BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

BLUE TONGUE (BTV)

PROFICIENCY TEST 2023-13

Sciensano/PT VET BLUE T/2023-13/E
Biological health risks
Quality of laboratories
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- EN: https://www.sciensano.be/en/quality-laboratories

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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 divided into two different parts: serology and virology:

- The aim of the **serology part** was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies specific to Blue Tongue Virus in serum using ELISA.
- The aim of the **virology part** was to evaluate the ability of the participating laboratories to identify Blue Tongue Virus in blood using Real-time Polymerase Chain Reaction (RT-PCR).

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of Blue Tongue - serology on serum samples. The names of the participating laboratories are:

- Sciensano, service of Exotic and vector-borne diseases
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LNCR / ACSEDIATE
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory, Exotic and vector-borne diseases, Infectious diseases in animals Directorate, Sciensano.

Six reference serum samples of bovidae origin, either free from detectable BTV-specific antibodies (n=2; coded PT2023BLTSER_NS1 and PT2023BLTSER_NS2) or containing detectable BTV-specific antibodies (n=4; coded PT2023BLTSER_PS1, PT2023BLTSER_PS2, PT2023BLTSER_PS3 and PT2023BLTSER_PS4), were used.

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 (i) the historical background of the animals and (ii) the results obtained during pre-verification, hereby using the ID Screen® Bluetongue Competition kit from ID.VET.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on ten aliquots (ten serum samples, each 500 µL) of each sample using ELISA before and 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.

Sample content	Pathogen	Background	Origin	Expected result
PT2023BLTSER_PS1	BTV	vaccinated	Ovine	POS
PT2023BLTSER_PS2	BTV	vaccinated	Ovine	POS
PT2023BLTSER_PS3	BTV	vaccinated	Bovine	POS
PT2023BLTSER_PS4	BTV	vaccinated	Bovine	POS
PT2023BLTSER_NS1	BTV	uninfected/unvaccinated	Bovine	NEG
PT2023BLTSER_NS2	BTV	uninfected/unvaccinated	Ovine	NEG

(POS = positive; NEG = negative; BTV = Blue Tongue Virus)

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 BLTSER_	97506	97507	97508	97510	97516
PS1 (1)	BTSER23-5	BTSER23-4	BTSER23-2	BTSER23-7	BTSER23-7
PS1 (2)	BTSER23-9	BTSER23-10	BTSER23-6	BTSER23-10	BTSER23-9
PS2	BTSER23-3	BTSER23-3	BTSER23-8	BTSER23-6	BTSER23-2
PS3 (1)	BTSER23-1	BTSER23-5	BTSER23-1	BTSER23-1	BTSER23-5
PS3 (2)	BTSER23-2	BTSER23-6	BTSER23-9	BTSER23-5	BTSER23-6
PS4 (1)	BTSER23-6	BTSER23-2	BTSER23-7	BTSER23-3	BTSER23-4
PS4 (2)	BTSER23-10	BTSER23-8	BTSER23-10	BTSER23-9	BTSER23-10
NS1	BTSER23-4	BTSER23-7	BTSER23-5	BTSER23-4	BTSER23-3
NS2 (1)	BTSER23-7	BTSER23-1	BTSER23-3	BTSER23-2	BTSER23-1
NS2 (2)	BTSER23-8	BTSER23-9	BTSER23-4	BTSER23-8	BTSER23-8

3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 reference samples is at least 90%.

3.2 Virology (blood)

3.2.1 THE PARTICIPANTS

Seven laboratories participated in the proficiency test of Blue Tongue - virology on EDTA blood samples. The names of the participating laboratories are:

- Sciensano, service of Exotic and vector-borne diseases
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- LNCR / ACSEDIATE
- Biosellal
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)

3.2.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory, Exotic and vector-borne diseases, Infectious diseases in animals Directorate, Sciensano.

Eight reference blood samples of bovidae origin, either free from detectable Blue Tongue Virus (n=2; coded PT2023BLTVIR_NB1 and PT2023BLTVIR_NB2) or containing detectable Blue Tongue Virus (n=6; coded PT2023BLTVIR_PB1, PT2023BLTVIR_PB2, PT2023BLTVIR_PB3, PT2023BLTVIR_PB4, PT2023BLTVIR_PB5 and PT2023BLTVIR_PB6), were used. In addition, reference blood sample PT2023BLTVIR_NB2 was spiked with inactivated EHDV.

