BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2025-2

BOVINE VIRAL DIARRHEA (BVD)

CORRECTED VERSION

Sciensano/PT-program BVD/2025-2/E-CV

Biological health risks
Quality of laboratories
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A draft version of this report was submitted to the experts on 30/07/2025.

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.

A corrected version of the global report has been published for the following reasons: an incorrect method was associated with laboratory 97621 in the quantitative results tables in the section *Serology – Serum*, specifically for samples PS1, PS2, and PS3. Also, extra information was provided on the interpretation and formula details of participant-used kits for the part *Serology – Serum*. In addition, a correction was made on page 28, where a zero was missing, resulting in the total score being 100 instead of 10.

The corresponding changes are highlighted in blue on pages 28, 33 and 35–37. This version replaces the previous report dated 06/10/2025.

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

Date of publication: 21/10/2025

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

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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 proficiency test was organised 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 against BVD in serum of cattle.
- The aim of the **virology part** was to evaluate the ability of the participating laboratories to identify BVD virus in serum, blood and ear notch of cattle.

3 MATERIALS AND METHODS

3.1 Serology (serum - ELISA)

3.1.1 THE PARTICIPANTS

Eight laboratories participated in the BVD serology proficiency test to detect antibodies in serum samples using the ELISA method. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509
- 97513
- 97541
- 97544
- 97621

3.1.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the BIOX BIO K 283 – Monoscreen AbELISA BVDV (EO) (Batch number BVD24C07). When a sample is send for a PT for the first time ever, 10 aliquots (250 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (250 µL each) of each sample are tested, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in OD-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDSER_PS1	POS
PT2025BVDSER_PS2	POS
PT2025BVDSER_PS3	POS
PT2025BVDSER_NS1	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate using the same ELISA as the pre-PT. The samples were deemed 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: PT2025BVDSER_	97505	97507	97508	97509
PS1 (1)	BVDSSE25-6	BVDSSE25-2	BVDSSE25-2	BVDSSE25-1
PS1 (2)	BVDSSE25-8	BVDSSE25-3	BVDSSE25-3	BVDSSE25-4
PS1 (3)	BVDSSE25-9	BVDSSE25-5	BVDSSE25-8	BVDSSE25-6
PS1 (4)	BVDSSE25-10	BVDSSE25-9	BVDSSE25-9	BVDSSE25-9
PS2 (1)	BVDSSE25-2	BVDSSE25-6	BVDSSE25-1	BVDSSE25-2
PS2 (2)	BVDSSE25-3	BVDSSE25-7	BVDSSE25-10	BVDSSE25-7
PS3 (1)	BVDSSE25-1	BVDSSE25-1	BVDSSE25-4	BVDSSE25-3

Sample content: PT2025BVDSER_	97505	97507	97508	97509
PS3 (2)	BVDSSE25-5	BVDSSE25-4	BVDSSE25-6	BVDSSE25-8
PS3 (3)	BVDSSE25-7	BVDSSE25-8	BVDSSE25-7	BVDSSE25-10
NS1	BVDSSE25-4	BVDSSE25-10	BVDSSE25-5	BVDSSE25-5

Sample content: PT2025BVDSER_	97513	97541	97544	97621
PS1 (1)	BVDSSE25-1	BVDSSE25-1	BVDSSE25-3	BVDSSE25-2
PS1 (2)	BVDSSE25-2	BVDSSE25-4	BVDSSE25-5	BVDSSE25-3
PS1 (3)	BVDSSE25-3	BVDSSE25-6	BVDSSE25-7	BVDSSE25-8
PS1 (4)	BVDSSE25-9	BVDSSE25-7	BVDSSE25-8	BVDSSE25-9
PS2 (1)	BVDSSE25-4	BVDSSE25-9	BVDSSE25-6	BVDSSE25-6
PS2 (2)	BVDSSE25-5	BVDSSE25-10	BVDSSE25-9	BVDSSE25-7
PS3 (1)	BVDSSE25-7	BVDSSE25-2	BVDSSE25-2	BVDSSE25-1
PS3 (2)	BVDSSE25-8	BVDSSE25-3	BVDSSE25-4	BVDSSE25-5
PS3 (3)	BVDSSE25-10	BVDSSE25-8	BVDSSE25-10	BVDSSE25-10
NS1	BVDSSE25-6	BVDSSE25-5	BVDSSE25-1	BVDSSE25-4

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 - ELISA)

3.2.1 THE PARTICIPANTS

Four laboratories participated in the Bovine Viral Diarrhea (BVD) virology proficiency test, which involved detecting viral antigen in serum samples using ELISA. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509

3.2.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus (IDEXX BVDV Ag/Serum Plus), Batch number AH251. When a sample is send for a PT for the first time ever, 10 aliquots (500µl) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in OD-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDAGVIR_PS1	POS
PT2025BVDAGVIR_PS2	POS
PT2025BVDAGVIR_PS3	POS
PT2025BVDAGVIR_PS4	POS
PT2025BVDAGVIR_NS1	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate using the same ELISA as the pre-PT. The samples PS1, PS2, PS3 and NS1 were deemed stable. However, the test results of sample PS4 were inconsistent: some indicated a positive result, others negative. Therefore this sample was classified as non-interpretable, meaning both positive and negative participant responses are accepted as correct.

Sample content	Expected result
PT2025BVDAGVIR_PS1	POS
PT2025BVDAGVIR_PS2	POS
PT2025BVDAGVIR_PS3	POS
PT2025BVDAGVIR_PS4	POS/NEG/NI
PT2025BVDAGVIR_NS1	NEG

(POS = positive; NEG = negative; NI = not interpretable)

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: PT2025BVDAGVIR_	97505	97507	97508	97509
PS1 (1)	BVDVSE25-4	BVDVSE25-2	BVDVSE25-4	BVDVSE25-2
PS1 (2)	BVDVSE25-5	BVDVSE25-7	BVDVSE25-8	BVDVSE25-4
PS2	BVDVSE25-8	BVDVSE25-1	BVDVSE25-9	BVDVSE25-10
PS3 (1)	BVDVSE25-2	BVDVSE25-4	BVDVSE25-1	BVDVSE25-5
PS3 (2)	BVDVSE25-9	BVDVSE25-8	BVDVSE25-3	BVDVSE25-7
PS4 (1)	BVDVSE25-1	BVDVSE25-6	BVDVSE25-6	BVDVSE25-3
PS4 (2)	BVDVSE25-7	BVDVSE25-10	BVDVSE25-7	BVDVSE25-8
NS1 (1)	BVDVSE25-6	BVDVSE25-3	BVDVSE25-5	BVDVSE25-6
NS1 (2)	BVDVSE25-10	BVDVSE25-9	BVDVSE25-10	BVDVSE25-9
NS2	BVDVSE25-3	BVDVSE25-5	BVDVSE25-2	BVDVSE25-1

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%.

3.3 Virology (serum - PCR)

3.3.1 THE PARTICIPANTS

Five laboratories took part in the BVD virology proficiency test using PCR for viral RNA detection in serum samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97514
- 97534

3.3.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.3.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the inhouse qPCR-method, able to differentiate between BVD1 and BVD2. When a sample is send for a PT for the first time ever, 10 aliquots (500 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in CT-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.3.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDVIR_PS1	POS
PT2025BVDVIR_PS2	POS
PT2025BVDVIR_PS3	POS
PT2025BVDVIR_PS4	POS
PT2025BVDVIR_NS1	NEG
PT2025BVDVIR_NS2	NEG

(POS = positive; NEG = negative)

3.3.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate. The samples were deemed stable.

3.3.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2025 BVDVIR_	97505	97507	97508	97514	97534
PS1	BVDVSP25-5	BVDVSP25-5	BVDVSP25-4	BVDVSP25-5	BVDVSP25-3
PS2 (1)	BVDVSP25-2	BVDVSP25-2	BVDVSP25-8	BVDVSP25-2	BVDVSP25-1
PS2 (2)	BVDVSP25-6	BVDVSP25-8	BVDVSP25-10	BVDVSP25-3	BVDVSP25-2
PS3 (1)	BVDVSP25-1	BVDVSP25-7	BVDVSP25-2	BVDVSP25-4	BVDVSP25-7
PS3 (2)	BVDVSP25-3	BVDVSP25-10	BVDVSP25-6	BVDVSP25-8	BVDVSP25-10
PS4 (1)	BVDVSP25-4	BVDVSP25-1	BVDVSP25-1	BVDVSP25-7	BVDVSP25-6
PS4 (2)	BVDVSP25-10	BVDVSP25-9	BVDVSP25-5	BVDVSP25-9	BVDVSP25-8
NS1 (1)	BVDVSP25-7	BVDVSP25-4	BVDVSP25-3	BVDVSP25-1	BVDVSP25-4
NS1 (2)	BVDVSP25-9	BVDVSP25-6	BVDVSP25-7	BVDVSP25-10	BVDVSP25-9
NS2	BVDVSP25-8	BVDVSP25-3	BVDVSP25-9	BVDVSP25-6	BVDVSP25-5

3.3.7 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%.

3.4 Virology (blood - ELISA)

3.4.1 THE PARTICIPANTS

Four laboratories participated in a virology proficiency test for BVD antigen detection in whole blood using the ELISA method. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509

3.4.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.4.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus (IDEXX BVDV Ag/Serum Plus), Batch number AH251. When a sample is send for a PT for the first time ever, 10 aliquots (500 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in OD-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.4.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDAgVIR_PB1	POS
PT2025BVDAgVIR_PB2	POS
PT2025BVDAgVIR_PB3	POS
PT2025BVDAgVIR_NB1	NEG
PT2025BVDAgVIR_NB2	NEG

(POS = positive; NEG = negative)

3.4.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate using the same ELISA as the pre-PT. The samples were deemed stable.

3.4.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2025BVDAgVIR_	97505	97507	97508	97509
PB1 (1)	BVDVBE25-1	BVDVBE25-1	BVDVBE25-6	BVDVBE25-2
PB1 (2)	BVDVBE25-4	BVDVBE25-4	BVDVBE25-8	BVDVBE25-9
PB2 (1)	BVDVBE25-2	BVDVBE25-5	BVDVBE25-2	BVDVBE25-1
PB2 (2)	BVDVBE25-3	BVDVBE25-8	BVDVBE25-9	BVDVBE25-3
PB3 (1)	BVDVBE25-7	BVDVBE25-7	BVDVBE25-5	BVDVBE25-5
PB3 (2)	BVDVBE25-8	BVDVBE25-9	BVDVBE25-10	BVDVBE25-10
NB1 (1)	BVDVBE25-5	BVDVBE25-2	BVDVBE25-4	BVDVBE25-4
NB1 (1)	BVDVBE25-9	BVDVBE25-6	BVDVBE25-7	BVDVBE25-6
NB2 (1)	BVDVBE25-6	BVDVBE25-3	BVDVBE25-1	BVDVBE25-7
NB2 (2)	BVDVBE25-10	BVDVBE25-10	BVDVBE25-3	BVDVBE25-8

3.4.7 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%.

