BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2025-4

BLUE TONGUE AND EPIZOOTIC HAEMORRHAGIC DISEASE (BT&EHD)

Sciensano/PT-program BT&EHD/2025-4/E

Biological health risks
Quality of laboratories
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ISSN: 2294-3498

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A draft version of this report was submitted to the experts on 01/08/2025.

The experts were invited to send their comments via e-mail.

Responsibilities:
The National Reference Laboratory (NRL) of Sciensano was consulted for advice about the content of the global report, the interpretation of the results and the evaluation criteria. The responsibility for the choice of the samples used was carried out by the NRL.

Authorization of the report: by Ynse Van de Maele, coordinator

Date of publication: 17/09/2025

All the global reports are also available on our webpage:

- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
- FR: https://www.sciensano.be/fr/evaluation-externe-de-la-qualite/sante-animale-pt-vet
- EN: https://www.sciensano.be/en/external-quality-assessment/animal-health-pt-vet

TABLE OF CONTENTS

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 proficiency test was organised according to the ISO17043 ‘Conformity assessment - General requirements for proficiency testing’ norm.

2 AIM

This proficiency test aimed to evaluate the performance of participating laboratories in detecting Bluetongue virus and Epizootic Haemorrhagic Disease virus, using ELISA to detect antibodies in serum samples and qPCR to detect viral RNA in blood samples.

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of Blue Tongue and Epizootic Haemorrhagic Disease serology on serum samples. The laboratory numbers of the participating laboratories are:

- 97506
- 97507
- 97508
- 97510
- 97516

3.1.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Exotic and vector-borne diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

A total of eleven reference serum samples of bovine origin were included in the panel. The panel consisted of two samples free from detectable BTV- and EHDV-specific antibodies (PT2025BTVEHDV_NS1 and PT2025BTVEHDV_NS2), five samples containing detectable BTV-specific antibodies (PT2025BTVEHDV_PS1 to PS5), three samples containing detectable EHDV-specific antibodies (PT2025BTVEHDV_PS6 to PS8), and one sample containing both BTV- and EHDV-specific antibodies (PT2025BTVEHDV_PS9).

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 and the ID Screen® EHDV Competition from ID.VET .

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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 the ID Screen® Bluetongue Competition kit and the ID Screen® EHDV Competition kit from ID.VET. In all cases, the NRL obtained the same qualitative result (positive or negative according to the kit cut-off) for each of the ten aliquots. This consistent outcome confirmed that there was no variability in the serological status across aliquots of the same sample. Therefore, the samples were considered 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
PT2025BTVEHDV_PS1	BTV/EHDV	Infected BTV-3	Ovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PS2	BTV/EHDV	Vaccinated BTV-8	Ovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PS3	BTV/EHDV	Vaccinated BTV-8	Ovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PS4	BTV/EHDV	Infected BTV-3	Ovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PS5	BTV/EHDV	Infected BTV-3	Ovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PS6	BTV/EHDV	Infected EHDV-8	Bovine	NEG BTV + POS EHDV
PT2025BTVEHDV_PS7	BTV/EHDV	Infected EHDV-8	Bovine	NEG BTV + POS EHDV
PT2025BTVEHDV_PS8	BTV/EHDV	Infected EHDV-8	Bovine	NEG BTV + POS EHDV
PT2025BTVEHDV_PS9	BTV/EHDV	Infected BTV-3+EHDV-8	Bovine	POS BTV + POS EHDV
PT2025BTVEHDV_NS1	BTV/EHDV	Uninfected/ Unvaccinated	Ovine	NEG BTV + NEG EHDV
PT2025BTVEHDV_NS2	BTV/EHDV	Uninfected/ Unvaccinated	Bovine	NEG BTV + NEG EHDV

(BTV = Bluetongue Virus; EHDV = Epizootic Haemorrhagic Disease Virus; 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. After completion of the proficiency test, three aliquots of each sample were additionally tested, and in all cases the qualitative result (positive or negative according to the kit cut-off) remained unchanged. The samples were therefore considered 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: PT2025BTV EHDV_	97506	97507	97508	97510	97516
PS1	BESER25-1	BESER25-13	BESER25-3	BESER25-5	BESER25-14
PS2 (1)	BESER25-5	BESER25-2	BESER25-4	BESER25-8	BESER25-8
PS2 (2)	BESER25-12	BESER25-12	BESER25-6	BESER25-14	BESER25-15
PS3	BESER25-15	BESER25-3	BESER25-2	BESER25-15	BESER25-1
PS4	BESER25-10	BESER25-1	BESER25-11	BESER25-3	BESER25-6
PS5	BESER25-2	BESER25-9	BESER25-8	BESER25-6	BESER25-3
PS6 (1)	BESER25-3	BESER25-6	BESER25-5	BESER25-2	BESER25-10
PS6 (2)	BESER25-13	BESER25-8	BESER25-14	BESER25-13	BESER25-12
PS7	BESER25-9	BESER25-4	BESER25-10	BESER25-12	BESER25-13
PS8	BESER25-4	BESER25-15	BESER25-13	BESER25-7	BESER25-4
PS9 (1)	BESER25-6	BESER25-10	BESER25-1	BESER25-1	BESER25-5
PS9 (2)	BESER25-8	BESER25-11	BESER25-9	BESER25-9	BESER25-7
NS1 (1)	BESER25-7	BESER25-7	BESER25-12	BESER25-4	BESER25-2
NS1 (2)	BESER25-14	BESER25-14	BESER25-15	BESER25-10	BESER25-9
NS2	BESER25-11	BESER25-5	BESER25-7	BESER25-11	BESER25-11

3.1.7 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%.

3.2 Virology (serum)

3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Blue Tongue and Epizootic Haemorrhagic Disease virology on blood samples. The laboratory numbers of the participating laboratories are:

- 97506
- 97507
- 97508
- 97509
- 97516
- 97531

3.2.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Exotic and vector-borne diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

A total of eleven reference blood samples of bovine origin were included in the panel. The panel consisted of two samples free from detectable Bluetongue virus (BTV) and Epizootic Haemorrhagic Disease virus (EHDV) (PT2025BTVEHDV_NB1 and PT2025BTVEHDV_NB2), five samples containing detectable BTV (PT2025BTVEHDV_PB1 to PT2025BTVEHDV_PB5), two samples containing detectable EHDV (PT2025BTVEHDV_PB6 and PT2025BTVEHDV_PB7), and two samples containing both BTV and EHDV (PT2025BTVEHDV_PB8 and PT2025BTVEHDV_PB9).

For each reference blood sample, a certificate containing the status of the sample (the “golden standard”) was established. The status of the reference samples was determined based on (i) the historical background of the animals and (ii) the results obtained during pre-verification testing. All samples were tested with an in-house pan-BTV RT-qPCR assay (detecting all BTV serotypes) and a pan-EHDV RT-qPCR assay (detecting all EHDV serotypes). In addition, for BTV, four serotype-specific RT-qPCR assays were applied, targeting BTV-1, BTV-3, BTV-4, and BTV-8.