For each reference blood sample, a certificate containing the status of the sample (= ‘golden standard’) was made. The status of the reference blood samples was based on (i) the background of the animals and (ii) the results obtained during pre-verification, hereby using 4 different in-house developed BTV RT-qPCR assays: one detecting all BTV serotypes, one detecting only BTV-1, one detecting only BTV-4 and one detecting only BTV-8. Reference blood samples were also tested with an in-house developed EHDV RT-qPCR assay.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on ten aliquots (ten blood samples, each 1 mL) of each sample using RT-PCR before 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.

Sample content	Strain	Background	Origin	Expected result
PT2023BLTVIR_PB1	BTV-1	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_PB2	BTV-1	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_PB3	BTV-4	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_PB4	BTV-4	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_PB5	BTV-8	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_PB6	BTV-8	Uninfected/blood spiked	Bovine	POS
PT2023BLTVIR_NB1	None	Uninfected	Bovine	NEG
PT2023BLTVIR_NB2	EHDV-8	Uninfected/blood spiked	Bovine	NEG

(POS = positive; NEG = negative; BTV = Blue Tongue Virus; EHDV = Epizootic Haemorrhagic Disease Virus)

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: PT2023 BLTVIR_	97506	97507	97508	97509	97510	97514	97516
PB1	BTVIR23-9	BTVIR23-4	BTVIR23-3	BTVIR23-10	BTVIR23-3	BTVIR23-10	BTVIR23-7
PB2 (1)	BTVIR23-4	BTVIR23-6	BTVIR23-7	BTVIR23-1	BTVIR23-1	BTVIR23-1	BTVIR23-5
PB2 (2)	BTVIR23-10	BTVIR23-10	BTVIR23-10	BTVIR23-5	BTVIR23-5	BTVIR23-3	BTVIR23-10
PB3	BTVIR23-7	BTVIR23-7	BTVIR23-1	BTVIR23-8	BTVIR23-7	BTVIR23-7	BTVIR23-1
PB4	BTVIR23-3	BTVIR23-1	BTVIR23-4	BTVIR23-6	BTVIR23-10	BTVIR23-9	BTVIR23-9
PB5	BTVIR23-1	BTVIR23-5	BTVIR23-9	BTVIR23-2	BTVIR23-2	BTVIR23-5	BTVIR23-4
PB6 (1)	BTVIR23-2	BTVIR23-3	BTVIR23-5	BTVIR23-7	BTVIR23-6	BTVIR23-6	BTVIR23-2
PB6 (2)	BTVIR23-8	BTVIR23-9	BTVIR23-6	BTVIR23-9	BTVIR23-8	BTVIR23-8	BTVIR23-3
NB1	BTVIR23-5	BTVIR23-2	BTVIR23-8	BTVIR23-4	BTVIR23-9	BTVIR23-4	BTVIR23-8
NB2	BTVIR23-6	BTVIR23-8	BTVIR23-2	BTVIR23-3	BTVIR23-4	BTVIR23-2	BTVIR23-6

3.2.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 reference samples is at least 90%.

4 TIMELINE

Transfer of the samples from NRL to QL: 13/11/2023
Randomisation of the samples by QL: 16/11/2023
Sending samples to participants: 20/11/2023

- Storage of the samples : frozen (- 20 °C)

Deadline for submitting the results: 08/12/2023
Individual report to the participants: 14/12/2023

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, positive samples PS1, PS3 and PS4 were replicated two times. Additionally, negative sample NS2 was repeated two times. Therefore, the panel included ten samples in total.