3.5 Virology (blood - PCR)

3.5.1 THE PARTICIPANTS

Six laboratories took part in the BVD virology proficiency test using PCR to detect viral RNA in whole blood samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97514
- 97516
- 97534

3.5.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.5.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the inhouse qPCR-method, able to differentiate between BVD1 and BVD2. When a sample is send for a PT for the first time ever, 10 aliquots (500 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in CT-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.5.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDVIR_PB1	POS
PT2025BVDVIR_PB2	POS
PT2025BVDVIR_PB3	POS
PT2025BVDVIR_PB4	POS
PT2025BVDVIR_NB1	NEG

(POS = positive; NEG = negative)

3.5.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate. The samples were deemed stable.

3.5.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2025BVDVIR_	97505	97507	97508	97514
PB1 (1)	BVDVBP25-1	BVDVBP25-7	BVDVBP25-1	BVDVBP25-1
PB1 (2)	BVDVBP25-3	BVDVBP25-8	BVDVBP25-10	BVDVBP25-7
PB2 (1)	BVDVBP25-2	BVDVBP25-3	BVDVBP25-6	BVDVBP25-3
PB2 (2)	BVDVBP25-6	BVDVBP25-5	BVDVBP25-8	BVDVBP25-9
PB3 (1)	BVDVBP25-5	BVDVBP25-2	BVDVBP25-3	BVDVBP25-4
PB3 (2)	BVDVBP25-7	BVDVBP25-4	BVDVBP25-7	BVDVBP25-6
PB4 (1)	BVDVBP25-4	BVDVBP25-1	BVDVBP25-2	BVDVBP25-5
PB4 (2)	BVDVBP25-9	BVDVBP25-6	BVDVBP25-5	BVDVBP25-10
NB1 (1)	BVDVBP25-8	BVDVBP25-9	BVDVBP25-4	BVDVBP25-2
NB1 (2)	BVDVBP25-10	BVDVBP25-10	BVDVBP25-9	BVDVBP25-8

Sample content: PT2025BVDVIR_	97516	97534
PB1 (1)	BVDVBP25-7	BVDVBP25-6
PB1 (2)	BVDVBP25-8	BVDVBP25-8
PB2 (1)	BVDVBP25-1	BVDVBP25-1
PB2 (2)	BVDVBP25-4	BVDVBP25-10
PB3 (1)	BVDVBP25-6	BVDVBP25-4
PB3 (2)	BVDVBP25-9	BVDVBP25-7
PB4 (1)	BVDVBP25-3	BVDVBP25-5
PB4 (2)	BVDVBP25-10	BVDVBP25-9
NB1 (1)	BVDVBP25-2	BVDVBP25-2
NB1 (2)	BVDVBP25-5	BVDVBP25-3

3.5.7 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%.

3.6 Virology (ear notch - ELISA)

3.6.1 THE PARTICIPANTS

Five laboratories participated in a BVD virology proficiency test for antigen detection in ear notch samples using the ELISA method. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509
- 97513

3.6.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.6.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the inhouse qPCR-method, able to differentiate between BVD1 and BVD2. When a sample is send for a PT for the first time ever, 10 aliquots (500 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in CT-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.6.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDAGVIR_PE1	POS
PT2025BVDAGVIR_PE2	POS
PT2025BVDAGVIR_PE3	POS
PT2025BVDAGVIR_PE4	POS
PT2025BVDAGVIR_PE5	POS
PT2025BVDAGVIR_NE1	NEG
PT2025BVDAGVIR_NE2	NEG
PT2025BVDAGVIR_NE3	NEG

Sample content	Expected result
PT2025BVDAGVIR_NE4	NEG
PT2025BVDAGVIR_NE5	NEG

(POS = positive; NEG = negative)

* = The positive sample PS6 represents a positive sample diluted to the limit of detection, implying that the result can be doubtful. Therefore, for this sample, POS. NEG or NI are accepted as correct results.

3.6.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate using the same ELISA as the pre-PT. The samples were deemed stable.

3.6.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2025 BVDAGVIR_	97505	97507	97508	97509	97513
PE1	BVDVENE25-6	BVDVENE25-6	BVDVENE25-9	BVDVENE25-1	BVDVENE25-9
PE2	BVDVENE25-1	BVDVENE25-2	BVDVENE25-5	BVDVENE25-6	BVDVENE25-4
PE3	BVDVENE25-5	BVDVENE25-4	BVDVENE25-2	BVDVENE25-7	BVDVENE25-6
PE4	BVDVENE25-8	BVDVENE25-10	BVDVENE25-4	BVDVENE25-3	BVDVENE25-2
PE5	BVDVENE25-3	BVDVENE25-7	BVDVENE25-10	BVDVENE25-9	BVDVENE25-10
NE1	BVDVENE25-7	BVDVENE25-9	BVDVENE25-7	BVDVENE25-4	BVDVENE25-3
NE2	BVDVENE25-9	BVDVENE25-3	BVDVENE25-8	BVDVENE25-8	BVDVENE25-7
NE3	BVDVENE25-10	BVDVENE25-8	BVDVENE25-3	BVDVENE25-2	BVDVENE25-8
NE4	BVDVENE25-2	BVDVENE25-5	BVDVENE25-1	BVDVENE25-5	BVDVENE25-5
NE5	BVDVENE25-4	BVDVENE25-1	BVDVENE25-6	BVDVENE25-10	BVDVENE25-1

3.6.7 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%.

3.7 Virology (ear notch - PCR)

3.7.1 THE PARTICIPANTS

Seven laboratories took part in the BVD virology proficiency test using PCR for viral RNA detection in ear notch samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97513
- 97514
- 97516
- 97534

3.7.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral Re-emerging Enzootic and Bee Diseases' in the department of 'Infectious diseases in animals Directorate', prepared the samples.

All samples are Belgian field samples, detected during the routine surveillance of BVD in function of the national eradication program, collected and stored frozen before aliquotation and transport.

3.7.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using the inhouse qPCR-method, able to differentiate between BVD1 and BVD2. When a sample is send for a PT for the first time ever, 10 aliquots (500 µL) are tested on the same day. When samples were used in a previous PT, three aliquots (500 µL each) of each sample are tested, both before and after the PT, via ELISA. the NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

The criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the positive values is < 10%. For negative samples the 10% rule does not always apply, as small differences in CT-value can easily result in higher CV%. The most important here is that the qualitative interpretation remains the same across all tested aliquots.

3.7.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2025BVDVIR_PE1	POS
PT2025BVDVIR_PE2	POS
PT2025BVDVIR_PE3	POS
PT2025BVDVIR_PE4	POS
PT2025BVDVIR_PE5	POS
PT2025BVDVIR_NE1	NEG
PT2025BVDVIR_NE2	NEG

Sample content	Expected result
PT2025BVDVIR_NE3	NEG
PT2025BVDVIR_NE4	NEG
PT2025BVDVIR_NE5	NEG

(POS = positive; NEG = negative)

3.7.5 STABILITY

The criteria for stability is that the status of the sample in post-PT remains the status assigned in pre-PT test. The post-PT was blinded by the NRL and performed in triplicate. The samples were deemed stable.

3.7.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2025BVDVIR_	97505	97507	97508	97513
PE1	BVDVENP25-2	BVDVENP25-10	BVDVENP25-10	BVDVENP25-6
PE2	BVDVENP25-10	BVDVENP25-4	BVDVENP25-5	BVDVENP25-5
PE3	BVDVENP25-7	BVDVENP25-9	BVDVENP25-7	BVDVENP25-2
PE4	BVDVENP25-4	BVDVENP25-6	BVDVENP25-9	BVDVENP25-4
PE5	BVDVENP25-1	BVDVENP25-2	BVDVENP25-1	BVDVENP25-9
NE1	BVDVENP25-5	BVDVENP25-7	BVDVENP25-2	BVDVENP25-8
NE2	BVDVENP25-8	BVDVENP25-5	BVDVENP25-3	BVDVENP25-7
NE3	BVDVENP25-9	BVDVENP25-1	BVDVENP25-8	BVDVENP25-10
NE4	BVDVENP25-6	BVDVENP25-8	BVDVENP25-4	BVDVENP25-3
NE5	BVDVENP25-3	BVDVENP25-3	BVDVENP25-6	BVDVENP25-1

Sample content: PT2025BVDVIR_	97514	97516	97534
PE1	BVDVENP25-7	BVDVENP25-1	BVDVENP25-1
PE2	BVDVENP25-2	BVDVENP25-2	BVDVENP25-8
PE3	BVDVENP25-8	BVDVENP25-5	BVDVENP25-2
PE4	BVDVENP25-9	BVDVENP25-10	BVDVENP25-4
PE5	BVDVENP25-5	BVDVENP25-7	BVDVENP25-6
NE1	BVDVENP25-1	BVDVENP25-6	BVDVENP25-5
NE2	BVDVENP25-4	BVDVENP25-9	BVDVENP25-7

Sample content: PT2025BVDVIR_	97514	97516	97534
NE3	BVDVENP25-10	BVDVENP25-3	BVDVENP25-3
NE4	BVDVENP25-6	BVDVENP25-8	BVDVENP25-10
NE5	BVDVENP25-3	BVDVENP25-4	BVDVENP25-9

3.7.7 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 April 3, 2025. The samples were then sent to the participants on April 14, 2025. The deadline for submitting the results was set for May 9, 2025. All participants submitted their results on time. Finally, the individual reports were provided to the participants on July 2, 2025.

5 RESULTS

5.1 Serology (serum – ELISA)

5.1.1 RESULTS PER SAMPLE

The panel consisted of four distinct samples. However, sample PS2 was included twice, PS3 three times, and PS1 four times. As a result, the panel comprised a total of ten samples.

One laboratory 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	Expected results	Total results	Observed results
PS1	POS	36	30 POS 1 NEG 5 NI
PS2	POS	18	18 POS
PS3	POS	27	27 POS
NS1	NEG	9	9 NEG

(POS = positive; NEG = negative, NI = not interpretable)

5.1.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Competition	Bio-X Diagnostics	Monoscreen Ab ELISA BVD	**3**	**30**	**30**	**100**
ELISA Competition	IDVet	ID screen BVD p80 antibody competition	**4**	**40**	**34**	**85**
ELISA Competition	Thermo Fisher Scientific	PrioCHECK Bovine BVDV Ab Plate Kit	**1**	**10**	**10**	**100**
ELISA Indirect	IDEXX	BVDV Ab X3	**1**	**10**	**10**	**100**
TOTAL			**9**	**90**	**84**	**93**

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

5.1.3 CONCLUSION

In 2025, eight laboratories participated in the proficiency test Bovine Viral Diarrhea serology (serum - ELISA) organised 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.