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-qPCR prior to the PT. All aliquots were analysed with the pan-BTV RT-qPCR and the pan-EHDV RT-qPCR assays. In each case, the NRL obtained the same qualitative result (positive or negative according to the assay used) across all aliquots of a given sample. This consistent outcome demonstrated the absence of variability within each batch, and therefore the samples were considered 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
PT2025BTVEHDV_PB1	BTV-1	Uninfected/ blood spiked	Bovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PB2	BTV-4	Uninfected/ blood spiked	Bovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PB3	BTV-8	Uninfected/ blood spiked	Bovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PB4	BTV-3	Uninfected/ blood spiked	Bovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PB5	BTV-3	Uninfected/ blood spiked	Bovine	POS BTV + NEG EHDV
PT2025BTVEHDV_PB6	EHDV-8	Uninfected/ blood spiked	Bovine	NEG BTV + POS EHDV
PT2025BTVEHDV_PB7	EHDV-8	Uninfected/ blood spiked	Bovine	NEG BTV + POS EHDV
PT2025BTVEHDV_PB8	BTV-3 and EHDV-8	Uninfected/ blood spiked	Bovine	POS BTV + POS EHDV
PT2025BTVEHDV_PB9	BTV-8 and EHDV-8	Uninfected/ blood spiked	Bovine	POS BTV + POS EHDV
PT2025BTVEHDV_NB1	None	Uninfected	Bovine	NEG BTV + NEG EHDV
PT2025BTVEHDV_NB2	None	Uninfected	Bovine	NEG BTV + NEG EHDV

(BTV = Bluetongue Virus; EHDV = Epizootic Haemorrhagic Disease Virus; 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. After completion of the proficiency test, three aliquots of each sample were additionally tested with the pan-BTV RT-qPCR and pan-EHDV RT-qPCR assays. In all cases, the qualitative result (positive or negative according to the assay used) remained unchanged. The samples were therefore considered 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: PT2025BTVEHDV_	97506	97507	97508
PB1	BEVIR25-4	BEVIR25-1	BEVIR25-6
PB2	BEVIR25-10	BEVIR25-8	BEVIR25-14
PB3	BEVIR25-1	BEVIR25-15	BEVIR25-3
PB4 (1)	BEVIR25-6	BEVIR25-4	BEVIR25-5
PB4 (2)	BEVIR25-11	BEVIR25-9	BEVIR25-9
PB5	BEVIR25-12	BEVIR25-3	BEVIR25-8
PB6 (1)	BEVIR25-2	BEVIR25-11	BEVIR25-4
PB6 (2)	BEVIR25-3	BEVIR25-14	BEVIR25-12
PB7	BEVIR25-14	BEVIR25-7	BEVIR25-2
PB8	BEVIR25-9	BEVIR25-5	BEVIR25-7
PB9 (1)	BEVIR25-5	BEVIR25-6	BEVIR25-11
PB9 (2)	BEVIR25-8	BEVIR25-10	BEVIR25-15
NB1 (1)	BEVIR25-7	BEVIR25-2	BEVIR25-10
NB1 (2)	BEVIR25-13	BEVIR25-13	BEVIR25-13
NB2	BEVIR25-15	BEVIR25-12	BEVIR25-1

Sample content: PT2025BTVEHDV_	97509	97516	97531
PB1	BEVIR25-3	BEVIR25-15	BEVIR25-9
PB2	BEVIR25-1	BEVIR25-8	BEVIR25-10
PB3	BEVIR25-13	BEVIR25-5	BEVIR25-4
PB4 (1)	BEVIR25-6	BEVIR25-12	BEVIR25-2
PB4 (2)	BEVIR25-7	BEVIR25-14	BEVIR25-11
PB5	BEVIR25-2	BEVIR25-13	BEVIR25-12
PB6 (1)	BEVIR25-5	BEVIR25-1	BEVIR25-3
PB6 (2)	BEVIR25-11	BEVIR25-11	BEVIR25-5
PB7	BEVIR25-15	BEVIR25-6	BEVIR25-15
PB8	BEVIR25-12	BEVIR25-10	BEVIR25-14
PB9 (1)	BEVIR25-4	BEVIR25-2	BEVIR25-6

Sample content: PT2025BTVEHDV_	97509	97516	97531
PB9 (2)	BEVIR25-14	BEVIR25-7	BEVIR25-13
NB1 (1)	BEVIR25-8	BEVIR25-3	BEVIR25-7
NB1 (2)	BEVIR25-10	BEVIR25-9	BEVIR25-8
NB2	BEVIR25-9	BEVIR25-4	BEVIR25-1

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

The randomisation of the samples by QL took place on June 6, 2025. The samples were then sent to the participants on June 10, 2025. The deadline for submitting the results was set for July 4, 2025. All participants submitted their results on time. Finally, the individual reports were provided to the participants on July 31, 2025.

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of eleven different samples. However, samples PS2, PS6, PS9 and NS1 were replicated twice. Therefore, the panel included fifteen samples in total.

Table of laboratories that used a combination of methods (one for BTV and one for EHDV)

Four laboratories used a combination of testing methods (one targeting BTV and another targeting EHDV) to analyse the panel of samples. The evaluation was based on the combined results from both methods.

Sample content	Expected results	Total results	Observed results
PS1	POS BTV + NEG EHDV	4	4 POS BTV + NEG EHDV
PS2	POS BTV + NEG EHDV	8	8 POS BTV + NEG EHDV
PS3	POS BTV + NEG EHDV	4	4 POS BTV + NEG EHDV
PS4	POS BTV + NEG EHDV	4	4 POS BTV + NEG EHDV
PS5	POS BTV + NEG EHDV	4	4 POS BTV + NEG EHDV
PS6	NEG BTV + POS EHDV	8	8 NEG BTV + POS EHDV
PS7	NEG BTV + POS EHDV	4	4 NEG BTV + POS EHDV
PS8	NEG BTV + POS EHDV	4	4 NEG BTV + POS EHDV
PS9	POS BTV + POS EHDV	8	8 POS BTV + POS EHDV
NS1	NEG BTV + NEG EHDV	8	8 NEG BTV + NEG EHDV
NS2	NEG BTV + NEG EHDV	4	4 NEG BTV + NEG EHDV

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

Table of laboratories that used only one method (method BTV)

One laboratory chose to apply two different BTV testing methods to the same samples, resulting in an additional BTV dataset being submitted. This extra dataset was evaluated separately. In addition, another laboratory submitted results exclusively for the BTV component of the test. Therefore, in the table below, samples that tested positive for EHDV only were excluded from the evaluation.

