BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

BOVINE VIRAL DIARRHEA (BVD)

PROFICIENCY TEST 2022/10

Sciensano/PT VET BVD/3-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:11/01/2023.

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YnVa2851 Digitally signed by YnVa2851 Date: 2023.01.19 10:11:42 +01'00'

Date of publication: 19/01/2023

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1 INTRODUCTION

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

2 AIM

This PT was 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

Seven laboratories participated in the proficiency test of BVD serology on serum (ELISA). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- ANSES Unité Pathologie et Bien-être des ruminants (PBER)-Site de Niort
- IDEXX Diavet (Switserland)
- LSI-Thermofisher Scientific (France)

3.1.2 THE SAMPLES

The samples (refrigerated serum) were prepared by the National Reference Laboratory (NRL), VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: CATZELE17
- Sample P2: wpos 16.01
- Sample P3: CATZELE1
- Sample P4: CATZELE2
- Sample P5: CATZELE6
- Sample N1: Glorieux 6030
- Sample N2: Glorieux 6027
- Sample N3: Glorieux 5893
- Sample N4: Glorieux n°5

All samples are field samples stored frozen before aliquotation and transport

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (250 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 9 different samples: 5 positive and 4 negative samples. Negative sample N3 was repeated twice.

Sample ID	Repetition	Status
PT2022BVDSERSP1	1	POS
PT2022BVDSERSP2	1	POS
PT2022BVDSERSP3	1	POS
PT2022BVDSERSP4	1	POS
PT2022BVDSERSP5	1	POS
PT2022BVDSERSN1	1	NEG
PT2022BVDSERSN2	1	NEG
PT2022BVDSERSN3	2	NEG
PT2022BVDSERSN4	1	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 stability check was conform.

3.1.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: BVDSERSER(E)	97505	97507	97508	97509	97513	97521	97541
22-1	P5	N1	N4	P3	N2	P3	P1
22-2	P4	N4	N1	N2	N4	P4	P4
22-3	P2	P4	P3	N3	N1	N1	P3
22-4	N3	N3	N3	P1	N3	N4	N4
22-5	N3	N2	P4	P4	N3	P1	N3
22-6	N1	N3	P1	P5	P3	P5	N2
22-7	P1	P1	P5	P2	P2	N3	N1

Sample ID: BVDSERSER(E)	97505	97507	97508	97509	97513	97521	97541
22-8	N2	P5	P2	N4	P1	N3	P5
22-9	N4	P2	N2	N3	P5	N2	P2
22-10	P3	P3	N3	N1	P4	P2	N3

3.2 Virology (serum - ELISA)

3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of BVD virology on serum (ELISA). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- ANSES Unité Pathologie et Bien-être des ruminants (PBER)-Site de Niort
- IDEXX Diavet (Switserland)

3.2.2 THE SAMPLES

The samples (frozen serum) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: CATRendac1
- Sample P2: CATMICHEL6210-026112012
- Sample P3: CAT DGZ1
- Sample P4: CATDOX1
- Sample P5: CATRendac1 - DIL 1/10
- Sample N1: CATZELE 18
- Sample N2: CATZELE 2
- Sample N3: CATZELE 6
- Sample N4: CATZELE 8

All samples are field samples stored frozen before aliquotation and transport.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (250 µl-500 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 9 different samples: 5 positive and 4 negative samples. Negative sample N2 was repeated twice.

Sample ID	Repetition	Status
PT2022BVDAgVIRSP1	1	POS
PT2022BVDAgVIRSP2	1	POS
PT2022BVDAgVIRSP3	1	POS
PT2022BVDAgVIRSP4	1	POS
PT2022BVDAgVIRSP5	1	POS
PT2022BVDAgVIRSN1	1	NEG
PT2022BVDAgVIRSN2	2	NEG
PT2022BVDAgVIRSN3	1	NEG
PT2022BVDAgVIRSN4	1	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 stability check was conform.

3.2.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: BVDVIRSER(E)	97505	97507	97508	97509	97513	97521
22-1	N3	N2	P4	N3	P1	N1
22-2	P5	P4	N3	P2	P4	N4
22-3	P2	N1	N2	P5	P3	P1
22-4	P1	N3	N4	N2	N2	P5
22-5	P4	P2	N1	P4	N4	P2
22-6	N1	P1	P5	P1	N2	N2
22-7	N2	P3	N2	N2	P5	P3
22-8	N2	N4	P1	P3	N1	N3
22-9	P3	P5	P2	N4	N3	N2
22-10	N4	N2	P3	N1	P2	P4

3.3 Virology (serum – RT-qPCR)

3.3.1 THE PARTICIPANTS

Seven laboratories participated in the proficiency test of BVD virology on serum (RT-qPCR). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LNCR / ACSEDIATE
- ANSES Unité Pathologie et Bien-être des ruminants (PBER)-Site de Niort
- IDEXX Diavet (Switserland)
- LSI-Thermofisher Scientific (France)

3.3.2 THE SAMPLES

The samples (frozen serum) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: CATRendac1 - DIL 1/10
- Sample P2: cat vanderperre
- Sample P3: BOBVDCER4
- Sample P4: CATDOX1
- Sample P5: CATBraem0032
- Sample N1: CATZELE20
- Sample N2: CATZELE8
- Sample N3: CATZELE9

All samples are field samples stored frozen before aliquotation and transport.