One laboratory, which submitted two datasets, scored 70% in each test. This laboratory used the ID Screen® BVD p80 Antibody Competition ELISA from IDvet for both analyses. The only difference was the incubation protocol: a short protocol was used in the first run, and a long protocol in the second. Three out of four PS1 samples were reported as either non-interpretable or negative in both runs. Additionally, a comment was added stating that three samples had a doubtful interpretation. These results were marked as incorrect, as the samples in question are not borderline or near the cut-off. In contrast, the two other laboratories that used the same test kit achieved a perfect score of 100%.

The other five laboratories achieved the maximum score of 100%, resulting in an overall accuracy of 93% across all participants.

5.2 Virology (serum – ELISA)

5.2.1 RESULTS PER SAMPLE

The panel consisted of six distinct samples. However, samples PS1, PS3, PS4 and NS1 were included twice. As a result, the panel comprised a total of ten samples.

Sample content	Expected results	Total results	Observed results
PS1	POS	8	8 POS
PS2	POS	4	4 POS
PS3	POS	8	8 POS
PS4	POS/NEG/NI	8	4 POS / 4 NEG
NS1	NEG	8	8 NEG
NS2	NEG	4	4 NEG

(POS = positive; NEG = negative, NI = not interpretable)

5.2.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Antigen	IDEXX	BVDV Ag/Serum Plus Test	**4**	**40**	**40**	**100**
TOTAL			**4**	**40**	**40**	**100**

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

5.2.3 CONCLUSION

In 2025, four laboratories participated in the proficiency test Bovine Viral Diarrhea virology (serum – antigen ELISA) organised 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 participating laboratories analysed the samples using the IDEXX BVDV Ag/Serum Plus Test and obtained a maximum score of 100%.

5.3 Virology (serum – PCR)

5.3.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, samples PS2, PS3, PS4 and NS1 were replicated twice. Therefore, the panel included ten samples in total.

Sample content	Expected results	Total results	Observed results
PS1	POS	5	5 POS
PS2	POS	10	10 POS
PS3	POS	10	10 POS
PS4	POS	10	10 POS
NS1	NEG	10	10 NEG
NS2	NEG	5	5 NEG

(POS = positive; NEG = negative)

5.3.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

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

Name producer	Name protocol/kit	N	NR	NCR	%
QIAGEN	QIAamp Viral RNA Mini Kit	**1**	**10**	**10**	**100**
Indical Bioscience	IndiMag Pathogen Kit	**2**	**20**	**20**	**100**
Biosellal	BioExtract SuperBall	**1**	**10**	**10**	**100**
Thermo Fisher Scientific	Extraction MagMA CORE Nucleic Acid Purification Kit	**1**	**10**	**10**	**100**
TOTAL		**5**	**50**	**50**	**100**

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

5.3.3 RESULTS PER USED PCR PROTOCOL/KIT

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

Name producer	Name PCR protocol/kit	N	NR	NCR	%
Homemade	Homemade	**1**	**10**	**10**	**100**
Thermo Fisher Scientific	VetMAX BVDV 4ALL Kit	**2**	**20**	**20**	**100**
Indical Bioscience	Virotype BVDV 2.0 RT PCR Kit	**1**	**10**	**10**	**100**
Biosellal	Bio-T kit BVDV-BDV Universal	**1**	**10**	**10**	**100**
TOTAL		**5**	**50**	**50**	**100**

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

5.3.4 CONCLUSION

In 2025, five laboratories participated in the proficiency test Bovine Viral Diarrhea virology (serum - PCR) organised 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 participating laboratories achieved the maximum score of 100%.

5.4 Virology (blood – ELISA)

5.4.1 RESULTS PER SAMPLE

The panel consisted of five distinct samples. However, all positive and negative samples were included twice. As a result, the panel comprised a total of ten samples.

Sample content	Expected results	Total results	Observed results
PB1	POS	8	8 POS
PB2	POS	8	8 POS
PB3	POS	8	8 POS
NB1	NEG	8	8 NEG
NB2	NEG	8	8 NEG

(POS = positive; NEG = negative)

5.4.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Antigen	IDEXX	BVDV Ag/Serum Plus Test	**4**	**40**	**40**	**100**
TOTAL			**4**	**40**	**40**	**100**

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

5.4.3 CONCLUSION

In 2025, four laboratories participated in the proficiency test Bovine Viral Diarrhea virology (blood – ELISA) organised 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 participating laboratories analysed the samples using the IDEXX BVDV Ag/Serum Plus Test and obtained a maximum score of 100%.

5.5 Virology (blood - PCR)

5.5.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, all negative and positive samples included in the panel were replicated twice. Therefore, the panel included ten samples in total.

Sample content	Expected results	Total results	Observed results
PB1	POS	12	12 POS
PB2	POS	12	12 POS
PB3	POS	12	12 POS
PB4	POS	12	12 POS
NB1	NEG	12	12 NEG

(POS = positive; NEG = negative)

5.5.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

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

Name producer	Name protocol/kit	N	NR	NCR	%
QIAGEN	QIAamp Viral RNA Mini Kit	**1**	**10**	**10**	**100**
Indical Bioscience	IndiMag Pathogen Kit	**2**	**20**	**20**	**100**
Biosellal	BioExtract SuperBall	**1**	**10**	**10**	**100**
IDVet	ID Gene Mag Universal Extraction kit	**1**	**10**	**10**	**100**
Thermo Fisher Scientifi	Extraction MagMAX™ CORE Nucleic Acid Purification Kit	**1**	**10**	**10**	**100**
TOTAL		**6**	**60**	**60**	**100**

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

5.5.3 RESULTS PER USED PCR PROTOCOL/KIT

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

Name producer	Name PCR protocol/kit	N	NR	NCR	%
Homemade	Homemade	**1**	**10**	**10**	**100**
Thermo Fisher Scientific	VetMAX BVDV 4ALL Kit	**2**	**20**	**20**	**100**
Indical Bioscience	Virotype BVDV 2.0 RT PCR Kit	**1**	**10**	**10**	**100**
Biosellal	Bio-T kit® BVDV-BDV Universal	**1**	**10**	**10**	**100**
IDVet	ID Gene™ BVDV/BDV Triplex 2.0	**1**	**10**	**10**	**100**
TOTAL		**6**	**60**	**60**	**100**

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

5.5.4 CONCLUSION

In 2025, six laboratories participated in the proficiency test Bovine Viral Diarrhea virology (blood – PCR) organised 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 participating laboratories achieved the maximum score of 100%.

5.6 Virology (ear notch – ELISA)

5.6.1 RESULTS PER SAMPLE

The panel consisted of ten unique samples, with no repetitions included.

Sample content	Expected results	Total results	Observed results
PE1	POS	5	5 POS
PE2	POS	5	5 POS
PE3	POS	5	5 POS
PE4	POS	5	5 POS
PE5	POS	5	5 POS
NE1	NEG	5	5 NEG
NE2	NEG	5	5 NEG
NE3	NEG	5	5 NEG
NE4	NEG	5	5 NEG
NE5	NEG	5	5 NEG

(POS = positive; NEG = negative)

5.6.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Antigen	IDEXX	BVDV Ag/Serum Plus Test	**5**	**50**	**50**	**100**
TOTAL			**5**	**50**	**50**	**100**

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

5.6.3 CONCLUSION

In 2025, five laboratories participated in the proficiency test Bovine Viral Diarrhea virology (ear notch – ELISA) organised 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 participating laboratories analysed the samples using the IDEXX BVDV Ag/Serum Plus Test and obtained a maximum score of 100%.

5.7 Virology (ear notch - PCR)

5.7.1 RESULTS PER SAMPLE

The panel consisted of ten unique samples, with no repetitions included.

Sample content	Expected results	Total results	Observed results
PE1	POS	7	7 POS
PE2	POS	7	7 POS
PE3	POS	7	7 POS
PE4	POS	7	7 POS
PE5	POS	7	6 POS 1 NEG
NE1	NEG	7	7 NEG
NE2	NEG	7	7 NEG
NE3	NEG	7	7 NEG
NE4	NEG	7	7 NEG
NE5	NEG	7	7 NEG

(POS = positive; NEG = negative)

5.7.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

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

Name producer	Name extraction protocol/kit	N	NR	NCR	%
QIAGEN	RNeasy Mini Kit	**1**	**10**	**10**	**100**
Indical Bioscience	IndiMag Pathogen Kit	**2**	**20**	**19**	**95**
Bio-X Diagnostics	ADIAMAG XL	**1**	**10**	**10**	**100**
Biosellal	BioExtract SuperBall	**1**	**10**	**10**	**100**
IDVet	ID Gene Mag Universal Extraction kit	**1**	**10**	**10**	**100**
Thermo Fisher Scientific	MagMAX Core Nucleic Acid Purification Kit	**1**	**10**	**10**	**100**
TOTAL		**7**	**70**	**69**	**99**

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

5.7.3 RESULTS PER USED PCR PROTOCOL/KIT

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

Name producer	Name PCR protocol/kit	N	NR	NCR	%
Homemade	Homemade	**1**	**10**	**10**	**100**
Thermo Fisher Scientific	LSI VetMAX BVDV 4ALL Detection Kit	**2**	**20**	**19**	**95**
QIAGEN	BVDV RT-PCR Kit	**1**	**10**	**10**	**100**
BioX-Adiagene	Adiavet BVD RealTime	**1**	**10**	**10**	**100**
Biosellal	Bio-T kit BVDV/BDV Universal	**1**	**10**	**10**	**100**
IDVet	ID Gene™ BVDV/BDV Triplex 2.0	**1**	**10**	**10**	**100**
TOTAL		**7**	**70**	**69**	**99**

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

5.7.4 CONCLUSION

In 2025, seven laboratories participated in the proficiency test Bovine Viral Diarrhea serology (ear notch – PCR) organised 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.

One laboratory scored 90% in each test. However, it met the minimum performance requirement of 90% and was therefore assessed as satisfactory. The remaining six laboratories achieved the maximum score of 100%, resulting in an overall accuracy of 99% across all participants.