Sample content	Expected results	Total results	Observed results
PS1	POS BTV + NEG EHDV	2	2 POS BTV
PS2	POS BTV + NEG EHDV	4	4 POS BTV
PS3	POS BTV + NEG EHDV	2	2 POS BTV
PS4	POS BTV + NEG EHDV	2	2 POS BTV
PS5	POS BTV + NEG EHDV	2	2 POS BTV
PS6	NEG BTV + POS EHDV	/	/
PS7	NEG BTV + POS EHDV	/	/
PS8	NEG BTV + POS EHDV	/	/
PS9	POS BTV + POS EHDV	4	4 POS BTV
NS1	NEG BTV + NEG EHDV	4	4 NEG BTV
NS2	NEG BTV + NEG EHDV	2	2 NEG BTV

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

5.1.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
Combination of two methods (BTV & EHDV)	IDVet	ID Screen BTV Competition ELISA	**4**	**60**	**60**	**100**
	IDVet	ID Screen EHDV Competition ELISA				
One method (BTV)	IDVet	ID Screen BTV Competition ELISA	**1**	**11**	**11**	**100**
One method (BTV)	IDEXX	Bluetongue Competition Ab Test	**1**	**11**	**11**	**100**
TOTAL			**6**	**82**	**82**	**100**

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

5.1.3 CONCLUSION

In 2025, five laboratories participated in the proficiency test Blue Tongue and Epizootic Haemorrhagic Disease serology (serum) organised by Sciensano. Four laboratories used a combination of testing methods (one targeting BTV and another targeting EHDV) to analyse the panel of samples. Additionally, two datasets focusing solely on BTV were evaluated separately.

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)

5.2.1 RESULTS PER SAMPLE

The panel consisted of eleven different samples. However, samples PB4, PB6, PB9 and NB1 were replicated twice. Therefore, the panel included fifteen samples in total.

Table of laboratories that used a combination of methods (one for BTV and one for EHDV)

Five laboratories used a combination of testing methods (one targeting BTV and another targeting EHDV) to analyse the panel of samples. The evaluation was based on the combined results from both methods.

Sample content	Expected results	Total results	Observed results
PB1	POS BTV + NEG EHDV	5	5 POS BTV + NEG EHDV
PB2	POS BTV + NEG EHDV	5	5 POS BTV + NEG EHDV
PB3	POS BTV + NEG EHDV	5	5 POS BTV + NEG EHDV
PB4	POS BTV + NEG EHDV	10	10 POS BTV + NEG EHDV
PB5	POS BTV + NEG EHDV	5	5 POS BTV + NEG EHDV
PB6	NEG BTV + POS EHDV	10	10 NEG BTV + POS EHDV
PB7	NEG BTV + POS EHDV	5	5 NEG BTV + POS EHDV
PB8	POS BTV + POS EHDV	5	5 POS BTV + POS EHDV
PB9	POS BTV + POS EHDV	10	10 POS BTV + POS EHDV
NB1	NEG BTV + NEG EHDV	10	10 NEG BTV + NEG EHDV
NB2	NEG BTV + NEG EHDV	5	5 NEG BTV + NEG EHDV

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

Table of laboratory that used only one method (method BTV)

One laboratory submitted results exclusively for the BTV component of the test. Therefore, in the table below, samples that tested positive for EHDV only were excluded from the evaluation.

Sample content	Expected results	Total results	Observed results
PB1	POS BTV + NEG EHDV	1	1 POS BTV + NEG EHDV
PB2	POS BTV + NEG EHDV	1	1 POS BTV + NEG EHDV
PB3	POS BTV + NEG EHDV	1	1 POS BTV + NEG EHDV
PB4	POS BTV + NEG EHDV	2	2 POS BTV + NEG EHDV
PB5	POS BTV + NEG EHDV	1	1 POS BTV + NEG EHDV
PB6	NEG BTV + POS EHDV	/	/
PB7	NEG BTV + POS EHDV	/	/
PB8	POS BTV + POS EHDV	/	/
PB9	POS BTV + POS EHDV	2	2 POS BTV + POS EHDV
PB1	NEG BTV + NEG EHDV	2	2 NEG BTV + NEG EHDV
PB2	NEG BTV + NEG EHDV	1	1 NEG BTV + NEG EHDV

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

5.2.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

Below, the table displays the results for each used extraction protocol/kit method.

Method	Name producer	Name extraction protocol/kit	N	NR	NCR	%
Used for both BTV and EHDV	QIAGEN	QIAamp Viral RNA Mini Kit	**1**	**15**	**15**	**100**
Used for both BTV and EHDV	Indical Bioscience	IndiMag Pathogen Kit	**3**	**45**	**45**	**100**
Used only for BTV	Indical Bioscience	IndiMag Pathogen Kit	**1**	**11**	**11**	**100**
Used for both BTV and EHDV	IDVet	ID Gene Mag Universal Extraction kit	**1**	**15**	**15**	**100**
	TOTAL		**6**	**86**	**86**	**100**

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

5.2.3 RESULTS PER USED PCR PROTOCOL/KIT

Below, the table displays the results for each used PCR protocol/kit method.

Method	Name producer	Name PCR protocol/kit	N	NR	NCR	%
Combination of two methods (BTV & EHDV)	Homemade	Homemade	1	15	15	100
	Homemade	Homemade				
Combination of two methods (BTV & EHDV)	Bio-X Diagnostics	Adiavet BTV Real Time	1	15	15	100
	IDVet	ID Gene EHDV Duplex				
Combination of two methods (BTV & EHDV)	Bio-X Diagnostics	Adiavet BTV Real Time	3	45	45	100
	Bio-X Diagnostics	Adiavet EHDV Real Time				
One method (BTV)	Indical Bioscience	BTV 2.0 RT-PCR	1	11	11	100
TOTAL			**6**	**86**	**86**	**100**

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

5.2.4 CONCLUSION

In 2025, six laboratories participated in the proficiency test Blue Tongue and Epizootic Haemorrhagic Disease virology (blood) organised by Sciensano. Five laboratories used a combination of testing methods (one targeting BTV and another targeting EHDV) to analyse the panel of samples. Additionally, one datasets focusing solely on BTV was evaluated separately.

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 covered by BELAC accreditation and is provided solely for the information of the laboratories.