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 content	Status	Number of repetitions (total results)	Observed result
PS1	POS	2 (12)	12 POS
PS2	POS	1 (6)	6 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

5.1.2 USED ELISA PROTOCOLS/KITS

In the table below, the ELISA protocols/kits used are listed along with the number of datasets and achieved score.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Competition	ID.VET	ID SCREEN® Blue Tongue Competition	**5**	**100**	**100**	**100**
ELISA Competition	IDEXX	VP7 Antibody Test Kit	**1**	**10**	**10**	**100**
TOTAL			**6**	**60**	**60**	**100**

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

5.1.3 CONCLUSION

In 2023, five laboratories participated in the proficiency test Blue Tongue 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 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 Virology (blood)

5.1.1 RESULTS PER SAMPLE

The panel consisted of eight different samples. However, positive samples PB2 and PB6 were replicated two times. Therefore, the panel included ten samples in total.

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 content	Status	Number of repetitions (total results)	Observed result
PB1	POS	1 (8)	8 POS
PB2	POS	2 (16)	16 POS
PB3	POS	1 (8)	7 POS 1 NEG
PB4	POS	1 (8)	8 POS
PB5	POS	1 (8)	8 POS
PB6	POS	2 (16)	16 POS
NB1	NEG	1 (8)	8 NEG
NB2	NEG	1 (8)	7 NEG 1 POS

5.1.2 USED EXTRACTION PROTOCOLS/KITS

In the table below, the extraction protocols/kits used are listed along with the number of datasets and achieved score.

Manufacturer extraction protocol / kit	Name extraction protocol / kit	N	NR	NCR	%
Machery nagel	Nucleospin RNA virus	1	10	10	100
Indical Bioscience	IndiMag Pathogen Kit	3	30	28	93
ThermoFisher Scientific	MagMAX Core Nucleic Acid Purification Kit	1	10	10	100
Biosellal	BioExtract® Column	1	10	10	100
IDVET	ID Gene Mag Universal Extraction kit	2	20	20	100
TOTAL		**8**	**80**	**78**	**98**

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

5.1.3 USED RT-PCR PROTOCLS/KITS

In the table below, the RT-PCR protocols/kits used are listed along with the number of datasets and achieved score.

Manufacturer RT-qPCR protocol / kit	Name RT-qPCR protocol / kit	N	NR	NCR	%
Homemade	Homemade	1	10	10	100
ADIAGENE	ADIAVET BTV real time	5	50	48	96
Thermofisher	Vetmax Bluetongue Virus NS3 - all genotype	1	10	10	100
Biosellal	Bio-T kit® BTV - all genotypes	1	10	10	100
TOTAL		**8**	**80**	**78**	**98**

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

5.1.4 CONCLUSION

In 2023, seven laboratories participated in the proficiency test Blue Tongue 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 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. Six laboratories succeeded in achieving the maximum score (100%) for this test. However , for one laboratory was observed that two samples were not in agreement with the status of the reference samples (80% of correct results). While the Ct-values were accurately determined, there was an inadvertent misassignment of interpretation by the laboratory. Consequently, this discrepancy is probably attributed to an encoding error. Based on the Ct-values for sample NB2, no cross-reaction with EHDV was detected. To conclude, an overall score of 98% was achieved.

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 SEROLOGY (SERUM)

PT2023BLTVIR-PS1

Lab number	97506	97507	97508 (1)	97508 (2)	97510	97516
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_1	M_1
S/P % (REP1)	9,00	10,53	8,49	30,43	6,39	8,80
S/P % (REP2)	9,00	11,50	9,65	30,86	6,10	9,10
Mean	9,00	11,02	9,07	30,64	6,25	8,95
SD	0,00	0,69	0,82	0,30	0,21	0,21
CV (%)	0,00	6,22	9,00	0,98	3,29	2,37

Numbers were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = ID.VET - ID SCREEN® BLUETONGUE COMPETITION, M_2 = IDEXX - VP7 Antibody Test Kit).