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 - ELISA)

Interpretation and formula details of participant-used kits

Kit		*Formula*	*Interpretation*
Bio-X Diagnostics - Monoscreen Ab ELISA BVD	ELISA Competition	S/P %= ($OD_{neg\ control}$ - OD_{sample}) / $OD_{neg\ control}$) × 100	<50% → Negative ≥50% → Positive
IDVet - ID screen BVD p80 antibody competition	ELISA Competition	PI % = 100 - ((OD_{sample} / $OD_{neg\ control\ mean}$) × 100) S/N % = (OD_{sample} / $OD_{neg\ control\ mean}$) × 100	LAB 97509 with PI% formula ≤50% → Negative 50-60% → Doubtful ≥60% → Positive LAB 97513 with S/N% formula >60% → Negative ≤60% → Positive LAB 97621 with S/N% formula ≥50% → Negative 40-50% → Doubtful ≤40% → Positive
Thermo Fisher - PrioCHECK™ Bovine BVDV Ab Plate Kit	ELISA Competition	PI % = 100 − (corrected OD_{sample} / corrected $OD_{neg\ control}$) × 100 corrected OD = raw OD - mean blank OD	<50% → Negative ≥50% → Positive
IDEXX - BVDV Ab X3	ELISA Indirect	S/P = OD_{sample} / $OD_{pos\ control\ mean}$	< 0.3 → Negative ≥ 0.3 → Positive

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 four replicate samples: PT2025BVDSER-PS1

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_2
Pos control	0,182	0,1695	0,1705	0,117	0,094
Neg control	1,934	2,4935	2,2905	1,438	1,257
OD (1)	0,589	0,485	0,645	0,451	0,453
OD (2)	0,671	0,467	0,574	0,434	0,48
OD (3)	0,675	0,475	0,566	0,473	0,489
OD (4)	0,712	0,594	0,552	0,502	0,462

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3	M_4	M_2	M_2
Pos control	0,564	0,525	0,174	0,124
Neg control	1,51	0,071	1,797	1,867
OD (1)	0,336	0,385	0,712	0,97
OD (2)	0,303	0,295	0,738	0,856
OD (3)	0,313	0,295	0,755	0,911
OD (4)	0,306	0,402	0,835	0,979

M_1 = Bio-X Diagnostics - Monoscreen Ab ELISA BVD; M_2 = IDVet - ID screen BVD p80 antibody competition; M_3 = Thermo Fisher Scientific - Priocheck bovine BVD Ab; M_4 = IDEXX - BVDV Ab X3

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1 (S/P%)	M_1 (S/P%)	M_1 (S/P%)	M_2 (PI%)	M_2 (S/N%)
(1)	69,57	80,549	71,84	68,668	36
(2)	65,31	81,271	74,94	69,795	38
(3)	65,1	80,95	75,289	67,117	39
(4)	63,19	76,178)	75,9	65,062	37
Mean	65,79	79,74	74,49	67,66	37,50
SD	2,69	2,39	1,81	2,05	1,29
CV (%)	4,09	3,00	2,43	3,03	3,44

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3 (PI%)	M_4 (S/P)	M_2 (S/N%)	M_2 (S/N%)
(1)	81	/	39,6	52
(2)	83	/	41	46
(3)	82	/	42	49
(4)	83	/	46	52
Mean	82,25	/	42,15	49,75
SD	0,96	/	2,75	2,87
CV (%)	1,16	/	6,52	5,77

Quantitative results for two replicate samples: PT2025BVDSER-PS2

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_2
Pos control	0,182	0,1695	0,1705	0,117	0,094
Neg control	1,934	2,4935	2,2905	1,438	1,257
OD (1)	0,163	0,119	0,12	0,122	0,123
OD (2)	0,162	0,121	0,126	0,131	0,129

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3	M_4	M_2	M_2
Pos control	0,564	0,525	0,174	0,124
Neg control	1,51	0,071	1,797	1,867
OD (1)	0,061	2,05	0,217	0,159
OD (2)	0,06	1,978	0,197	0,159

M_1 = Bio-X Diagnostics - Monoscreen Ab ELISA BVD; M_2 = IDVet - ID screen BVD p80 antibody competition; M_3 = Thermo Fisher Scientific - Priocheck bovine BVD Ab; M_4 = IDEXX - BVDV Ab X3

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1 (S/P%)	M_1 (S/P%)	M_1 (S/P%)	M_2 (PI%)	M_2 (S/N%)
(1)	91,55	95,228	94,761	91,486	10
(2)	91,61	95,147	94,499	90,921	10
Mean	91,58	95,19	94,63	91,20	10,00
SD	0,04	0,06	0,19	0,40	0,00
CV (%)	0,05	0,06	0,20	0,44	0,00

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3 (PI%)	M_4 (S/P)	M_2 (S/N%)	M_2 (S/N%)
(1)	100	/	12	9
(2)	100	/	11	9
Mean	100,00	/	11,50	9,00
SD	0,00	/	0,71	0,00
CV (%)	0,00	/	6,15	0,00

Quantitative results for three replicate samples: PT2025BVDSER-PS3

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1	M_1	M_1	M_2	M_2
Pos control	0,182	0,1695	0,1705	0,117	0,094
Neg control	1,934	2,4935	2,2905	1,438	1,257
OD (1)	0,373	0,325	0,316	0,235	0,236
OD (2)	0,29	0,306	0,298	0,232	0,229
OD (3)	0,359	0,304	0,296	0,256	0,237

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3	M_4	M_2	M_2
Pos control	0,564	0,525	0,174	0,124
Neg control	1,51	0,071	1,797	1,867
OD (1)	0,248	1,464	0,421	0,421
OD (2)	0,271	1,488	0,431	0,406
OD (3)	0,256	1,431	0,409	0,434

M_1 = Bio-X Diagnostics - Monoscreen Ab ELISA BVD; M_2 = IDVet - ID screen BVD p80 antibody competition; M_3 = Thermo Fisher Scientific - Priocheck bovine BVD Ab; M_4 = IDEXX - BVDV Ab X3

Lab number	97505	97507	97508	97509	97513
Method (ELISA protocol/kit)	M_1 (S/P%)	M_1 (S/P%)	M_1 (S/P%)	M_2 (PI%)	M_2 (S/N%)
(1)	80,7	86,966	86,204	83,666	19
(2)	85	87,728	86,99	83,897	18
(3)	81,45	87,808	87,077	82,177	19
Mean	82,38	87,50	86,76	83,25	18,67
SD	2,30	0,46	0,48	0,93	0,58
CV (%)	2,79	0,53	0,55	1,12	3,09

Lab number	97541	97544	97621 (1)	97621 (2)
Method (ELISA protocol/kit)	M_3 (PI%)	M_4 (S/P)	M_2 (S/N%)	M_2 (S/N%)
(1)	87	/	23	23
(2)	85	/	24	22
(3)	86	/	23	23
Mean	86,00	/	23,33	22,67
SD	1,00	/	0,58	0,58
CV (%)	1,16	/	2,47	2,55

6.1.2 VIROLOGY (SERUM - ELISA)

Interpretation and formula details of participant-used kits

Kit	*Formula*	*Interpretation*
IDEXX - BVDV Ag/Serum Plus Test	S-N = OD_{sample} - $OD_{mean\ neg\ control}$	S-N ≤ 0.3 → Negative S-N > 0.3 → Positive

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

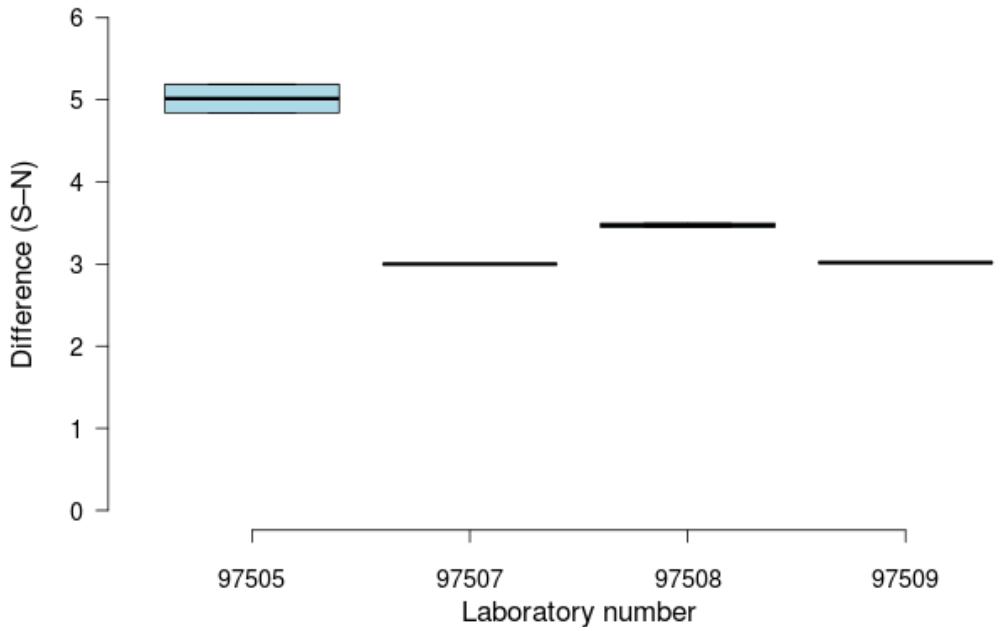
CV(%)	*Interpretation*
≤ 10%	Excellent reproducibility
10% – 15%	Acceptable, but moderate variation
15% – 20%	Questionable, should be reviewed
> 20%	Poor reproducibility - not reliable

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 two replicate samples: PT2025BVDAgVIR-PS1

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	5,2205	3,136	3,543	3,1
OD (2)	4,8703	3,135	3,493	3,1

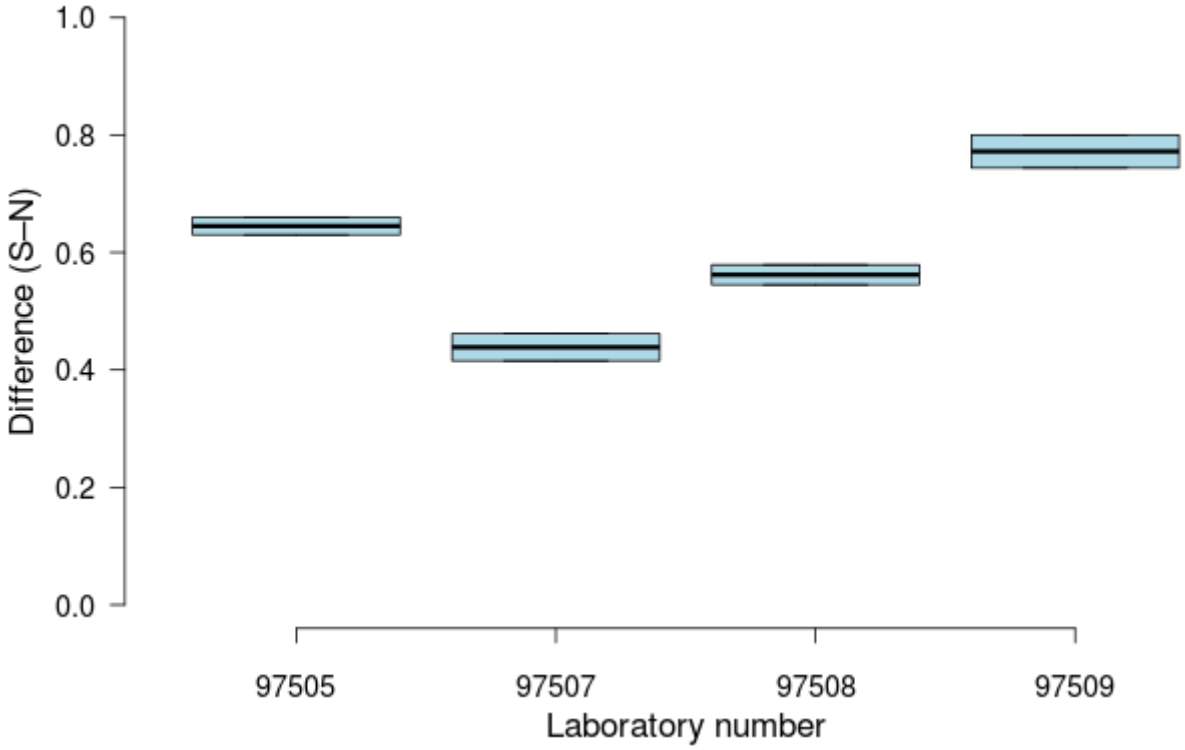
Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	5,19	3,0005	3,497	3,017
S-N (2)	4,84	2,9995	3,447	3,017
Mean	5,015	3	3,472	3,017
SD	0,247	0,00071	0,0354	0
CV (%)	4,935	0,0236	1,018	0