6.1 Annex: 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)

Interpretation and formula details of participant-used kits

Kit	*Formula*	*Interpretation*
IDVet - ID Screen® BTV Competition ELISA	S/N% = (OD_{sample} / $OD_{negative\ control}$) * 100	Interpretation English manual: ≤ 40% → Positive ≥ 40% → Negative Interpretation French manual: ≤ 35% → Positive 35–45% → Doubtful ≥ 45% → Negative
IDEXX - VP7 Antibody Test Kit BTV	S/N% = (OD_{sample} / $OD_{negative\ control}$) * 100	≤ 70% → Positive 70–80% → Doubtful ≥ 80% → Negative
IDVet - ID Screen EHDV Competition ELISA	S/N% = (OD_{sample} / $OD_{negative\ control}$) * 100	≤ 30% → Positive 30–40% → Doubtful ≥ 40% → Negative

Interpretation of CV(%) in ELISA tests for positive samples

CV(%)	*Interpretation*
≤ 10%	Excellent reproducibility
10% – 15%	Acceptable, but moderate variation
15% – 20%	Questionable, should be reviewed
> 20%	Poor reproducibility - not reliable

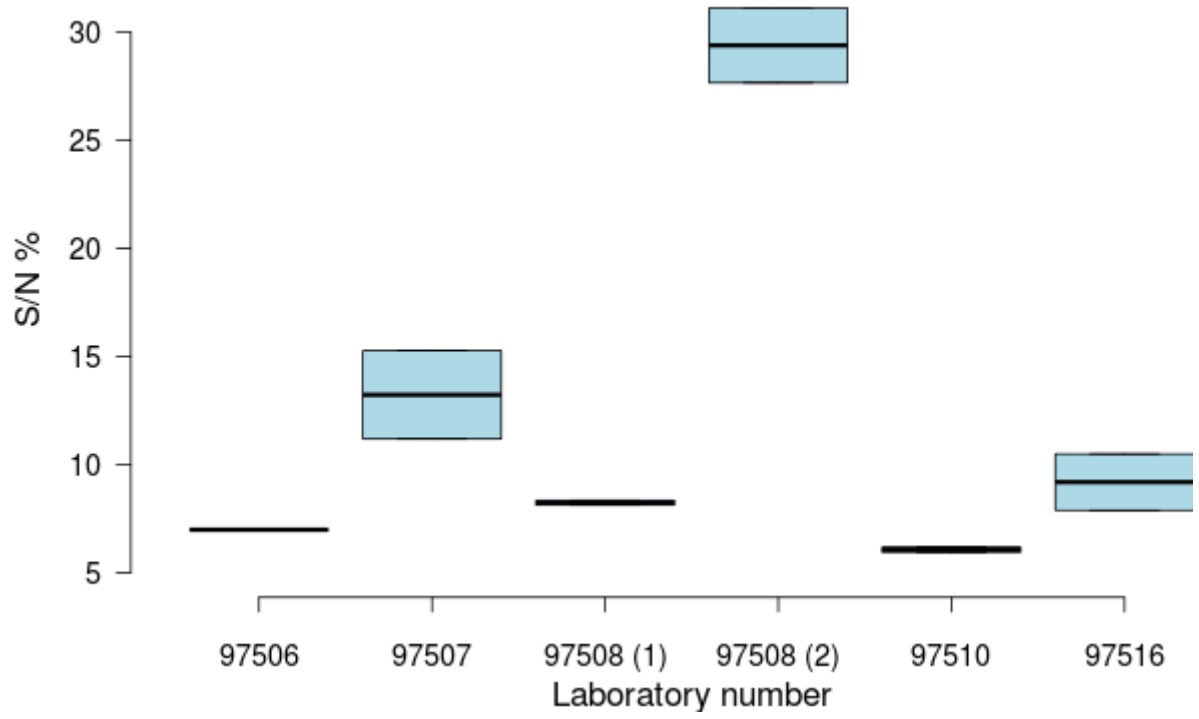
Note: When the number of replicates is low, the CV(%) may not provide a reliable estimate of assay precision and should be interpreted accordingly.

Quantitative results for duplicate samples: PT2025BTVEHDV-PS2 (BTV POS)

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
Pos control	0,048	0,106	0,079	0,058	0,0465	0,058
Neg control	1,138	1,25	1,388	1,392	1,291	1,531
OD (1)	0,075	0,14	0,116	0,385	0,077	0,121
OD (2)	0,075	0,191	0,113	0,433	0,08	0,161

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/N % (1)	7	11,196	8,3573	27,658	5,964	7,9
S/N % (2)	7	15,274	8,1412	31,106	6,197	10,5
Mean	7,00	13,24	8,25	29,38	6,08	9,20
SD	0,00	2,88	0,15	2,44	0,16	1,84
CV (%)	0,00	21,79	1,85	8,30	2,71	19,98

S/N % = Signal-to-Noise Percentage; SD = standard deviation; CV = coefficient of variation; M_1 = IDVet - ID Screen BTV Competition ELISA; M_2 = IDEXX - VP7 Antibody Test Kit (IDEXX Bluetongue Competition.

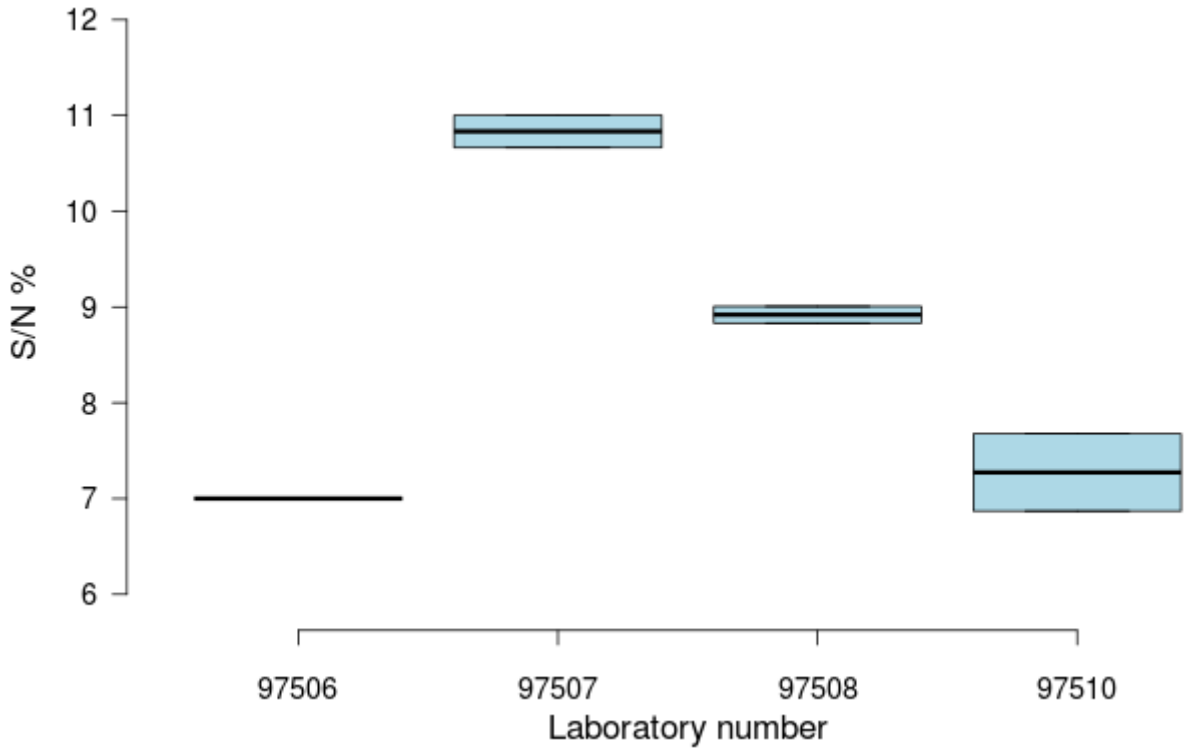


Quantitative results for duplicate samples: PT2025BTVEHDV-PS6 (EHDV POS)