3.3.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (500 µl) of each sample using RT-qPCR method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.3.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 8 different samples: 5 positive and 3 negative samples. Negative samples N1 and N2 were repeated twice.

Sample ID	Repetition	Status
PT2022BVDVIRSP1	1	POS
PT2022BVDVIRSP2	1	POS
PT2022BVDVIRSP3	1	POS
PT2022BVDVIRSP4	1	POS
PT2022BVDVIRSP5	1	POS

Sample ID	Repetition	Status
PT2022BVDVIRSN1	2	NEG
PT2022BVDVIRSN2	2	NEG
PT2022BVDVIRSN3	1	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 stability check was conform.

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 ID: BVDVIRSER(P)	97505	97507	97508	97510	97513	97521	97534
22-1	N2	P4	N1	P2	P5	N1	P3
22-2	P1	N1	N2	N1	P3	N1	P4
22-3	P2	N3	P3	N1	N2	N3	N2
22-4	P4	P1	P2	P1	N2	N2	N1
22-5	N1	P5	P5	N2	P1	P4	P1
22-6	P5	P3	P4	P4	N1	P3	N3
22-7	N3	N2	N2	P5	P4	P2	N1
22-8	P3	N2	N3	P3	N1	N2	P5
22-9	N1	P2	P1	N3	N3	P1	N2
22-10	N2	N1	N1	N2	P2	P5	P2

3.4 Virology (blood - ELISA)

3.4.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of BVD virology on blood (ELISA). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- IDEXX Diavet (Switserland)

3.4.2 THE SAMPLES

The samples (frozen blood) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE.

Information about the **origin** of the samples:

- Sample P1: CATBraem0032
- Sample P2: CATstoffels 1
- Sample P3: CATMICHEL6210
- Sample P4: cat vanderperre
- Sample N1: CATZELE8
- Sample N2: CATZELE9
- Sample N3: CATZELE 12
- Sample N4: CATZELE 20

All samples are field samples stored frozen before aliquotation and transport.

3.4.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (500 µl) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.4.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 8 different samples: 4 positive and 4 negative samples. Positive sample P2 and negative sample N3 were repeated twice.

Sample ID	Repetition	Status
PT2022BVDAgVIRBP1	1	POS
PT2022BVDAgVIRBP2	2	POS
PT2022BVDAgVIRBP3	1	POS
PT2022BVDAgVIRBP4	1	POS
PT2022BVDAgVIRBN1	1	NEG
PT2022BVDAgVIRBN2	1	NEG
PT2022BVDAgVIRBN3	2	NEG
PT2022BVDAgVIRBN4	1	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 stability check was conform.

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 ID: BVDVIRBLOOD(E)	97505	97507	97508	97509	97521
22-1	P3	N3	P1	N3	N2
22-2	P1	P1	P2	P4	N3
22-3	N4	N2	N3	N1	P1
22-4	N3	P2	P4	P3	P2
22-5	P2	P2	N2	N4	N3
22-6	N1	N3	P2	P1	P2
22-7	N2	N1	N3	N2	N4f
22-8	P2	P4	P3	N3	N1
22-9	P4	N4	N4	P2	P3
22-10	N3	P3	N1	P2	P4

3.5 Virology (blood - RT-qPCR)

3.5.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of BVD virology on blood (RT-qPCR). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
- IDEXX Diavet (Switserland)
- LSI-Thermofisher Scientific (France)

3.5.2 THE SAMPLES

The samples (frozen blood) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: CATRendac1 - DIL 1/50
- Sample P2: CAT vanderperre
- Sample P3: CATstoffels 1
- Sample P4: CATBraem0032
- Sample N1: Catzele 12
- Sample N2: Catzele 20
- Sample N3: Catzele 8
- Sample N4: Catzele 9

All samples are field samples stored frozen before aliquotation and transport.

3.5.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (500 µl) of each sample using RT-qPCR method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.5.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 8 different samples: 4 positive and 4 negative samples. Positive sample P1 and negative sample N2 were repeated twice.

Sample ID	Repetition	Status
PT2022BVDVIRBP1	2	POS
PT2022BVDVIRBP2	1	POS
PT2022BVDVIRBP3	1	POS
PT2022BVDVIRBP4	1	POS
PT2022BVDVIRBN1	1	NEG
PT2022BVDVIRBN2	2	NEG
PT2022BVDVIRBN3	1	NEG
PT2022BVDVIRBN4	1	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 stability check was conform.