Quantitative results for two replicate samples: PT2025BVDAgVIR-PS3

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	0,6593	0,551	0,625	0,827
OD (2)	0,6887	0,597	0,591	0,883

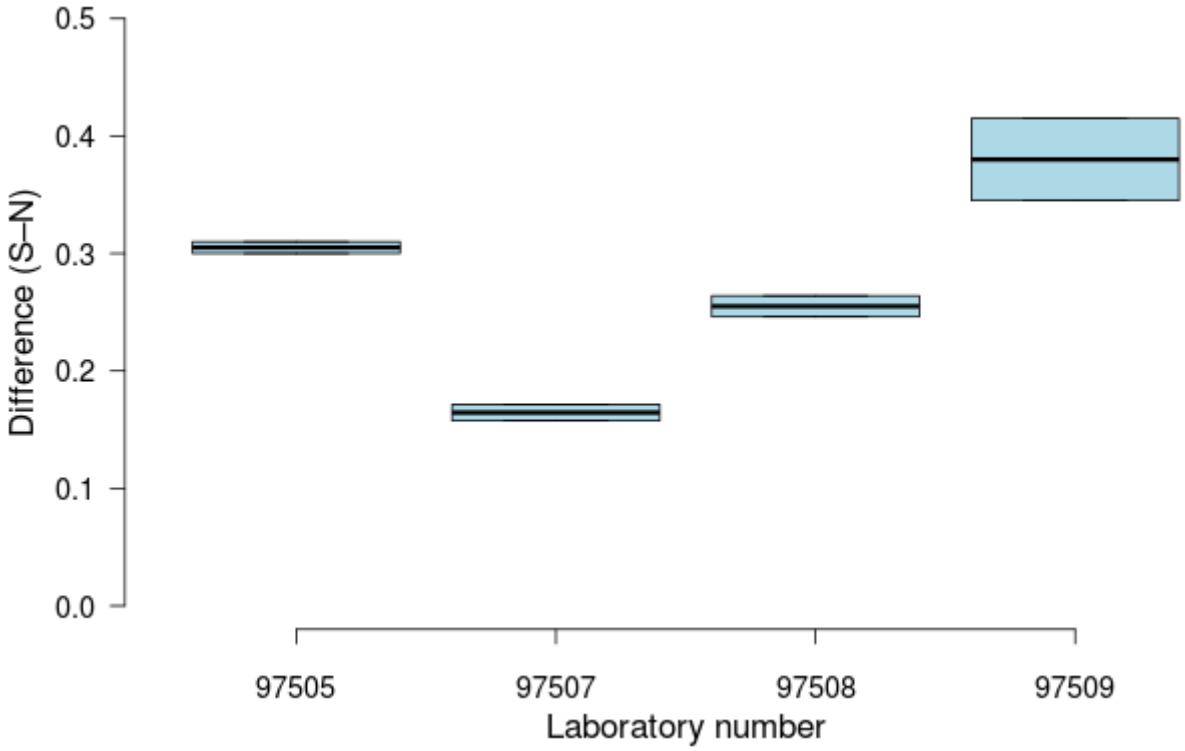
Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	0,63	0,4155	0,579	0,744
S-N (2)	0,66	0,4615	0,545	0,8
Mean	0,645	0,439	0,562	0,772
SD	0,021	0,033	0,024	0,040
CV (%)	3,289	7,418	4,278	5,129



Quantitative results for two replicate samples: PT2025BVDAgVIR-PS4

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	0,3276	0,293	0,292	0,428
OD (2)	0,3351	0,307	0,31	0,499

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	0,30	0,1575	0,246	0,345
S-N (2)	0,31	0,1715	0,264	0,415
Mean	0,305	0,165	0,255	0,380
SD	0,007	0,010	0,013	0,049
CV (%)	2,318	6,018	4,991	13,026



6.1.3 VIROLOGY (SERUM - PCR)

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

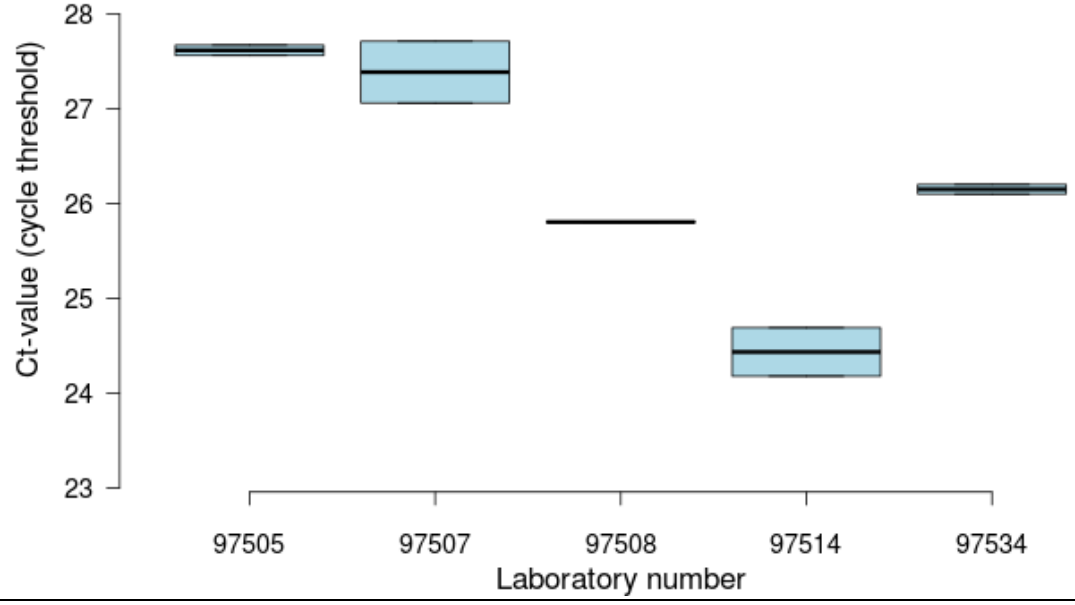
CV(%)	*Interpretation*
< 5%	Excellent – High repeatability and precision between replicates
5% – 10%	Good – Acceptable level of variation
10% – 15%	Moderate – May indicate pipetting error, extraction issues or instrument variability
> 15%	Poor – High variability, results may not be reliable or reproducible

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 two replicate samples: PT2025BVDAgVIR-PS2

Lab number	97505	97507	97508	97514	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_2
Pos control	25,34	27,89	29,33	26,17	27,6
Neg control	45	45	40	40	45
Cut-off for pos result	45	45	34	40	45
Cut-off for neg result	>35	>45	>40	>40	>45
Ct-value (1)	27,67	27,71	25,81	24,18	26,1
Ct-value (2)	27,56	27,06	25,8	24,69	26,2
Mean	27,615	27,385	25,805	24,435	26,150
SD	0,078	0,460	0,007	0,361	0,071
CV (%)	0,282	1,678	0,027	1,476	0,270

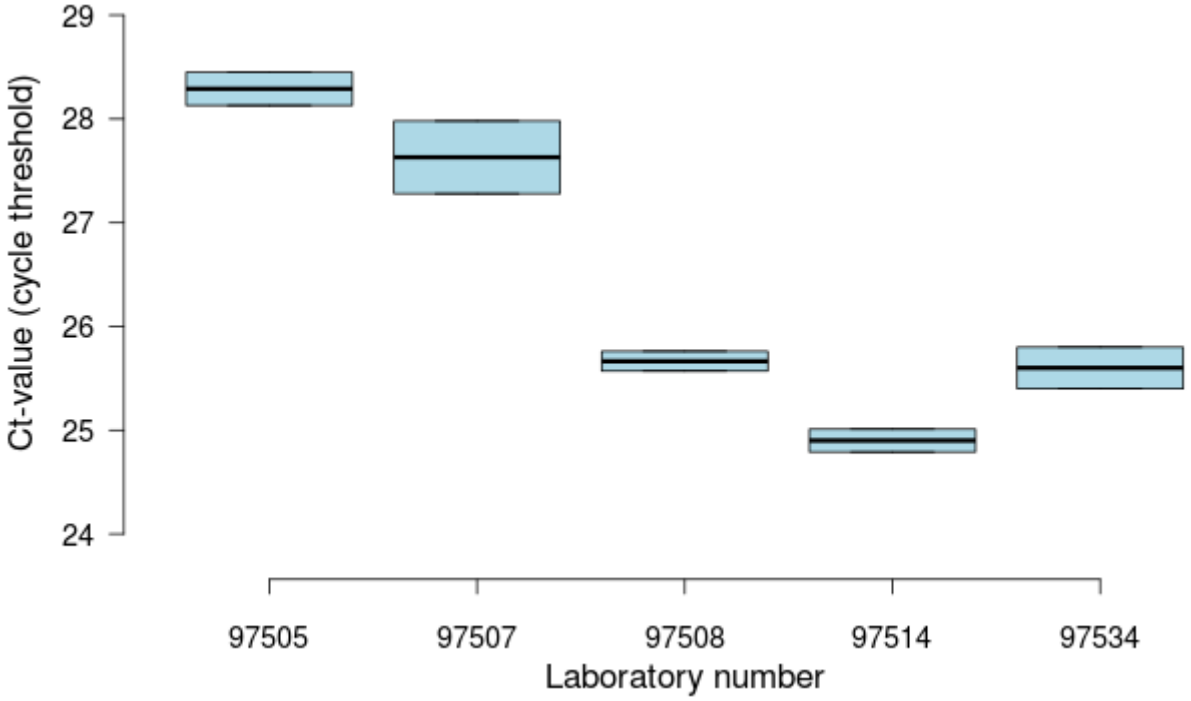
M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal



Quantitative results for two replicate samples: PT2025BVDVIR-PS3

Lab number	97505	97507	97508	97514	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_2
Pos control	25,34	27,89	29,33	26,17	27,6
Neg control	45	45	40	40	45
Cut-off for pos result	45	45	34	40	45
Cut-off for neg result	>35	>45	>40	>40	>45
Ct-value (1)	28,45	27,98	25,57	24,79	25,8
Ct-value (2)	28,13	27,28	25,76	25,01	25,4
Mean	28,290	27,630	25,665	24,900	25,600
SD	0,226	0,495	0,134	0,156	0,283
CV (%)	0,800	1,791	0,523	0,625	1,105

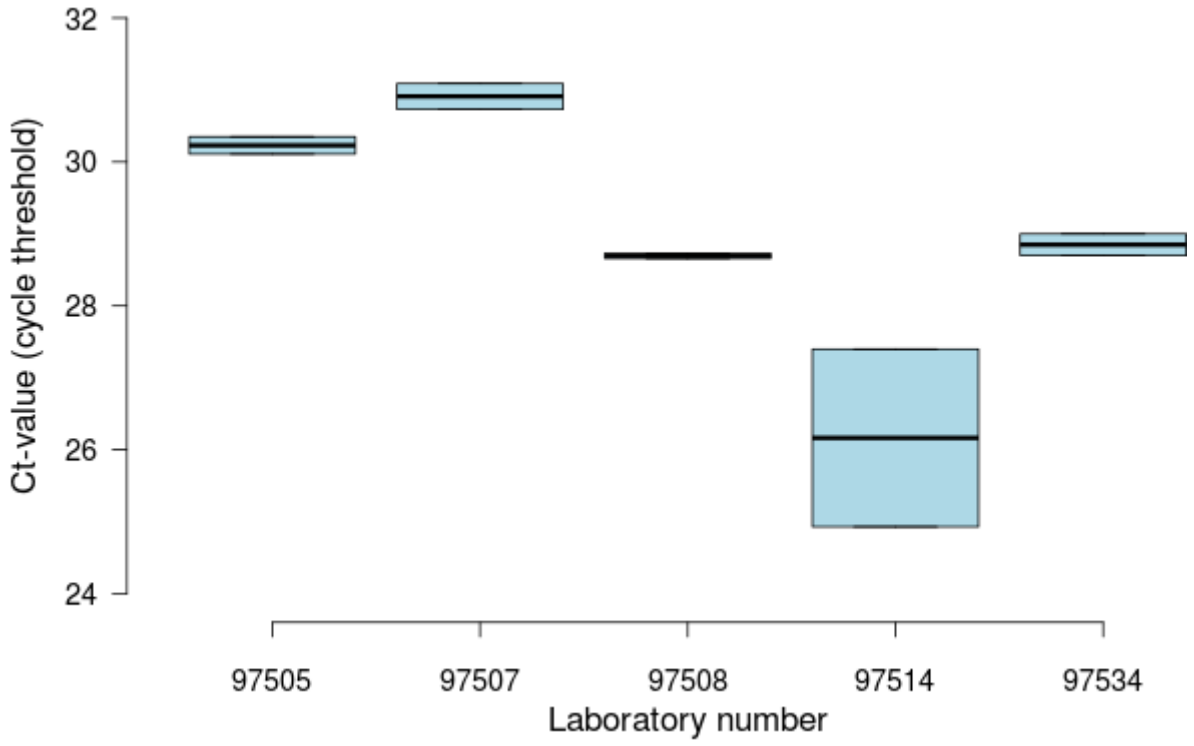
M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal



Quantitative results for two replicate samples: PT2025BVDVIR-PS4

Lab number	97505	97507	97508	97514	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_2
Pos control	25,34	27,89	29,33	26,17	27,6
Neg control	45	45	40	40	45
Cut-off for pos result	45	45	34	40	45
Cut-off for neg result	>35	>45	>40	>40	>45
Ct-value (1)	30,11	30,73	28,73	27,4	29
Ct-value (2)	30,35	31,09	28,66	24,93	28,7
Mean	30,23	30,91	28,695	26,165	28,85
SD	0,170	0,255	0,049	1,747	0,212
CV (%)	0,561	0,824	0,172	6,675	0,735

M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal



6.1.4 VIROLOGY (BLOOD - ELISA)

Interpretation and formula details of participant-used kits

Kit	*Formula*	*Interpretation*
IDEXX - BVDV Ag/Serum Plus Test	S-N = OD_{sample} - $OD_{mean\ neg\ control}$	S-N ≤ 0.3 → Negative S-N > 0.3 → Positive

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

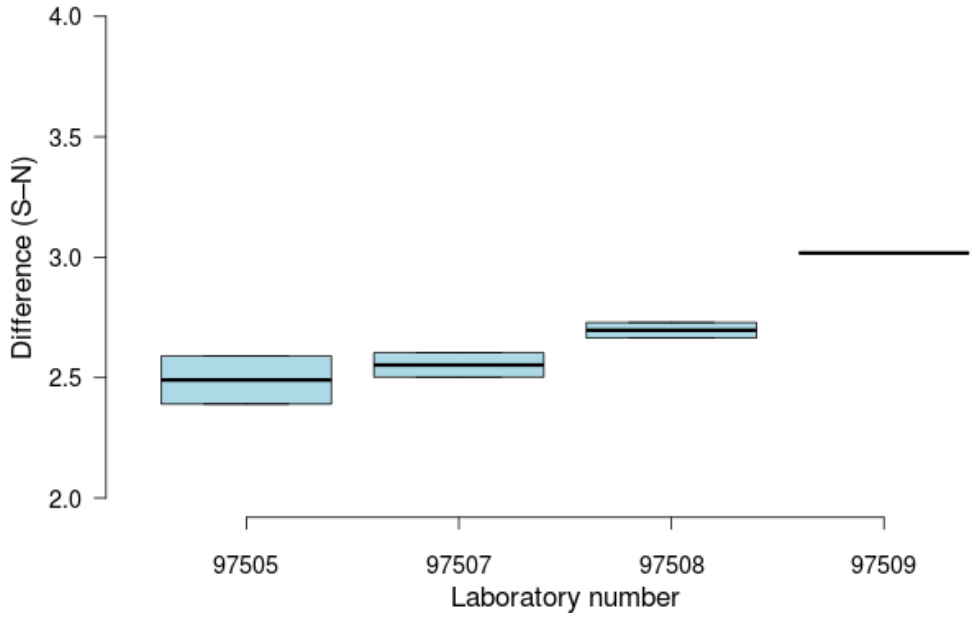
CV(%)	*Interpretation*
≤ 10%	Excellent reproducibility
10% – 15%	Acceptable, but moderate variation
15% – 20%	Questionable, should be reviewed
> 20%	Poor reproducibility - not reliable

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 two replicate samples: PT2025BVDAgVIR-PB1

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	2,6196	2,738	2,775	3,1
OD (2)	2,4185	2,638	2,71	3,1

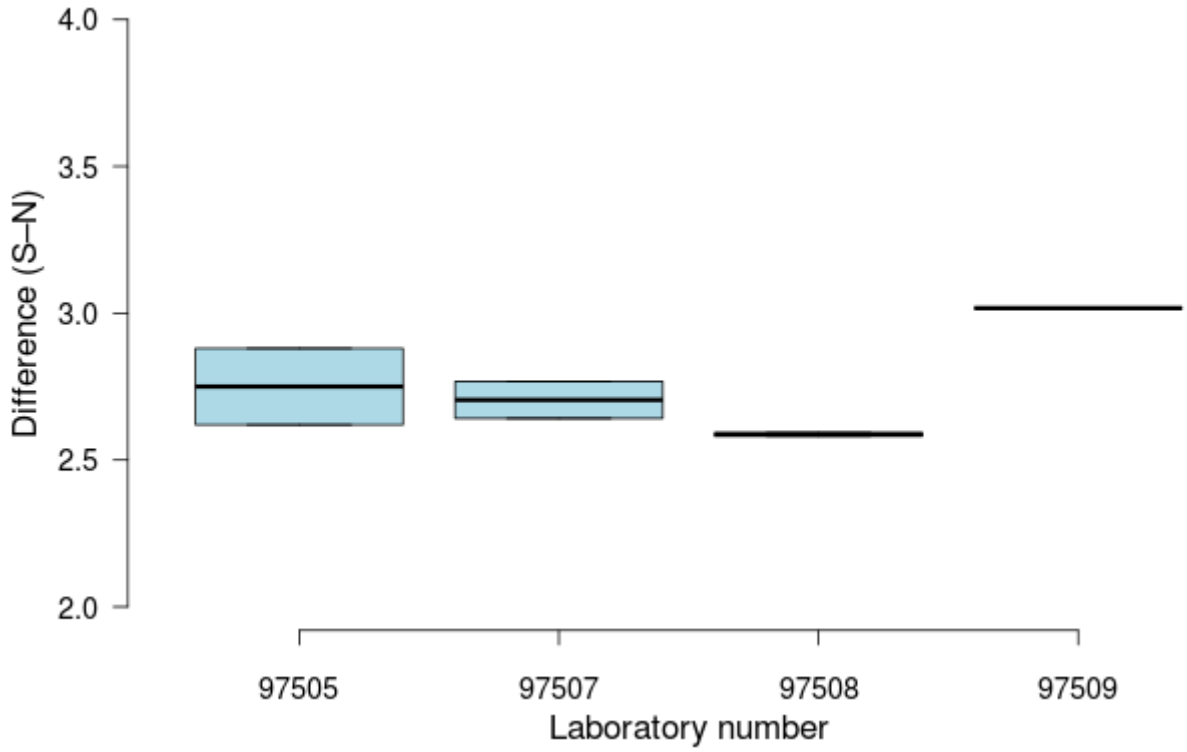
Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	2,59	2,6025	2,729	3,017
S-N (2)	2,39	2,5025	2,664	3,017
Mean	2,490	2,553	2,697	3,017
SD	0,141	0,071	0,046	0
CV (%)	5,680	2,770	1,705	0



Quantitative results for two replicate samples: PT2025BVDAgVIR-PB2

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	2,6515	2,777	2,639	3,1
OD (2)	2,9101	2,903	2,627	3,1