Lab number	97506	97507	97508	97510
Method (ELISA protocol/kit)	IDVet - ID Screen EHDV Competition ELISA			
Pos control	0,116	0,165	0,167	0,171
Neg control	1,708	1,482	1,721	2,11
OD (1)	0,123	0,158	0,152	0,162
OD (2)	0,128	0,163	0,155	0,145

Lab number	97506	97507	97508	97510
Method (ELISA protocol/kit)	IDVet - ID Screen EHDV Competition ELISA			
S/N % (1)	7	10,665	8,8321	7,676
S/N % (2)	7	11,002	9,0064	6,87
Mean	7,00	10,83	8,92	7,27
SD	0	0,24	0,12	0,57
CV (%)	0	2,20	1,38	7,84

S/N % = Signal-to-Noise Percentage; SD = standard deviation; CV = coefficient of variation



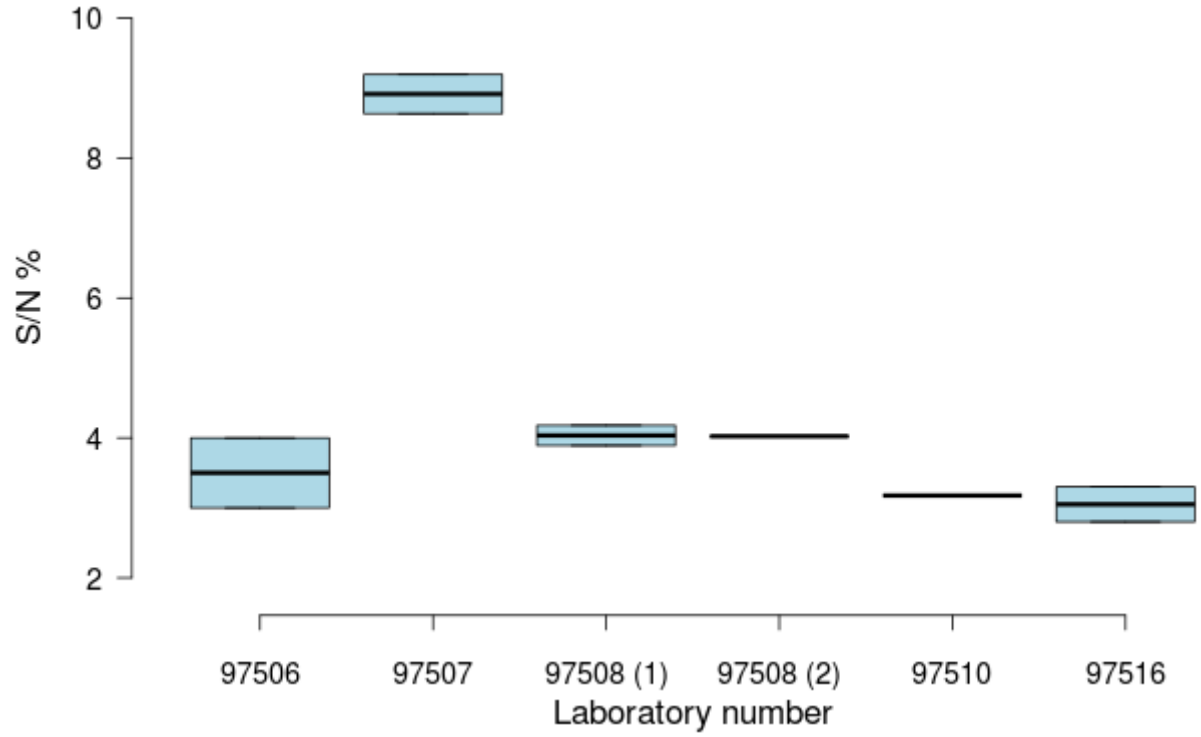
Quantitative results for duplicate samples: PT2025BTVEHDV-PS9 (BTV POS+EHDV POS)

BTV

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
Pos control	0,048	0,106	0,079	0,058	0,0465	0,058
Neg control	1,138	1,25	1,388	1,392	1,291	1,531
OD (1)	0,047	0,108	0,058	0,056	0,041	0,043
OD (2)	0,036	0,115	0,054	0,056	0,041	0,05

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/N % (1)	4	8,6365	4,1787	4,023	3,176	2,8
S/N % (2)	3	9,1963	3,8905	4,023	3,176	3,3
Mean	3,50	8,92	4,03	4,02	3,18	3,05
SD	0,71	0,40	0,20	0,00	0,00	0,35
CV (%)	20,20	4,44	5,05	0,00	0,00	11,59

S/N % = Signal-to-Noise Percentage; SD = standard deviation; CV = coefficient of variation; M_1 = IDVet - ID Screen BTV Competition ELISA; M_2 = IDEXX - VP7 Antibody Test Kit (IDEXX Bluetongue Competition.