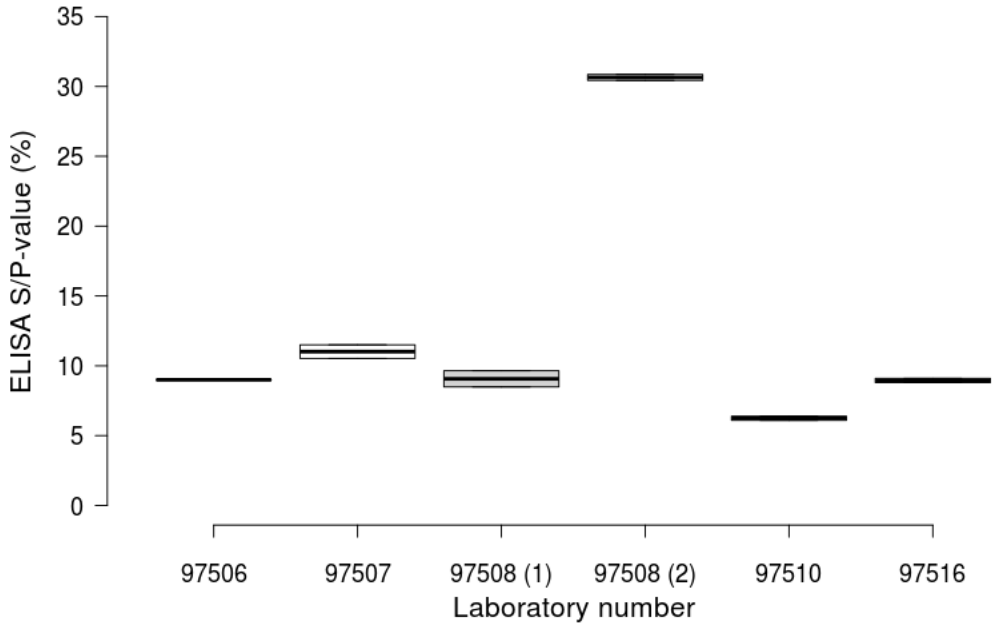


Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97506	97507	97508 (1)	97508 (2)	97510	97516
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_1	M_1
S/P % (REP1)	5,00	6,58	4,89	15,45	4,29	5,90
S/P % (REP2)	5,00	6,65	5,03	14,39	5,30	6,20
Mean	5,00	6,62	4,96	14,92	4,79	6,05
SD	0,00	0,05	0,10	0,75	0,72	0,21
CV (%)	0,00	0,75	1,94	5,03	14,99	3,51

Numbers were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = ID.VET - ID SCREEN® BLUETONGUE COMPETITION; M_2 = IDEXX - VP7 Antibody Test Kit).