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 ID: BVDVIRBLOOD(P)	97505	97507	97508	97516	97521	97534
22-1	P2	N2	P1	N1	P3	N2
22-2	P1	N3	N1	P3	P1	N1
22-3	N2	N1	N2	P1	P1	N4
22-4	P1	P1	P3	P1	N4	P3
22-5	N4	P2	P2	P4	P4	P2
22-6	P3	N2	N2	N2	N2	P4
22-7	N3	P4	P1	N4	P2	P1
22-8	P4	P3	N4	P2	N1	N2

Sample ID: BVDVIRBLOOD(P)	97505	97507	97508	97516	97521	97534
22-9	N1	P1	P4	N3	N3	P1
22-10	N2	N4	N3	N2	N2	N3

3.6 Virology (ear notch - ELISA)

3.6.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of BVD virology on ear notch (RT-qPCR). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- ANSES Unité Pathologie et Bien-être des ruminants (PBER)-Site de Niort
- IDEXX Diavet (Switserland)

3.6.2 THE SAMPLES

The samples (frozen ear notch) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: ear TO-16-042472
- Sample P2: ear BE352562185
- Sample P3: ear BE556226867
- Sample P4: ear TO-16 042479
- Sample P5: ear BE2565673545
- Sample N1: ear BE512670801
- Sample N2: BE815561529
- Sample N3: ear BE912665305
- Sample N4: ear BE512666893
- Sample N5: ear BE956555629

All samples are ear samples collected from IPI and non-IPI animals in Belgium, kept frozen at Sciensano.

3.6.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.6.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 10 different samples: 5 positive and 5 negative samples. No repetitions were included.

Sample ID	Repetition	Status
PT2022BVDAgVIREP1	1	POS
PT2022BVDAgVIREP2	1	POS
PT2022BVDAgVIREP3	1	POS
PT2022BVDAgVIREP4	1	POS
PT2022BVDAgVIREP5	1	POS
PT2022BVDAgVIREN1	1	NEG
PT2022BVDAgVIREN2	1	NEG
PT2022BVDAgVIREN3	1	NEG
PT2022BVDAgVIREN4	1	NEG
PT2022BVDAgVIREN5	1	NEG

(POS = positive; NEG = negative)

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 stability check was conform.

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 ID: BVDVIREN(E)	97505	97507	97508	97509	97513	97521
22-1	P3	P5	N5	P2	P5	P1
22-2	N1	N5	P3	N1	P4	P5
22-3	P4	N4	N1	N5	P3	N4
22-4	N2	P4	N3	P5	N3	P1
22-5	N4	P3	N2	P3	P1	N2
22-6	P1	N1	P5	N4	N4	N5
22-7	N5	P1	P1	N3	N5	P4
22-8	P2	N3	N4	P4	N2	N1
22-9	N3	P2	P2	N2	N1	N3
22-10	P5	N2	P4	P1	P2	P2

3.7 Virology (ear notch - RT-qPCR)

3.7.1 THE PARTICIPANTS

Seven laboratories participated in the proficiency test of BVD virology on ear nothc (RT-qPCR). The names of the participating laboratories are:

- Sciensano, department of Veterinary Virology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- ANSES Unité Pathologie et Bien-être des ruminants (PBER)-Site de Niort
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
- IDEXX Diavet (Switserland)
- LSI-Thermofisher Scientific (France)

3.7.2 THE SAMPLES

The samples (frozen ear notch) were prepared by the National Reference Laboratory (NRL), Service of VIRENBEE, Sciensano.

Information about the **origin** of the samples:

- Sample P1: ear TO-16-042472
- Sample P2: ear BE352562185
- Sample P3: ear BE556226867
- Sample P4: ear BE156573541
- Sample P5: ear BE456129869
- Sample N1: ear BE512670801
- Sample N2: ear BE112386491
- Sample N3: ear BE815561529
- Sample N4: ear BE512687176
- Sample N5: ear BE 471474094

All samples are ear samples collected from IPI and non-IPI animals in Belgium, kept frozen at Sciensano.

3.7.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots of each sample using RT-qPCR method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.7.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 10 different samples: 5 positive and 5 negative samples. No repetitions were included.

Sample ID	Repetition	Status
PT2022BVDVIREP1	1	POS
PT2022BVDVIREP2	1	POS
PT2022BVDVIREP3	1	POS

Sample ID	Repetition	Status
PT2022BVDVIREP4	1	POS
PT2022BVDVIREP5	1	POS
PT2022BVDVIREN1	1	NEG
PT2022BVDVIREN2	1	NEG
PT2022BVDVIREN3	1	NEG
PT2022BVDVIREN4	1	NEG
PT2022BVDVIREN5	1	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 stability check was conform.