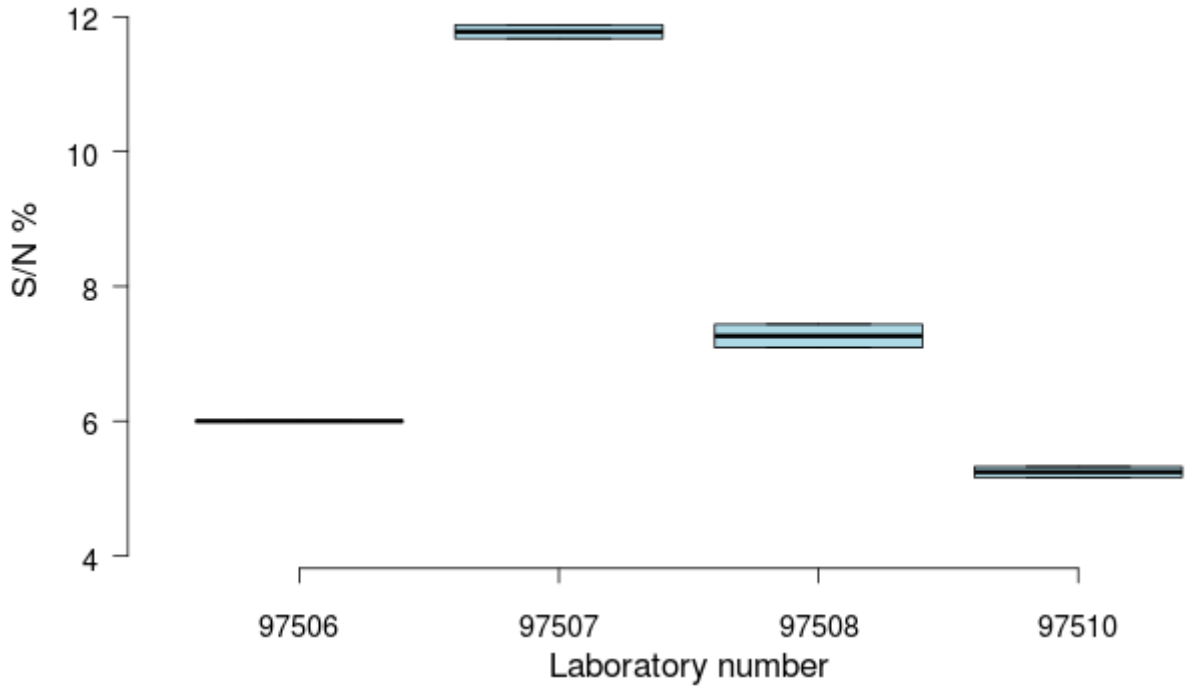


EHDV

Lab number	97506	97507	97508	97510
Method (ELISA protocol/kit)	IDVet - ID Screen EHDV Competition ELISA			
Pos control	0,116	0,165	0,167	0,171
Neg control	1,708	1,482	1,721	2,11
OD (1)	0,101	0,173	0,128	0,113
OD (2)	0,104	0,176	0,122	0,109

Lab number	97506	97507	97508	97510
Method (ELISA protocol/kit)	IDVet - ID Screen EHDV Competition ELISA			
S/N % (1)	6	11,677	7,4375	5,324
S/N % (2)	6	11,88	7,0889	5,165
Mean	6,00	11,78	7,26	5,24
SD	0,00	0,14	0,25	0,11
CV (%)	0,00	1,22	3,39	2,14

S/N % = Signal-to-Noise Percentage; SD = standard deviation; CV = coefficient of variation



6.1.2 VIROLOGY (BLOOD)

Interpretation of CV (%) in qPCR assays for positive samples

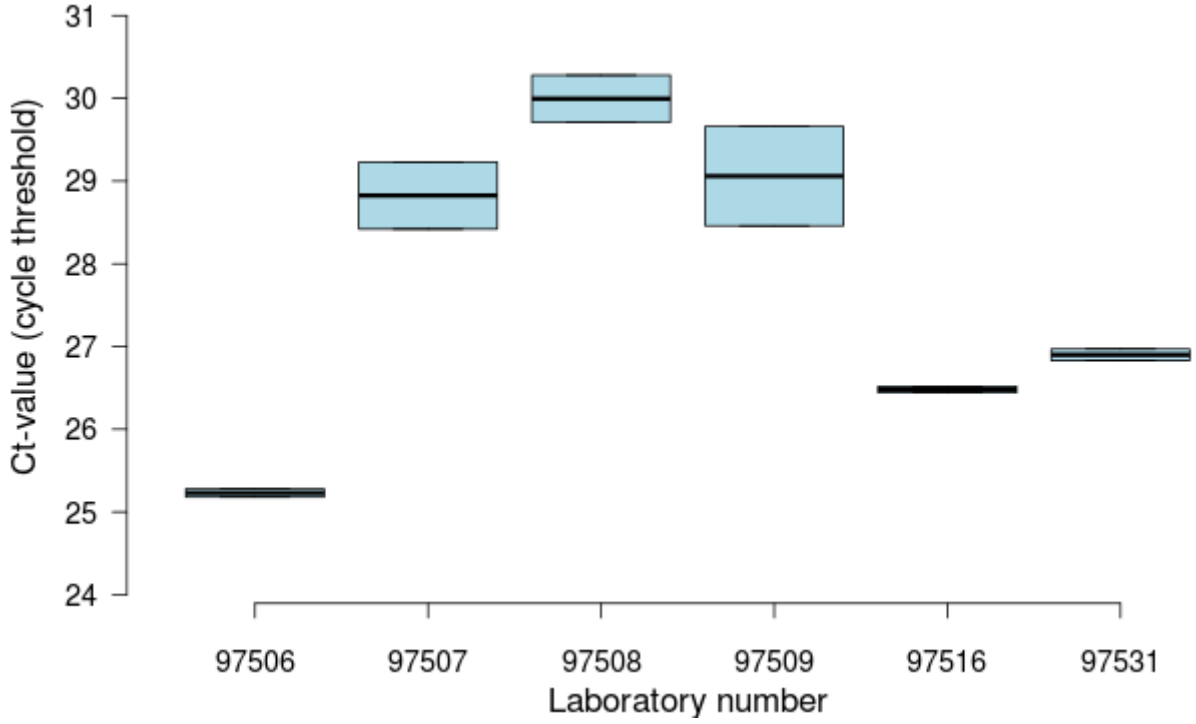
CV(%)	*Interpretation*
< 5%	Excellent – High repeatability and precision between replicates
5% – 10%	Good – Acceptable level of variation
10% – 15%	Moderate – May indicate pipetting error, extraction issues or instrument variability
> 15%	Poor – High variability, results may not be reliable or reproducible

Note: When the number of replicates is low, the CV(%) may not provide a reliable estimate of assay precision and should be interpreted accordingly.

Quantitative results for duplicate samples: PT2025BTVEHDV-PB4 (BTV POS)

Lab number	97505	97507	97508	97509	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_2	M_2	M_2	M_3
Pos control	30,74	29	29,55	28,45	24,51	29,19
Neg control	45	45	40	40	40	40
Cut-off	45	45	34	40	40	40
Ct-value (1)	25,28	28,42	29,71	28,46	26,44	26,97
Ct-value (2)	25,18	29,23	30,28	29,66	26,52	26,83
Mean	25,230	28,825	29,995	29,060	26,480	26,900
SD	0,071	0,573	0,403	0,849	0,057	0,099
CV (%)	0,280	1,987	1,344	2,920	0,214	0,368