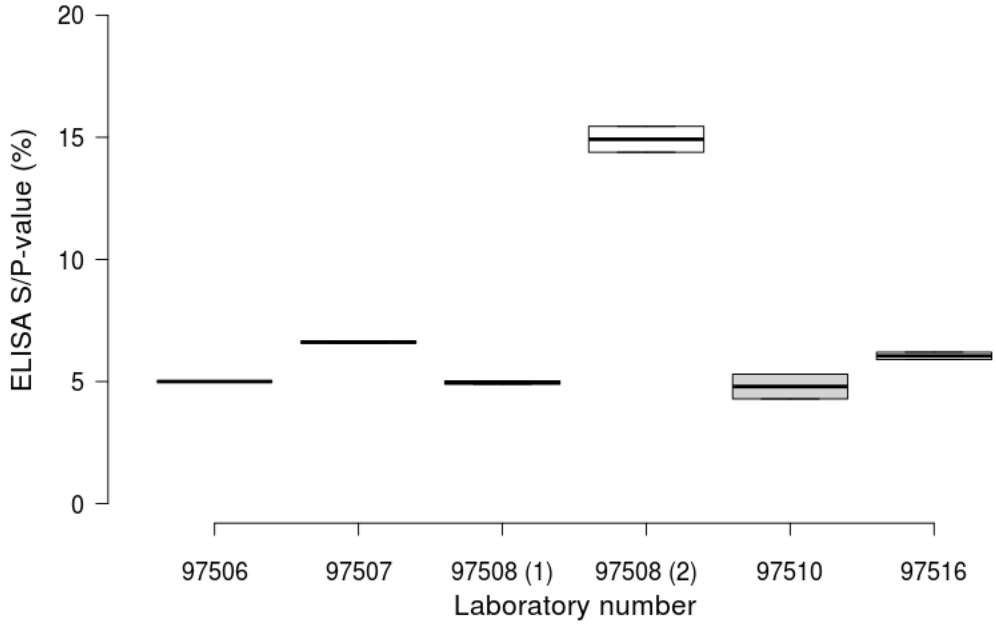


Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97506	97507	97508 (1)	97508 (2)	97510	97516
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_1	M_1
S/P % (REP1)	19,00	23,15	20,18	49,28	17,86	18,90
S/P % (REP2)	20,00	23,29	19,98	46,36	16,56	19,70
Mean	19,50	23,22	20,08	47,82	17,21	19,30
SD	0,71	0,10	0,14	2,07	0,92	0,57
CV (%)	3,63	0,42	0,71	4,32	5,34	2,93

Numbers were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = ID.VET - ID SCREEN® BLUETONGUE COMPETITION; M_2 = IDEXX - VP7 Antibody Test Kit).

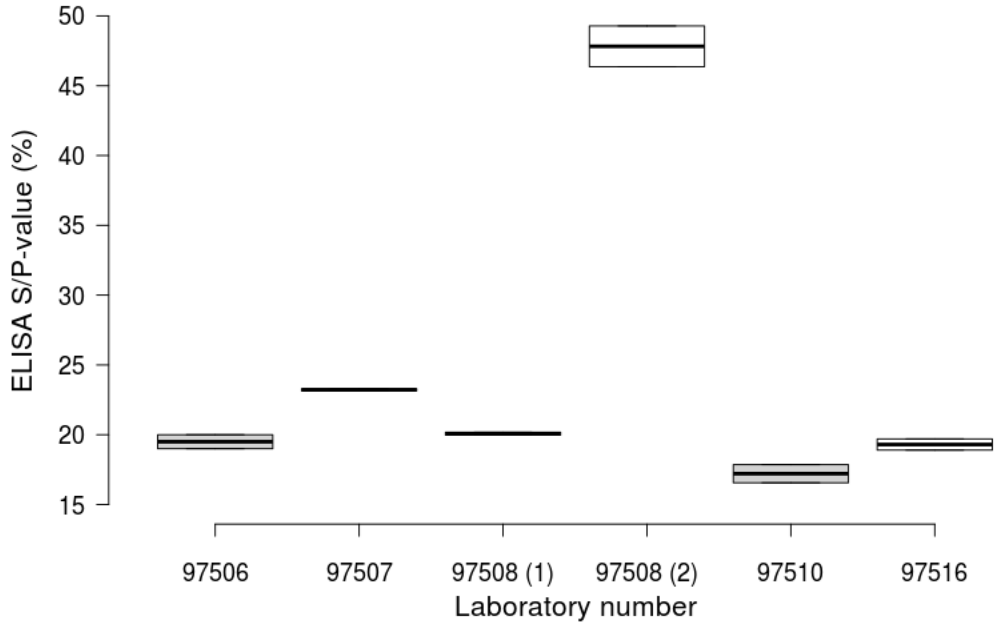


Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.

6.1.2 VIROLOGY (BLOOD)

PT2023BLTVIR-PB2

Lab number	97506	97507	97508	97509	97510	97514	97516 (1)	97516 (2)
Method (RT-qPCR protocol/kit)	M_1	M_2	M_2	M_2	M_3	M_4	M_2	M_2
Ct (REP1)	29,21	26,10	26,36	26,46	25,80	25,15	27,25	29,32
Ct (REP2)	29,11	26,10	26,92	26,26	25,50	25,25	27,75	29,11
Mean	29,16	26,10	26,64	26,36	25,65	25,20	27,50	29,22
SD	0,071	0,00	0,40	0,14	0,21	0,071	0,35	0,15
CV (%)	0,24	0,00	1,49	0,54	0,83	0,28	1,29	0,51

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade, M_2 = ADIAGENE - ADIAVET BTV real time, M_3 = Thermofisher - Vetmax Bluetongue Virus NS3 all genotype; M_4 = Biosellal - Bio-T kit® BTV all genotypes).