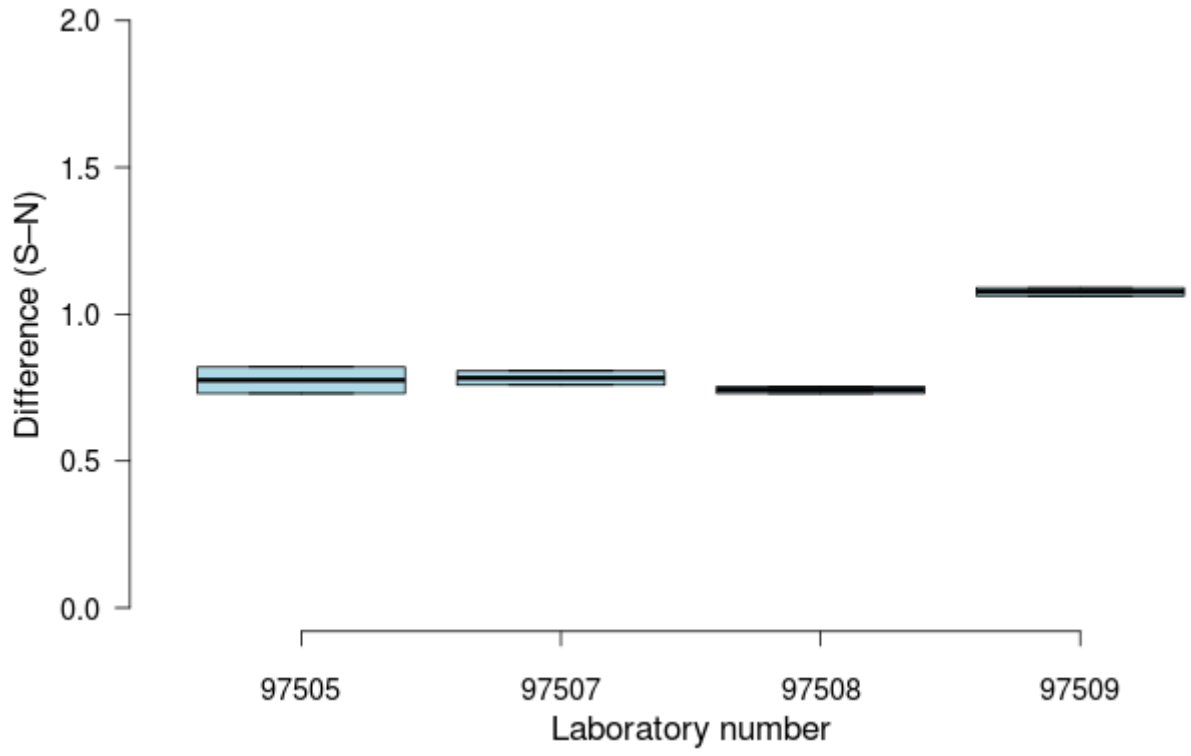
Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	2,62	2,6415	2,593	3,017
S-N (2)	2,88	2,7675	2,581	3,017
Mean	2,750	2,705	2,587	3,017
SD	0,184	0,089	0,008	0
CV (%)	6,685	3,294	0,328	0



Quantitative results for two replicate samples: PT2025BVDAgVIR-PB3

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
Pos control	0,98525	0,9645	1,2075	1,636
Neg control	0,0288	0,1355	0,046	0,084
OD (1)	0,8481	0,894	0,8	1,173
OD (2)	0,7617	0,942	0,776	1,146

Lab number	97505	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - BVDV Ag/Serum Plus Test			
S-N (1)	0,82	0,7585	0,754	1,09
S-N (2)	0,73	0,8065	0,73	1,062
Mean	0,775	0,783	0,742	1,076
SD	0,064	0,034	0,017	0,020
CV (%)	8,212	4,338	2,287	1,840



6.1.5 VIROLOGY (BLOOD - PCR)

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.

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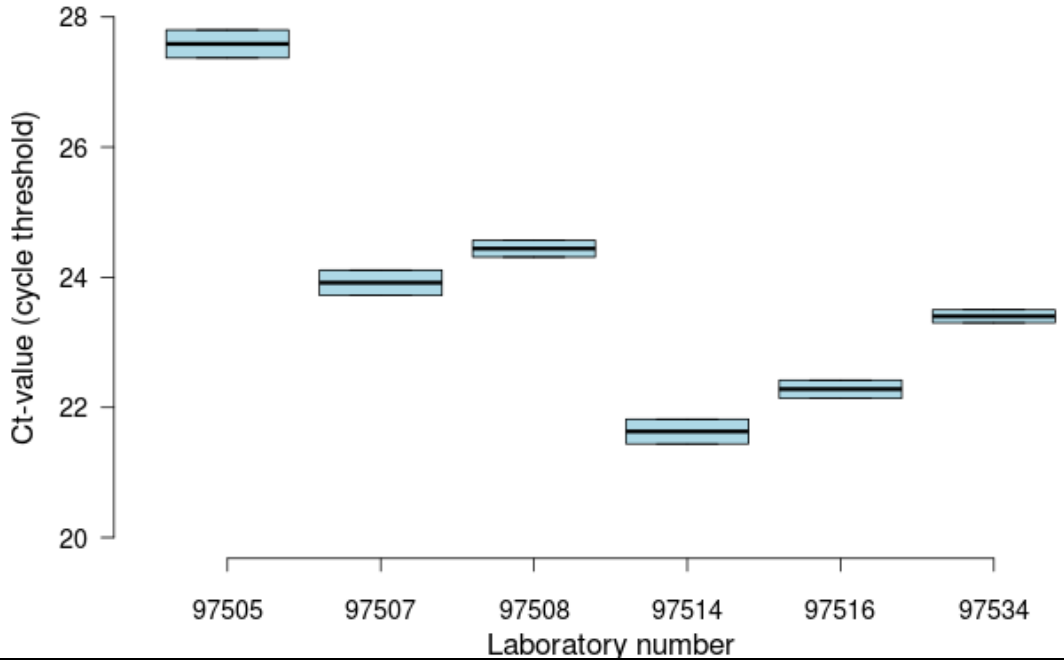
Quantitative results for two replicate samples: PT2025BVDVIR-PB1

Lab number	97505	97507	97508	97514	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_5	M_2
Pos control	25,34	27,89	29,33	26,17	33,66	27,6
Neg control	45	45	40	40	40	45
Cut-off for pos result	35	45	34	40	40	45
Cut-off for neg result	>45	>45	>40	>40	>40	>45
Ct-value (1)	27,37	23,72	24,57	21,44	22,42	23,5
Ct-value (2)	27,8	24,11	24,31	21,82	22,14	23,3
Mean	27,585	23,915	24,44	21,63	22,28	23,40
SD	0,304	0,276	0,184	0,269	0,198	0,141
CV (%)	1,102	1,153	0,752	1,242	0,889	0,604

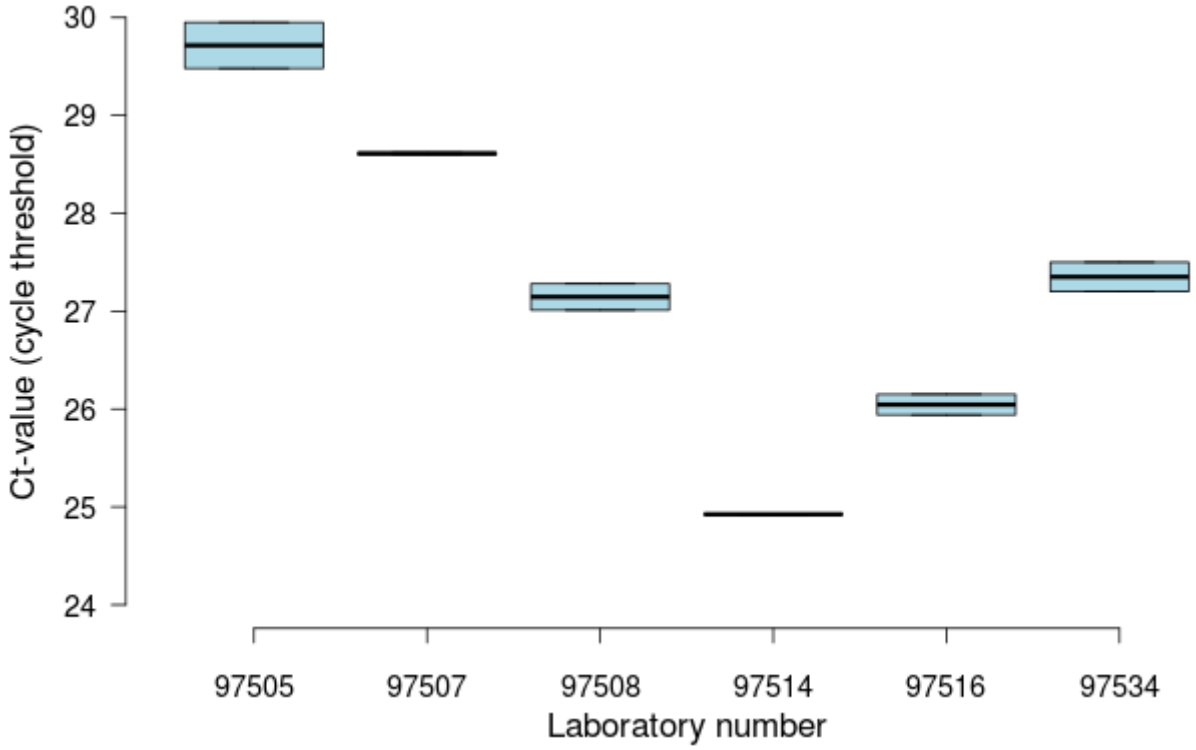
M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal; M_5 = IDVet - ID Gene BVDV/BDV Triplex 2.0



Quantitative results for two replicate samples: PT2025BVDVIR-PB2

Lab number	97505	97507	97508	97514	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_5	M_2
Pos control	25,34	27,89	29,33	26,17	33,66	27,6
Neg control	45	45	40	40	40	45
Cut-off for pos result	35	45	34	40	40	45
Cut-off for neg result	>45	>45	>40	>40	>40	>45
Ct-value (1)	29,95	28,6	27,01	24,93	25,94	27,5
Ct-value (2)	29,48	28,62	27,28	24,92	26,15	27,2
Mean	29,715	28,61	27,145	24,925	26,045	27,35
SD	0,332	0,014	0,191	0,007	0,148	0,212
CV (%)	1,118	0,049	0,703	0,028	0,57	0,776