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 ID: BVDVIREN(P)	97505	97507	97508	97513	97516	97521	97534
22-1	P4	P1	P1	N3	N3	N5	P4
22-2	P3	N4	N3	P5	N2	P1	P3
22-3	N5	N3	N5	P3	N4	P2	P5
22-4	N1	P3	P4	N2	P5	N3	N2
22-5	P5	P4	N1	N5	P1	P5	N4
22-6	N4	N2	P3	N1	P2	N1	P2
22-7	N3	N5	P5	P1	P3	N2	N1
22-8	N2	P5	N2	N4	N1	N4	N3
22-9	P2	N1	N4	P4	N5	P3	P1
22-10	P1	P2	P2	P2	P4	P4	N5

4 TIMELINE

Transfer of the samples from NRL to QL: 03/10/2022
Randomization of the samples by QL: 03/10/2022
Sending samples to participants: 10/10/2022

- Samples serology: cooled at 4 °C
- Samples virology: frozen at -20 °C

Deadline for submitting the results: 04/11/2022
Preliminary report: 10/01/2023

5 RESULTS

5.1 Serology (serum - ELISA)

5.1.1 RESULTS PER SAMPLE

The panel consisted of 9 different samples. Negative sample N3 was repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

One lab had chosen to test two different methods on the same samples, implying that there were two datasets submitted. These additional results are included in the tables below.

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (8)	8 POS
P2	POS	1 (8)	7 POS 1 NI
P3	POS	1 (8)	8 POS
P4	POS	1 (8)	8 POS
P5	POS	1 (8)	8 POS
N1	NEG	1 (8)	8 NEG
N2	NEG	1 (8)	8 NEG
N3	NEG	2 (16)	16 NEG
N4	NEG	1 (8)	8 NEG

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

5.1.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Indirect	Bio-X Diagnostics - Monoscreen Ab ELISA BVD	Short	**2**	**20**	**20**	**100**
ELISA Indirect	Idexx - BVD Total Ab	Short	**1**	**10**	**9**	**90**
ELISA Competition	ID.VET - Idscreen BVD p80 antibody competition	Short	**3**	**30**	**30**	**100**
ELISA Competition	Bio-X Diagnostics - Monoscreen Ab ELISA BVD	Short	**1**	**10**	**10**	**100**
ELISA Competition	Thermofisher - BVDV Ab ref 7588940	Short	**1**	**10**	**10**	**100**
TOTAL			**8**	**80**	**79**	**99**

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

5.1.3 CONCLUSION

In 2022, seven laboratories participated in proficiency test of BVD serology (serum - ELISA) organized by Sciensano. Two indirect ELISA and three blocking ELISA methods were selected by the laboratories for the detection of antibodies against BVD in serum.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Only the method of Idexx - BVD Total Ab failed to achieve a total score of 100%. The laboratory mentioned on the misreported sample that the sample was quite near to cut off to not interpretable. Also they mentioned that in the daily routine they would have sent it to the Reference Laboratory. Nevertheless, this laboratory obtained a score of 90% which is still in agreement with the guidelines. To conclude; an overall score of 99% was achieved implying that all the five methods used are suitable options for antibody detection against BVD in serum.

5.2 Virology (serum - ELISA)

5.2.1 RESULTS PER SAMPLE

The panel consisted of 9 different samples. Negative sample N2 was repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (6)	6 POS
P2	POS	1 (6)	6 POS
P3	POS	1 (6)	6 POS
P4	POS	1 (6)	6 POS
P5	POS	1 (6)	6 POS
N1	NEG	1 (6)	6 NEG
N2	NEG	2 (12)	12 NEG
N3	NEG	1 (6)	6 NEG
N4	NEG	1 (6)	6 NEG

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Method		Short or long incubation protocol	Formula	N	NR	NCR	%
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Short	$(OD_{sample}-OD_{NC})/(OD_{PC}-OD_{NC})$	**2**	**20**	**20**	**100**
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Short	Sample OD - Negative control mean OD	**4**	**40**	**40**	**100**
TOTAL				**6**	**60**	**60**	**100**

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

5.2.3 CONCLUSION

In 2022, six laboratories participated in proficiency test of BVD virology (serum - ELISA) organized by Sciensano. The method Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus from Idexx was selected by the participants. Only there was a difference in the procedure (another formula was used), therefore a distinction between these two was made.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test. As a results, it can be concluded that the method from Idexx is a suitable option for antibody detection against BVD in serum.

5.3 Virology (serum – RT-qPCR)

5.3.1 RESULTS PER SAMPLE

The panel consisted of 8 different samples. Negative samples N1 and N2 were repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

One lab had chosen to test two different methods on the same samples, implying that there were two datasets submitted. These additional results are included in the tables below.