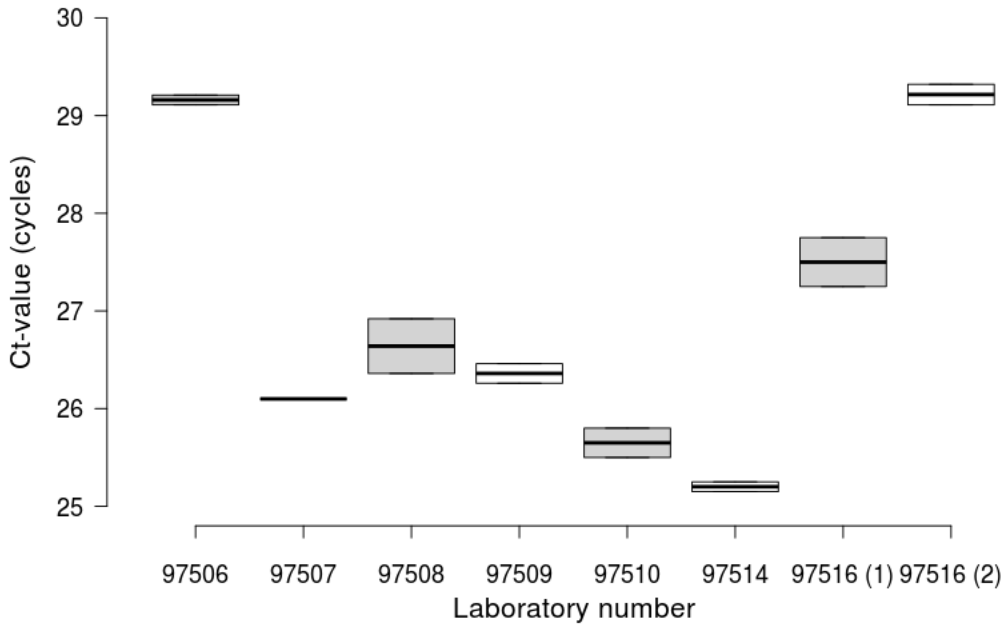


Figure 4. Distribution of the Ct-values (box-plots) per laboratory.

Lab number	97506	97507	97508	97509	97510	97514	97516 (1)	97516 (2)
Method (RT-qPCR protocol/kit)	M_1	M_2	M_2	M_2	M_3	M_4	M_2	M_2
Ct (REP1)	27,13	26,21	26,53	26,31	26,00	25,97	27,93	29,40
Ct (REP2)	27,23	26,61	26,73	26,28	26,00	26,13	27,57	30,14
Mean	27,18	26,41	26,63	26,30	26,00	26,05	27,75	29,77
SD	0,071	0,28	0,14	0,021	0,00	0,11	0,26	0,52
CV (%)	0,26	1,071	0,53	0,081	0,00	0,43	0,92	1,76

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Homemade, M_2 = ADIAGENE - ADIAVET BTV real time, M_3 = Thermofisher - Vetmax Bluetongue Virus NS3 all genotype; M_4 = Biosellal - Bio-T kit® BTV all genotypes).