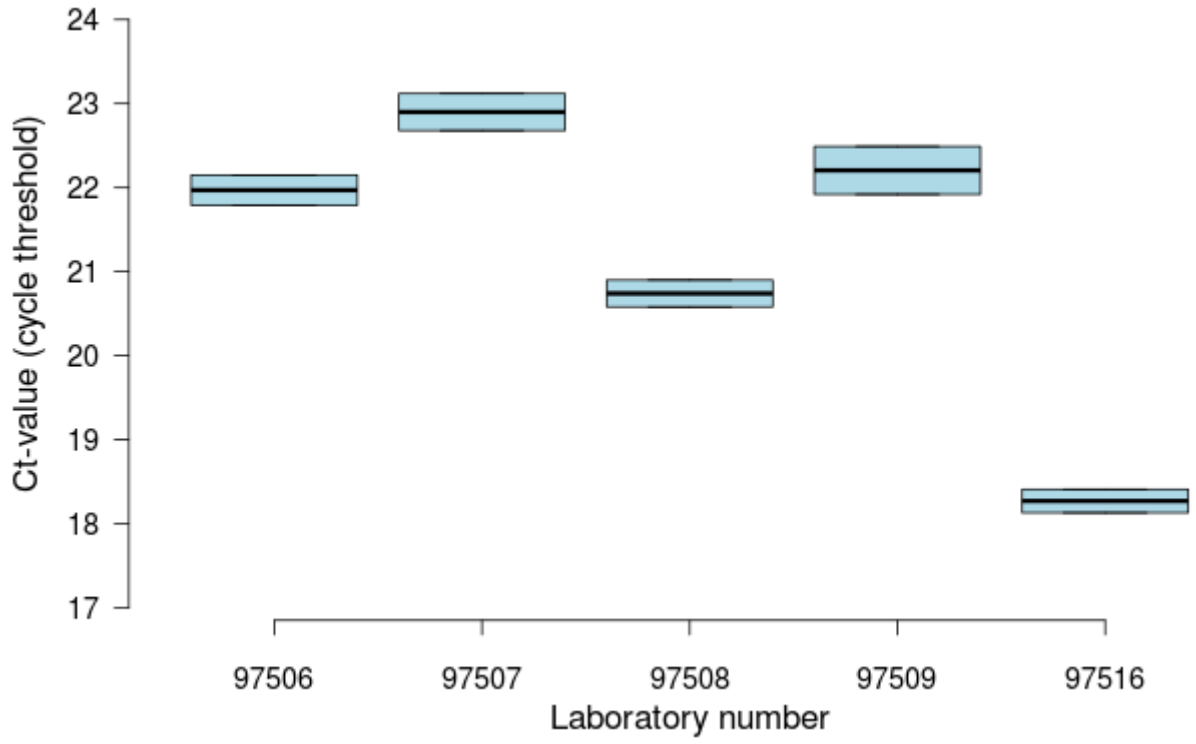
SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = Bio-X Diagnostics - Adiavet™ BTV Real Time; M_3 = Indical BTV 2.0 RT-PCR)


Ct-value (cycle threshold)
31
30
29
28
27
26
25
24
97506
97507
97508
97509
97516
97531
Laboratory number

Quantitative results for duplicate samples: PT2025BTVEHDV-PB6 (POS EHDV)

Lab number	97506	97507	97508	97509	97516
Method (PCR protocol/kit)	M_1	M_2	M_3	M_3	M_3
Pos control	28,62	28,99	26,41	26,14	23,77
Neg control	45	45	40	40	40
Cut-off	45	40	34	40	40
Ct-value (1)	22,15	22,68	20,58	21,92	18,13
Ct-value (2)	21,79	23,12	20,9	22,49	18,41
Mean	21,970	22,900	20,740	22,205	18,270
SD	0,255	0,311	0,226	0,403	0,198
CV (%)	1,159	1,359	1,091	1,815	1,084

SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = IDVet - ID Gene EHDV Duplex; M_3 = Bio-X Diagnostics - Adiavet EHDV Real Time

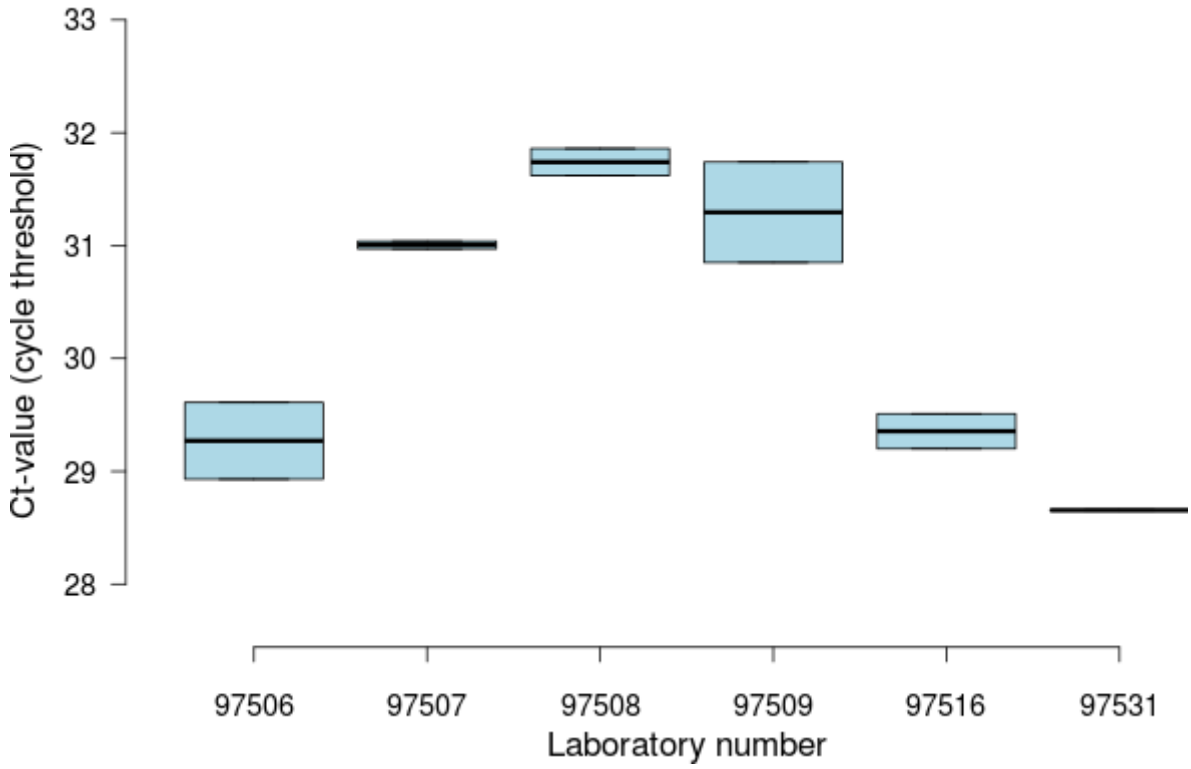


Quantitative results for duplicate samples: PT2025BTVEHDV-PB9 (BTV POS+EHDV POS)

BTV POS

Lab number	97506	97507	97508	97509	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_2	M_2	M_2	M_3
Pos control	30,74	29	29,55	28,45	24,51	29,19
Neg control	45	45	40	40	40	40
Cut-off	45	45	34	40	40	40
Ct-value (1)	28,93	30,97	31,62	30,85	29,51	28,65
Ct-value (2)	29,61	31,04	31,86	31,74	29,2	28,66
Mean	29,270	31,005	31,740	31,295	29,355	28,655
SD	0,481	0,049	0,170	0,629	0,219	0,007
CV (%)	1,643	0,160	0,535	2,011	0,747	0,025