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (8)	7 POS 1 NEG
P2	POS	1 (8)	8 POS 1 NEG
P3	POS	1 (8)	6 POS 2 NEG
P4	POS	1 (8)	6 POS 2 NEG
P5	POS	1 (8)	7 POS 1 NEG

Sample ID	Status	Number of repetitions (total results)	Observed result
N1	NEG	2 (16)	15 NEG 1 POS
N2	NEG	2 (16)	15 NEG 1 POS
N3	NEG	1 (8)	8 NEG

(POS = positive; NEG = negative)

5.3.2 USED METHOD

Manufacturer extraction protocol / kit	Name extraction protocol / kit	RT-qPCR protocol / kit	N	NR	NCR	%
Qiagen	QIAamp DNA Mini kit	Home made	2	20	15	75
Indical	IndiMag Pathogen Kit	Kit Thermofisher BVD4ALL	2	20	16	80
ThermoFisher Scientific	MagMAX CORE nucleic acid purification kit	Thermofisher vetMAX BVDV screening kit	2	20	20	100
BioX-Adiagene	ADIAMAG XL	Adiavet BVD real time (protocole court) 10K4TRI94	1	10	10	100
TOTAL			**7**	**70**	**61**	**87**

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

5.3.3 CONCLUSION

In 2022, seven laboratories participated in proficiency test of BVD virology (serum – RT-qPCR) organized by Sciensano. Different methods were selected by the participants for the identification of the BVD virus in serum of cattle.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Two laboratories did not achieve the minimum score of 90%. For one laboratory, this was partly due to two coding errors in the Toolkit and partly because they used two methods, one for the detection of BVD I and another for the detection of BVD II. For the second laboratory, an explanation could be found for the poor score of 60%. This laboratory inadvertently entered BVD blood RT-qPCR results instead of their BVD serum RT-qPCR results. After the lab was informed of this, they were able to prove they did have the correct answers, which implies that we can conclude that this is not a bad way of working or that the method is not suitable for this test. Since this concerns a coding error and not an analysis error, this lab does not have to take any further action. However, according to the quality guidelines, they do have to report this in their quality system.

Unlike the two laboratories discussed above, all other laboratories achieved the maximum score of 100%.

5.4 Virology (blood – ELISA)

5.4.1 RESULTS PER SAMPLE

The panel consisted of 8 different samples. Positive sample P2 and negative sample N3 were repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (5)	5 POS
P2	POS	2 (10)	10 POS
P3	POS	1 (5)	5 POS
P4	POS	1 (5)	5 POS
N1	NEG	1 (5)	5 NEG
N2	NEG	1 (5)	5 NEG
N3	NEG	2 (10)	10 NEG
N4	NEG	1 (5)	5 NEG

(POS = positive; NEG = negative)

5.4.2 USED METHOD

Method		Short or long incubation protocol	Formula	N	NR	NCR	%
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Short	$(OD_{sample}-OD_{NC})/(OD_{PC}-OD_{NC})$	**1**	**10**	**10**	**100**
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Short	Sample OD - Negative control mean OD	**4**	**40**	**40**	**100**
TOTAL				**5**	**50**	**50**	**100**

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

5.4.3 CONCLUSION

In 2022, five laboratories participated in proficiency test of BVD virology (blood - ELISA) organized by Sciensano. The method Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus from Idexx was selected by the participants. Only there was a difference in the procedure (another formula was used), therefore a distinction between these two was made.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test. As a results, it can be concluded that the method from Idexx is a suitable option for antibody detection against BVD in blood of cattle.

5.5 Virology (blood - RT-qPCR)

5.5.1 RESULTS PER SAMPLE

The panel consisted of 8 different samples. Positive sample P1 and negative sample N2 were repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	2 (12)	12 POS
P2	POS	1 (6)	6 POS
P3	POS	1 (6)	6 POS
P4	POS	1 (6)	6 POS
N1	NEG	1 (6)	6 NEG
N2	NEG	2 (12)	12 NEG
N3	NEG	1 (6)	6 NEG
N4	NEG	1 (6)	6 NEG

(POS = positive; NEG = negative)

5.5.2 USED METHOD

Manufacturer extraction protocol / kit	Name extraction protocol / kit	RT-qPCR protocol / kit	N	NR	NCR	%
Qiagen	RNEASY mini kit	Home made	1	10	10	100
Qiagen	QIAamp DNA Mini kit	Home made	1	10	10	100
Indical	IndiMag Pathogen Kit	Kit Thermofisher BVD4all	2	20	20	100
ThermoFisher Scientific	ThermoFisher Scientific - MagMaxCore	ThermoFisher Scientific - VetMAX BVDV4ALL	2	20	20	100
TOTAL			**6**	**60**	**60**	**100**

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

5.5.3 CONCLUSION

In 2022, six laboratories participated in proficiency test of BVD virology (blood – RT-qPCR) organized by Sciensano. Four different methods, from Qiagen, Indical and ThermoFisher Scientific were selected by the participants for the identification of the BVD virus in blood of cattle.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test. As a results, it can be concluded that these methods are suitable options the identification of the BVD virus in blood of cattle.

