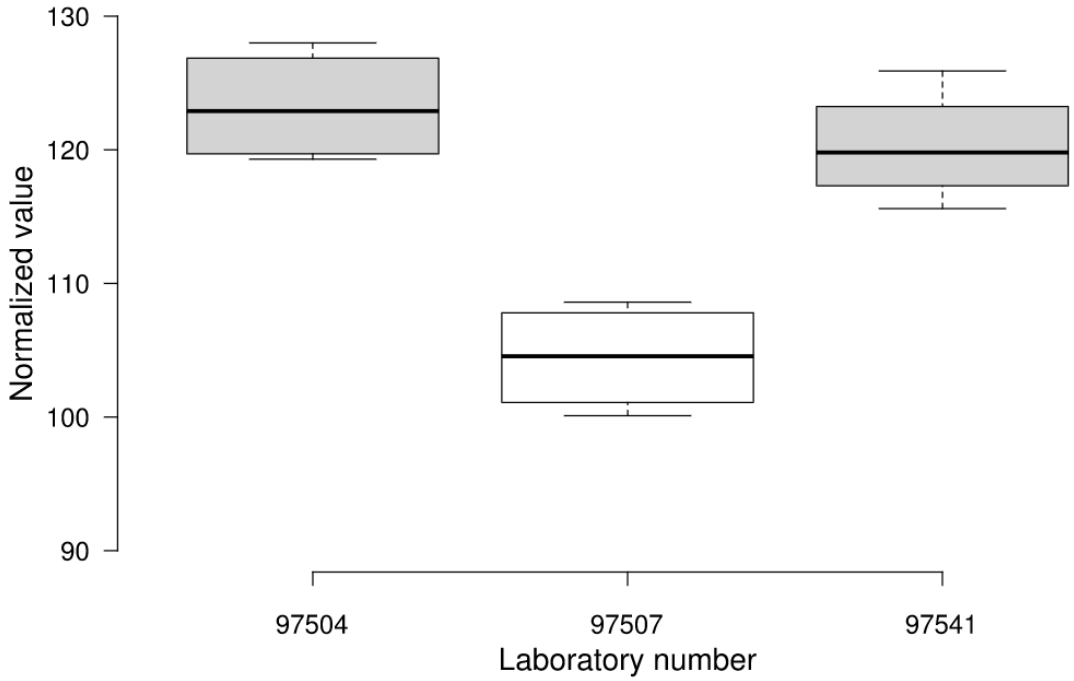


Figure 10. Distribution of the normalized values (box-plots) per laboratory.

PT2022QFSERMILKNM1

Lab number	97504	97507	97541
Method	Thermofisher - PrioCheck Ruminant Q Fever Ab Plate Kit		
REP1	-2.8	-9.2	-1.9
REP2	-2.7	-9.5	-2.2
REP3	-2.6	-9.8	-2.1
REP4	-2.7	-8.5	-2.0
Mean	-2.7	-9.2	-2.0
SD	0.1	0.5	0.1
CV (%)	-2.7	-5.8	-6.8

(REP = repetition; SD = standard deviation; CV = coefficient of variation)