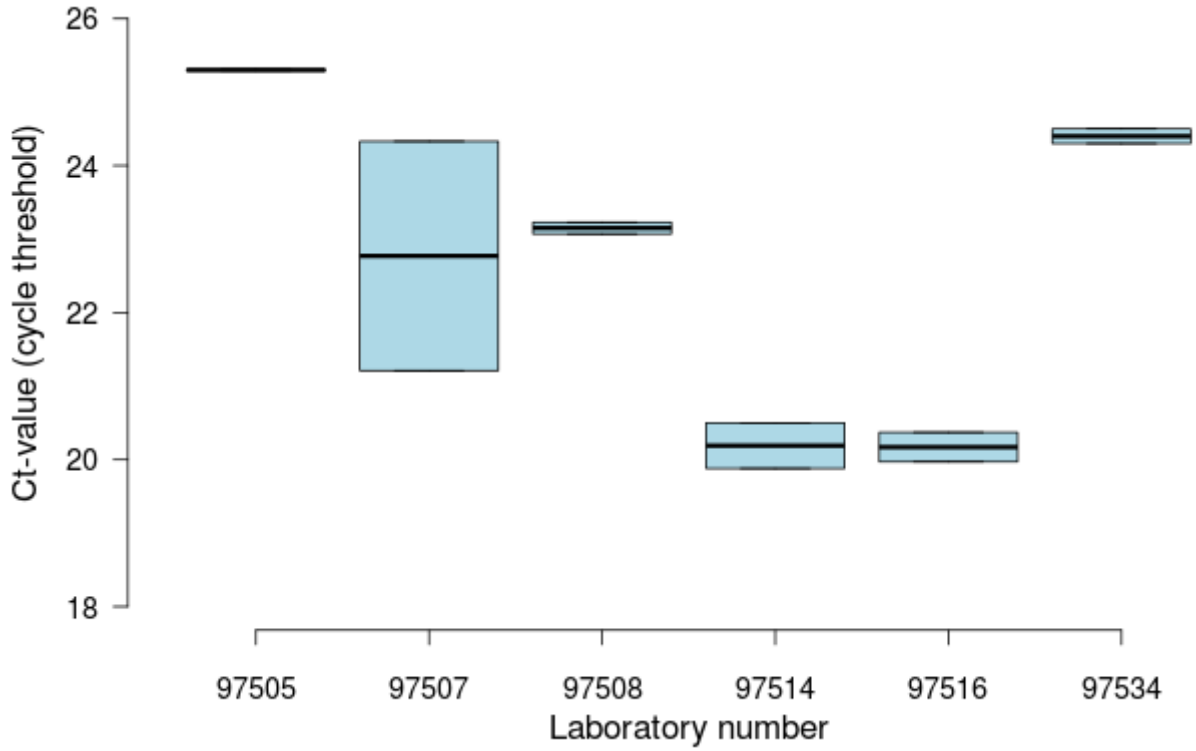
M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal; M_5 = IDVet - ID Gene BVDV/BDV Triplex 2.0



Quantitative results for two replicate samples: PT2025BVDVIR-PB3

Lab number	97505	97507	97508	97514	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_5	M_2
Pos control	25,34	27,89	29,33	26,17	33,66	27,6
Neg control	45	45	40	40	40	45
Cut-off for pos result	35	45	34	40	40	45
Cut-off for neg result	>45	>45	>40	>40	>40	>45
Ct-value (1)	25,29	24,33	23,23	19,88	19,97	24,3
Ct-value (2)	25,31	21,21	23,07	20,5	20,37	24,5
Mean	25,30	22,77	23,15	20,19	20,17	24,40
SD	0,014	2,206	0,113	0,438	0,283	0,141
CV (%)	0,056	9,689	0,489	2,171	1,402	0,58

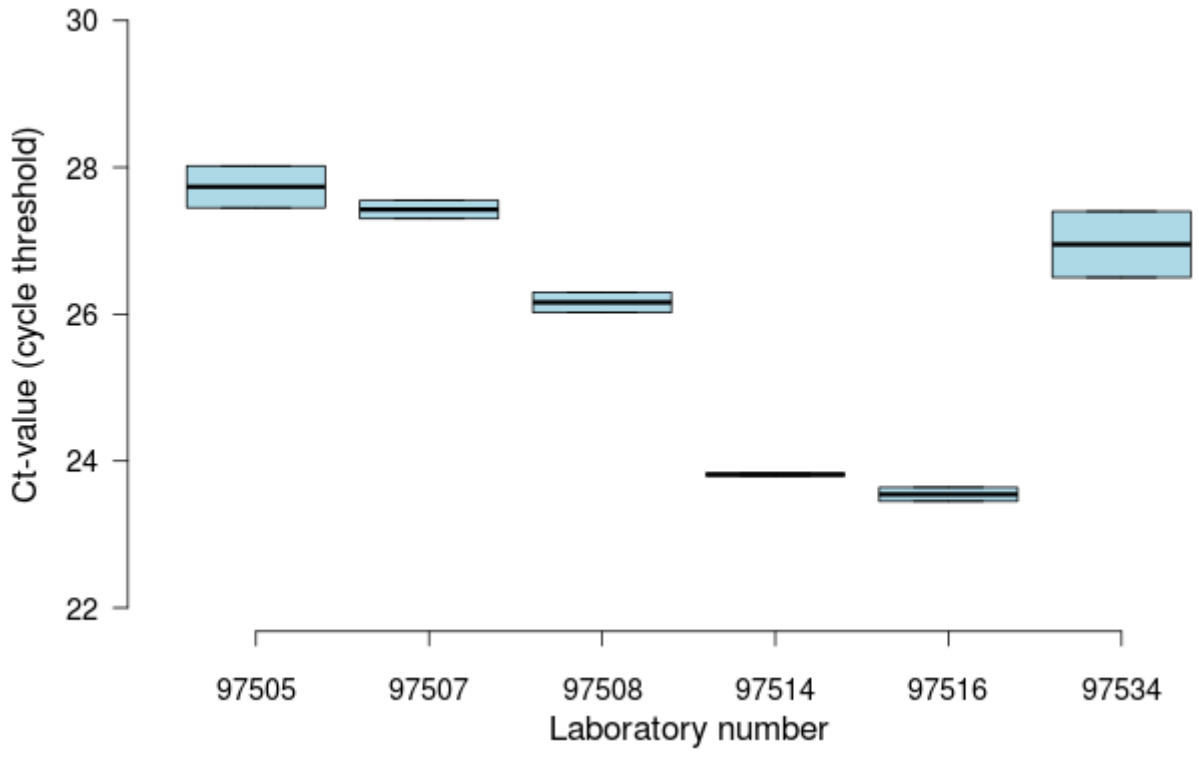
M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal; M_5 = IDVet - ID Gene BVDV/BDV Triplex 2.0



Quantitative results for two replicate samples: PT2025BVDVIR-PB4

Lab number	97505	97507	97508	97514	97516	97534
Method (PCR protocol/kit)	M_1	M_2	M_3	M_4	M_5	M_2
Pos control	25,34	27,89	29,33	26,17	33,66	27,6
Neg control	45	45	40	40	40	45
Cut-off for pos result	35	45	34	40	40	45
Cut-off for neg result	>45	>45	>40	>40	>40	>45
Ct-value (1)	27,45	27,3	26,02	23,83	23,64	27,4
Ct-value (2)	28,02	27,55	26,3	23,8	23,45	26,5
Mean	27,735	27,425	26,16	23,815	23,545	26,95
SD	0,403	0,177	0,198	0,021	0,134	0,636
CV (%)	1,453	0,645	0,757	0,089	0,571	2,361

M_1 = Homemade; M_2 = Thermo Fisher Scientific - VetMAX BVDV 4ALL Kit; M_3 = Indical Bioscience - Virotype BVDV 2.0 RT PCR Kit; M_4 = Biosellal - Bio-T kit BVDV-BDV Universal; M_5 = IDVet - ID Gene BVDV/BDV Triplex 2.0



6.2 Annex: Additional information

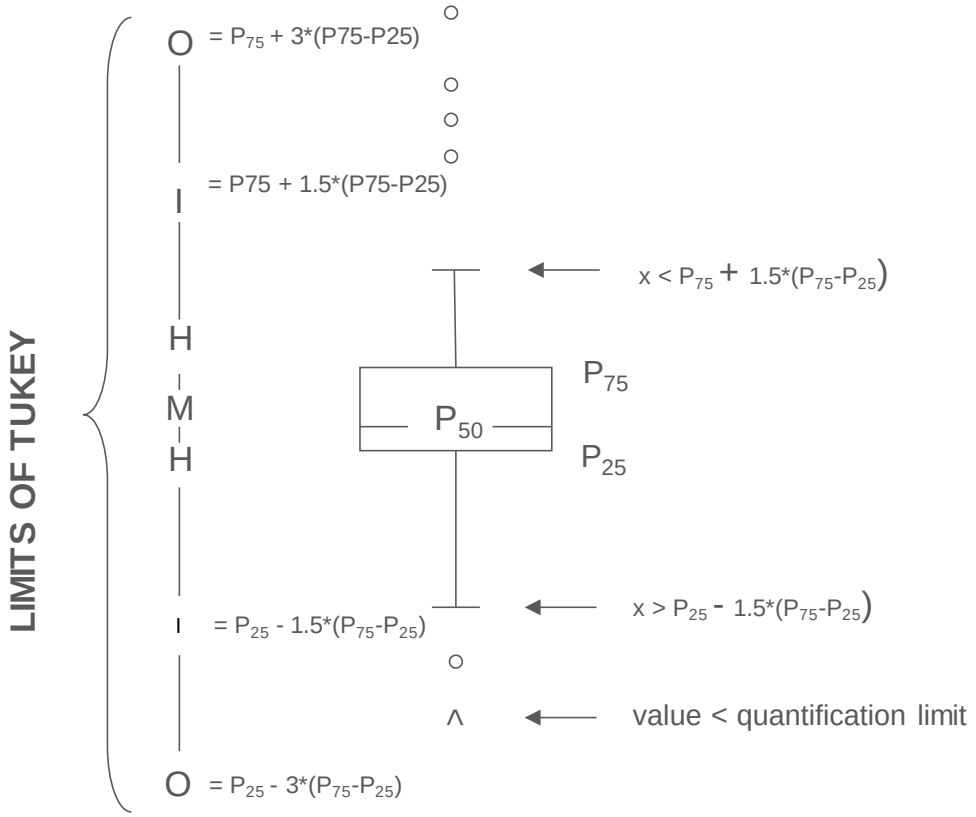
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

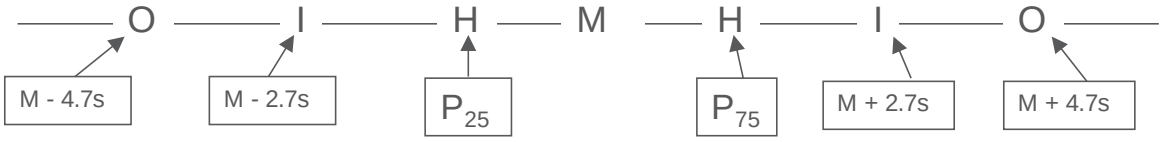
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2025
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2025
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2025

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