5.6 Virology (ear notch - ELISA)

5.6.1 RESULTS PER SAMPLE

The panel consisted of 10 different samples. No repetitions were included. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (6)	6 POS
P2	POS	1 (6)	6 POS
P3	POS	1 (6)	6 POS
P4	POS	1 (6)	6 POS
P5	POS	1 (6)	6 POS
N1	NEG	1 (6)	6 NEG
N2	NEG	1 (6)	6 NEG
N3	NEG	1 (6)	6 NEG
N4	NEG	1 (6)	6 NEG
N5	NEG	1 (6)	6 NEG

(POS = positive; NEG = negative)

5.6.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Long	**2**	**20**	**20**	**100**
ELISA Indirect	Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus	Short	**4**	**40**	**40**	**100**
TOTAL			**6**	**60**	**60**	**100**

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

5.6.3 CONCLUSION

In 2022, six laboratories participated in proficiency test of BVD virology (ear notch - ELISA) organized by Sciensano. The method Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus from Idexx was selected by all the participants for the detection of antibodies against BVD in ear notch.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test. As a results, it can be concluded that the method from Idexx is a suitable option for antibody detection against BVD in ear notch.

5.7 Virology (ear notch - RT-qPCR)

5.7.1 RESULTS PER SAMPLE

The panel consisted of 10 different samples. No repetitions were included. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
P1	POS	1 (6)	6 POS
P2	POS	1 (6)	6 POS
P3	POS	1 (6)	6 POS
P4	POS	1 (6)	6 POS
P5	POS	1 (6)	6 POS
N1	NEG	1 (6)	5 NEG 1 POS
N2	NEG	1 (6)	6 NEG
N3	NEG	1 (6)	6 NEG
N4	NEG	1 (6)	6 NEG
N5	NEG	1 (6)	6 NEG

(POS = positive; NEG = negative)

5.7.2 USED METHOD

Manufacturer extraction protocol / kit	Name extraction protocol / kit	RT-qPCR protocol / kit	N	NR	NCR	%
Qiagen	RNEASY mini kit	Home made	1	10	10	100
Indical	IndiMag Pathogen Kit	Thermofisher - LSIVETMAX BVD4ALL	1	10	10	100
IDVET	Direct lysis buffer	Virotype BVDV RT-PCR kit	1	10	10	100
BioX-Adiagene	ADIAMAG XL	Adiavet BVD RealTime	1	10	10	100
ThermoFisher Scientific	MagMAX™ CORE Nucleic Acid Purification Kit	LSIVETMAX BVD4ALL	2	20	19	95
Idexx	RealPCR Rapid Lysis Buffer	Home made	1	10	10	100
TOTAL						

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

5.7.3 CONCLUSION

In 2022, seven laboratories participated in proficiency test of BVD virology (ear notch – RT-qPCR) organized by Sciensano. Different methods were selected by the participants for the detection of antibodies against BVD in ear notch.

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the minimum score (90%) for this test.

6 ANNEXES (NOT UNDER ACCREDITATION)

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

6.1 Annex 1: Quantitative results

6.1.1 VIROLOGY BLOOD (ELISA)

PT2022BVDAgVIRBP2

Lab number	97505	97507	97508	97509	97521
Method	M_2	M_2	M_1	M_2	M_2
OD (REP1)	3,57	3,49	3,49	3,18	3,41
OD (REP2)	3,54	3,45	3,45	3,17	3,45
Mean	3,55	3,16	3,47	3,17	3,43
SD	0,02	0,05	0,03	0,01	0,02
CV (%)	0,58	1,48	0,75	0,36	0,72

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = ELISA indirect - Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus – formula Sciensano; M_2 = ELISA - Idexx - Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus – other formula).