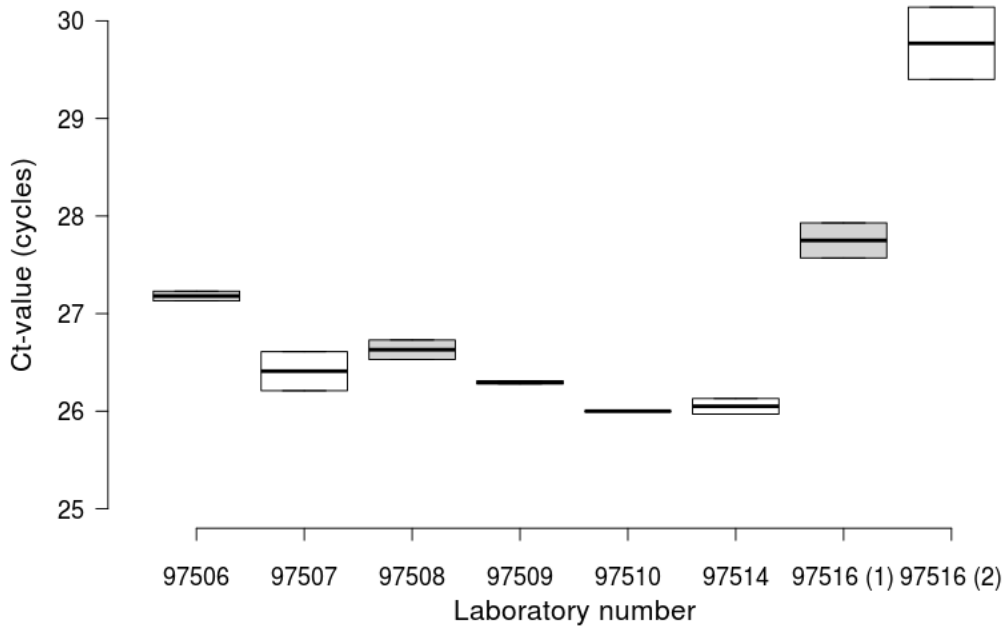


Figure 4. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

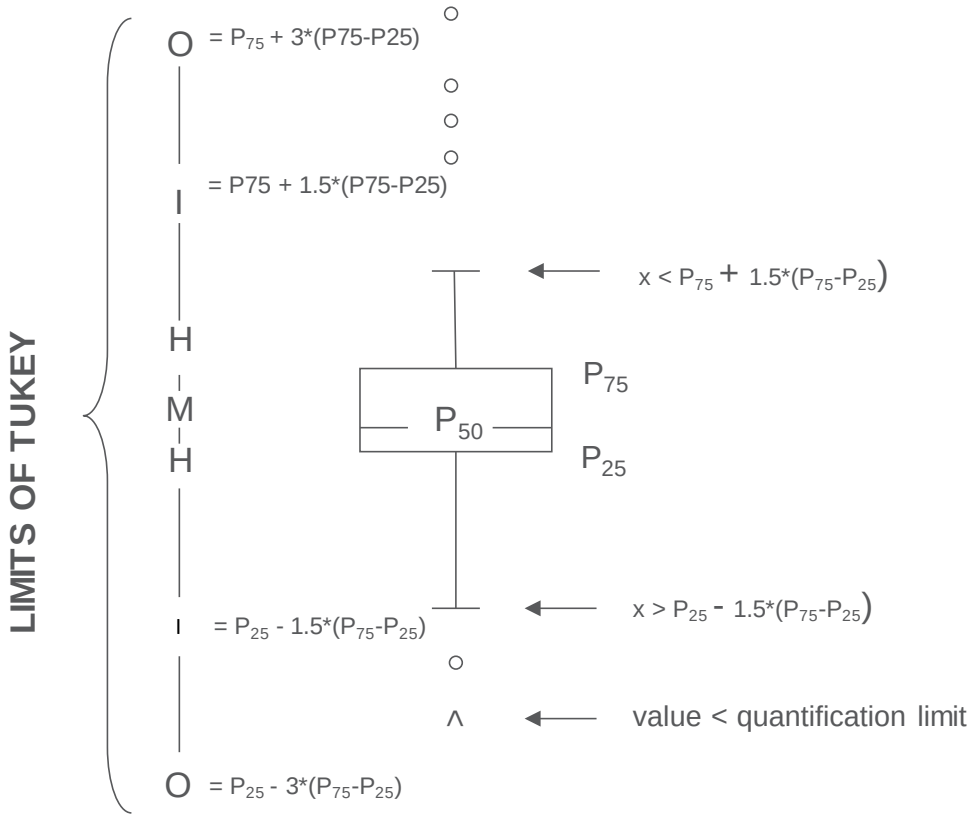
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

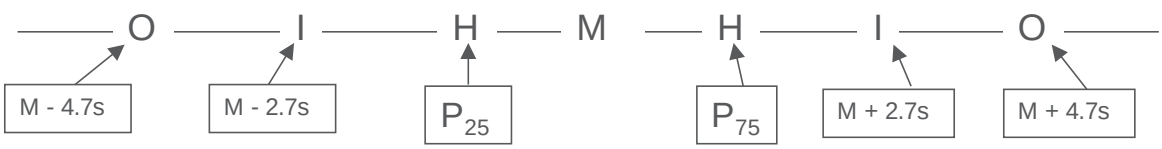
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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