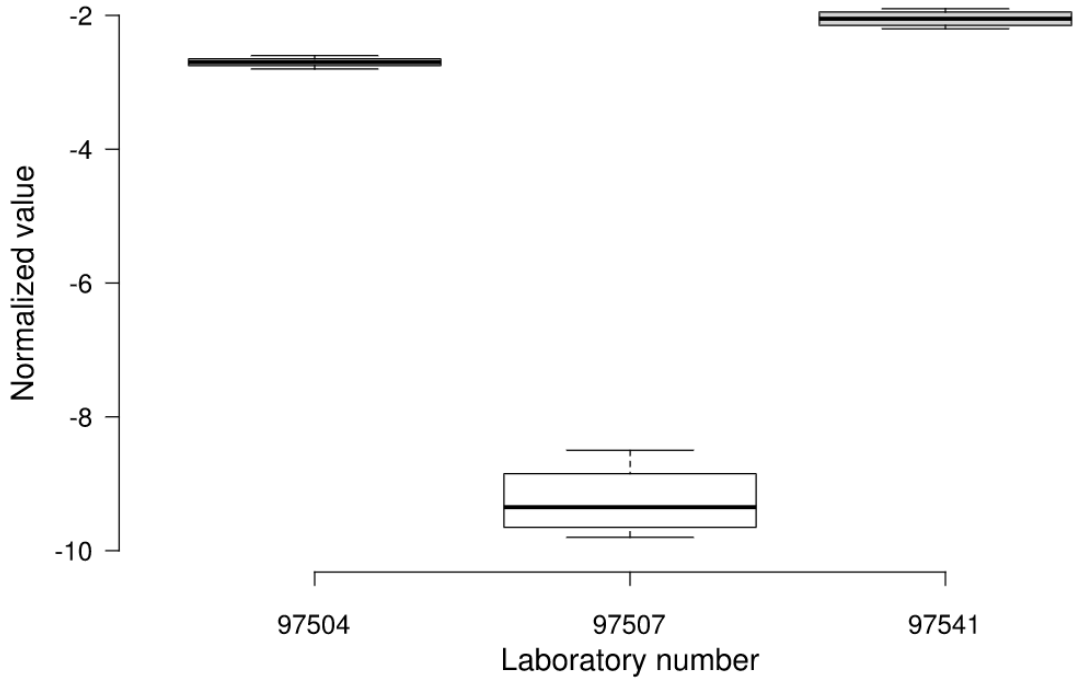


Figure 11. Distribution of the normalized values (box-plots) per laboratory.

6.2 Annex 2: Additional information

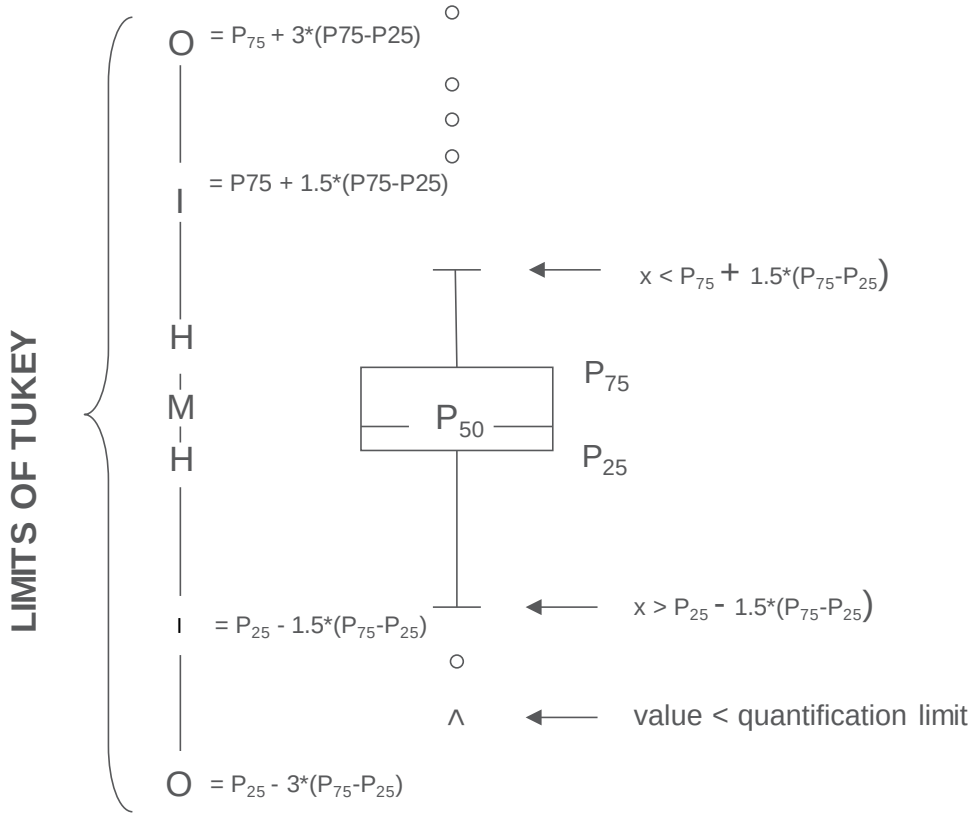
The preliminary report of this survey is available on our website via the following link:
https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

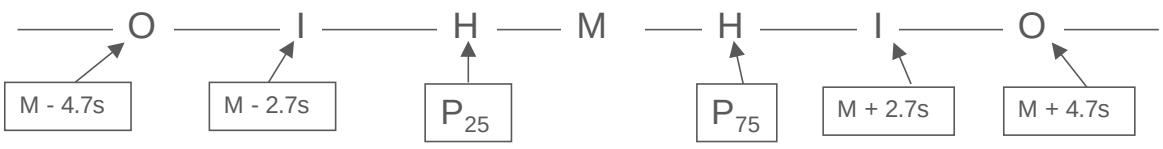
The calendar for Proficiency Testing in Veterinary diagnosis is available on our website:
https://www.wiv-isp.be/QML/activities/external_quality/calendar/kalender.htm

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

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