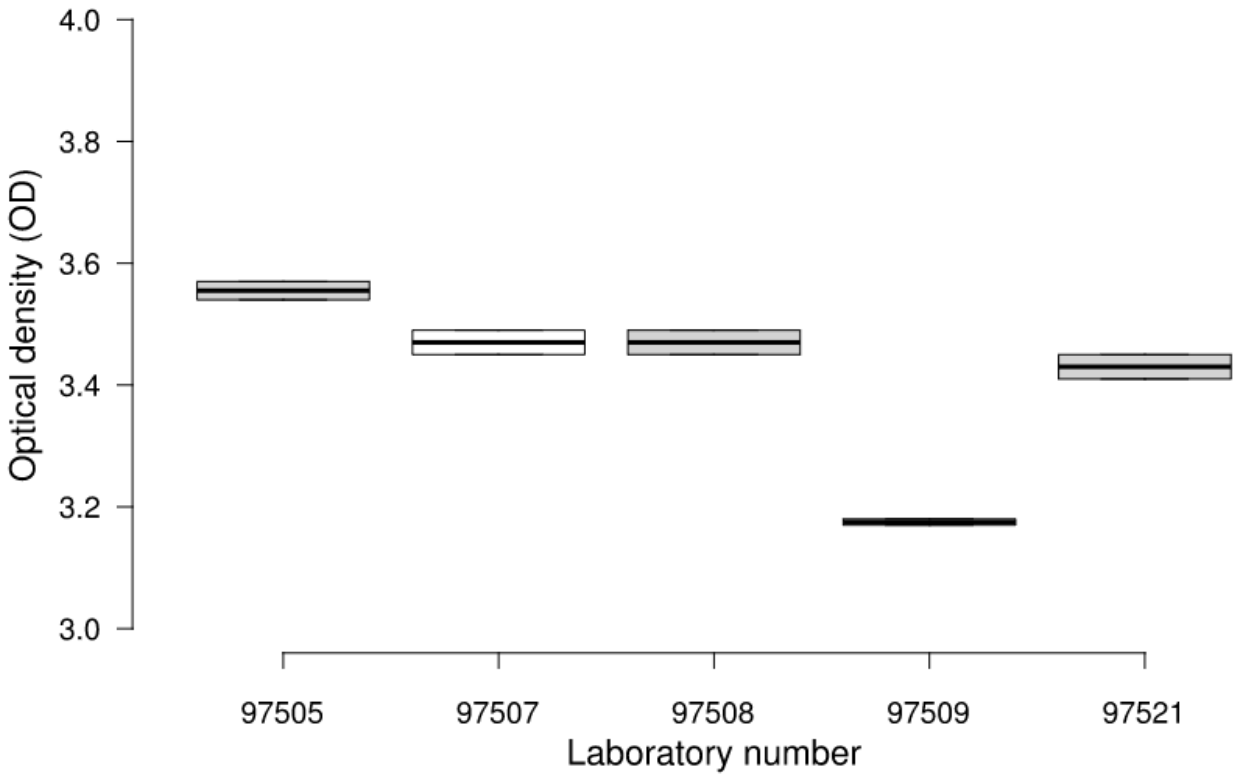


Figure 1. Distribution of the optical densities (box-plots) per laboratory.

6.1.2 VIROLOGY BLOOD (RT-QPCR)

PT2022BVDVIRBP1

Lab number	97505	97507	97508	97516	97521	97534
Method	M_1	M_2	M_2	M_3	M_4	M_3
Ct (REP1)	33,14	31,75	33,44	28,66	31,34	30,90
Ct (REP2)	32,93	32,00	33,45	29,04	30,94	30,90
Mean	33,04	31,87	33,45	28,85	31,14	30,90
SD	0,15	0,18	0,01	0,27	0,28	0,00
CV (%)	0,45	0,56	0,02	0,93	0,91	0,00

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Qiagen - RNEASY mini kit - Homemade; M_2 = Indical - IndiMag Pathogen Kit - Kit Thermofisher BVD4all; M_3 = ThermoFisher Scientific - MagMaxCore - VetMAX BVDV4ALL ; M_4 = Qiagen - QIAamp DNA Mini kit - Homemade).