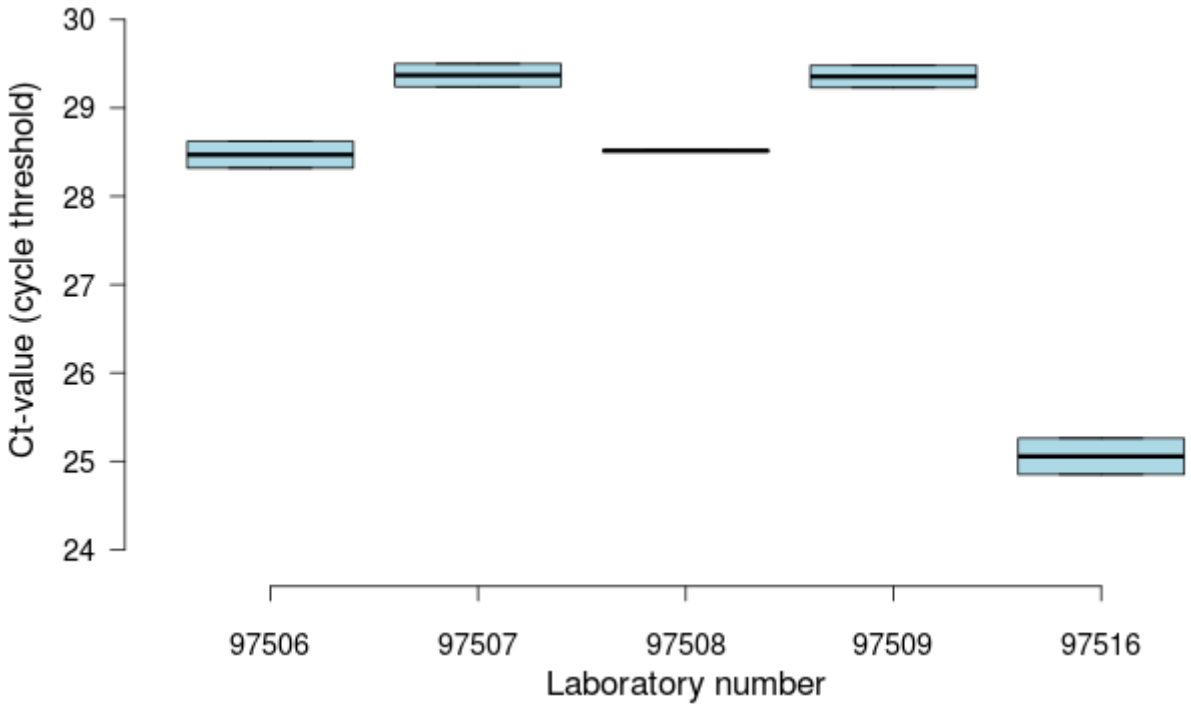
SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = Bio-X Diagnostics - Adiavet™ BTV Real Time; M_3 = Indical BTV 2.0 RT-PCR)



EHDV POS

Lab number	97506	97507	97508	97509	97516
Method (PCR protocol/kit)	M_1	M_2	M_3	M_3	M_3
Pos control	28,62	28,99	26,41	26,14	23,77
Neg control	45	45	40	40	40
Cut-off	45	40	34	40	40
Ct-value (1)	28,32	29,24	28,51	29,23	24,85
Ct-value (2)	28,62	29,5	28,52	29,48	25,26
Mean	28,470	29,370	28,515	29,355	25,055
SD	0,212	0,184	0,007	0,177	0,290
CV (%)	0,745	0,626	0,025	0,602	1,157

SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = IDVet - ID Gene EHDV Duplex; M_3 = Bio-X Diagnostics - Adiavet EHDV Real Time



6.2 Annex: Additional information

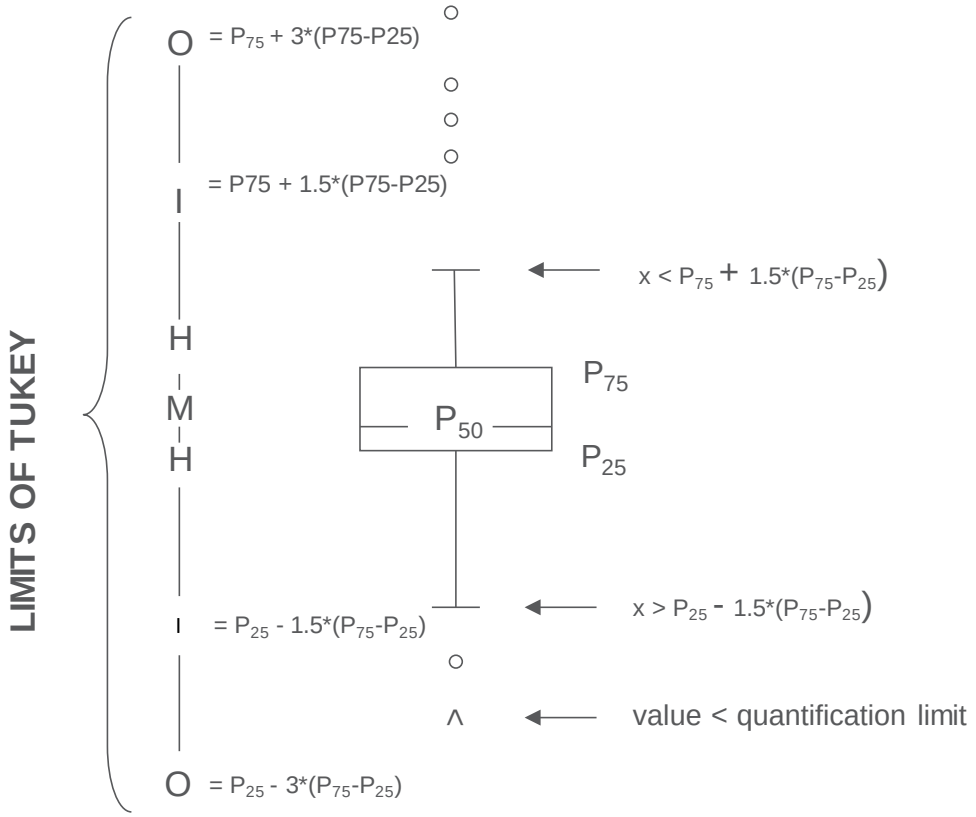
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

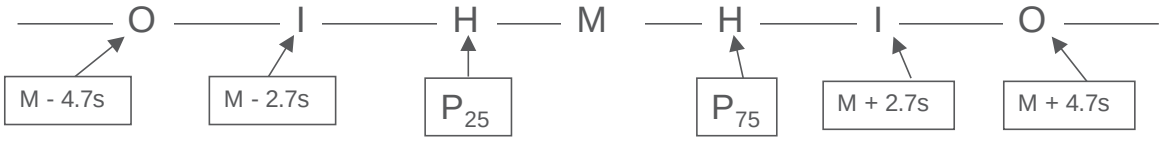
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2025
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2025
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2025

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