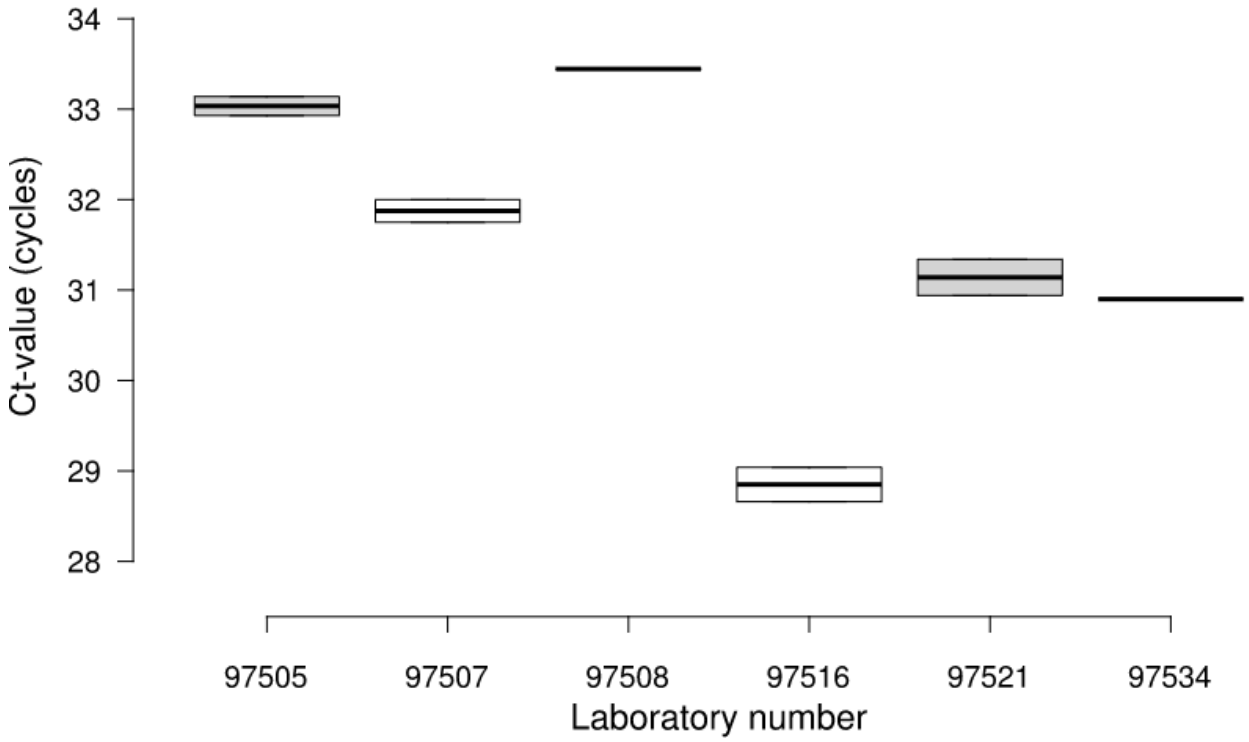


Figure 2. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

The **preliminary report** of this proficiency test is available on our website via the following link:

- 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

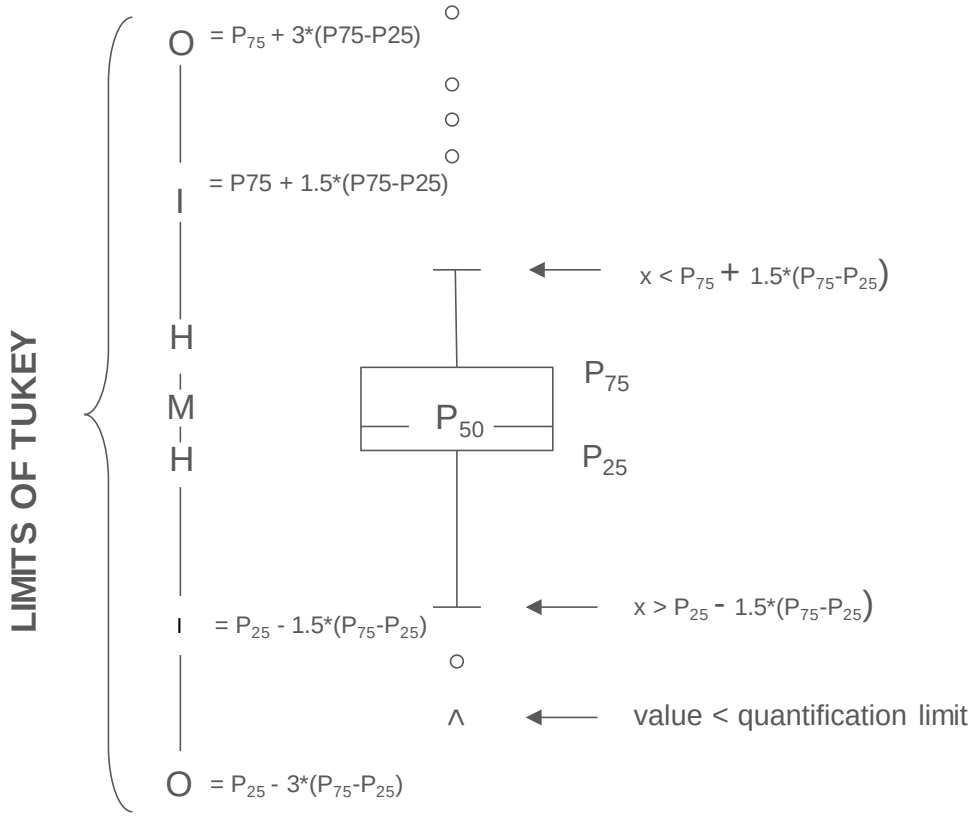
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

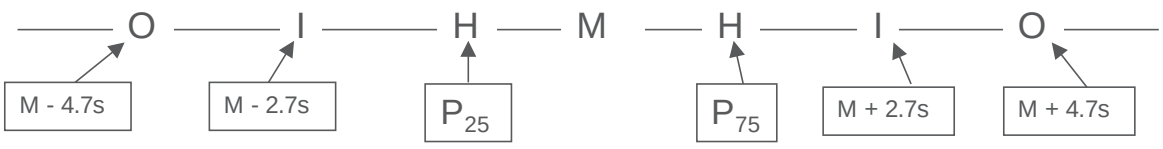
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 3 participants:

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





Corresponding limits in case of normal distribution

END