EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

Proficiency Testing in Veterinary Diagnosis
Blue Tongue Virus (BTV)

SURVEY 2021/10

Sciensano/PT VET BTV/2-E

Expertise and service provision
Quality of laboratories
J. Wytsmanstreet, 14
1050 Bruxelles | Belgique

www.sciensano.be

ISSN: 2294-3498

COMMITTEE OF EXPERTS

Sciensano					
Secretariat		PHONE:	02/642.55.22	FAX:	02/642.56.45
Name scheme coordinator	Bernard China	PHONE:	02 642 53 85		
		e-mail:	Bernard.China@sciensano.be		
Coordinator in formation	Ynse Van De Maele	PHONE	02/6425584		
		email	Ynse.vandemaele@sciensano.be		
Name alternate scheme coordinator	Arnaud Capron	PHONE:	02/6425397		
		e-mail:	Arnaud.Capron@sciensano.be		
Experts	**Institute**				
Ilse De Leeuw	**Sciensano**				

A preliminary version of this report was submitted to the National reference laboratory: 10/12/2021

Authorization to release the report: By Bernard China, scheme coordinator,10/01/2022.

Bernard China

All the reports are also available on our webpage:
https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm
https://www.wiv-isp.be/QML/activities/PT%20VET/nl/originaux/rapports_annee.htm

Table of Contents

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 '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.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of BTV-specific antibodies in serum of bovidae origin by ELISA and/or BTV RNA in blood of bovidae origin by RT-qPCR.

III. Materials and methods

3.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples were tested by means of a BTV antibody ELISA test and/or predefined reference blood samples were tested by means of RT-qPCR. The procedures for the ELISA tests and the RT-qPCR assays must be fully described in the SOPs of the participating laboratories.

3.2. Reference samples

The samples were prepared by the National Reference Laboratory, Exotic viruses and particular diseases, Infectious diseases in animals Directorate, Sciensano.

3.2.1. Reference serum samples

3.2.1.1. Origin of the serum samples

Seven reference serum samples of bovidae origin, either free from detectable BTV-specific antibodies (n=2; coded 'PT2021BLTSERNS1' and 'PT2021BLTSERNS2') or containing detectable BTV-specific antibodies (n=5; coded 'PT2021BLTSERPS1', 'PT2021BLTSERPS2', 'PT2021BLTSERPS3' 'PT2021BLTSERPS4' and 'PT2021BLTSERPS5'), were used. In total, 140 aliquots were distributed to 7 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference samples PT2021BLTSERNS1, PT2021BLTSERNS2, PT2021BLTSERPS1, PT2021BLTSERPS3, PT2021BLTSERPS4 and PT2021BLTSERPS5 and 2 aliquots of the reference sample PT2021BLTSERPS2. The positions of the reference serum samples in the blocks sent, were randomized for each participant (Table 2).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification, hereby using the ID Screen® Bluetongue Competition kit from ID.VET.

Table 1. Overview of the reference serum samples

Sample ID	Origine	Background	Repetition	Status
PT2021BLTSERNS1	bovine	uninfected/unvaccinated	3	negative
PT2021BLTSERNS2	ovine	uninfected/unvaccinated	3	negative
PT2021BLTSERPS1	ovine	vaccinated	3	positive
PT2021BLTSERPS2	ovine	vaccinated	2	positive
PT2021BLTSERPS3	bovine	infected	3	positive
PT2021BLTSERPS4	bovine	vaccinated	3	positive
PT2021BLTSERPS5	bovine	vaccinated	3	positive

3.2.1.2. Homogeneity

The homogeneity of the samples was tested by the NRL on 10 replicates of each sample using IDVET ID Screen Bluetongue Competition (BTC) ELISA, batch G81 before the survey. The samples were considered as homogeneous.

3.2.1.3. Stability

The stability was determined by comparison of the pre-survey results with the results obtained by the NRL during and after the survey. The samples were considered as stable.

3.2.1.4. The participants

7 laboratories participated to this survey:
Sciensano, ARSIA, DGZ, Lavetan, IDVET (France), LMVE (GD Lux.), ULG

3.2.1.5. Randomisation and panel composition

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

Table 2. Panel composition serology

Sample ID	97506	97507	97508	97509
BTSER21-1	PT2021BLTSERPS4	PT2021BLTSERPS1	PT2021BLTSERPS1	PT2021BLTSERNS2
BTSER21-2	PT2021BLTSERNS1	PT2021BLTSERNS2	PT2021BLTSERPS3	PT2021BLTSERPS2
BTSER21-3	PT2021BLTSERNS1	PT2021BLTSERNS2	PT2021BLTSERPS5	PT2021BLTSERPS1
BTSER21-4	PT2021BLTSERPS1	PT2021BLTSERPS4	PT2021BLTSERPS4	PT2021BLTSERPS5
BTSER21-5	PT2021BLTSERNS2	PT2021BLTSERNS1	PT2021BLTSERPS2	PT2021BLTSERPS3
BTSER21-6	PT2021BLTSERNS2	PT2021BLTSERPS4	PT2021BLTSERNS1	PT2021BLTSERPS3
BTSER21-7	PT2021BLTSERPS5	PT2021BLTSERPS5	PT2021BLTSERPS2	PT2021BLTSERPS3
BTSER21-8	PT2021BLTSERPS4	PT2021BLTSERPS1	PT2021BLTSERPS4	PT2021BLTSERPS1
BTSER21-9	PT2021BLTSERNS2	PT2021BLTSERNS1	PT2021BLTSERNS1	PT2021BLTSERPS4
BTSER21-10	PT2021BLTSERPS3	PT2021BLTSERPS5	PT2021BLTSERPS5	PT2021BLTSERPS1
BTSER21-11	PT2021BLTSERPS3	PT2021BLTSERPS5	PT2021BLTSERPS1	PT2021BLTSERPS4
BTSER21-12	PT2021BLTSERPS5	PT2021BLTSERPS3	PT2021BLTSERNS2	PT2021BLTSERPS5
BTSER21-13	PT2021BLTSERPS2	PT2021BLTSERPS4	PT2021BLTSERPS1	PT2021BLTSERNS2
BTSER21-14	PT2021BLTSERPS1	PT2021BLTSERPS1	PT2021BLTSERPS3	PT2021BLTSERPS4
BTSER21-15	PT2021BLTSERPS1	PT2021BLTSERPS3	PT2021BLTSERNS1	PT2021BLTSERNS1
BTSER21-16	PT2021BLTSERNS1	PT2021BLTSERPS2	PT2021BLTSERNS2	PT2021BLTSERNS2
BTSER21-17	PT2021BLTSERPS3	PT2021BLTSERNS1	PT2021BLTSERPS3	PT2021BLTSERNS1
BTSER21-18	PT2021BLTSERPS5	PT2021BLTSERPS2	PT2021BLTSERPS5	PT2021BLTSERPS2
BTSER21-19	PT2021BLTSERPS4	PT2021BLTSERNS2	PT2021BLTSERPS4	PT2021BLTSERNS1
BTSER21-20	PT2021BLTSERPS2	PT2021BLTSERPS3	PT2021BLTSERNS2	PT2021BLTSERPS5

Sample ID	97516	97522	97527
BTSER21-1	PT2021BLTSERNS2	PT2021BLTSERPS3	PT2021BLTSERPS3
BTSER21-2	PT2021BLTSERNS2	PT2021BLTSERPS5	PT2021BLTSERNS1
BTSER21-3	PT2021BLTSERPS3	PT2021BLTSERNS2	PT2021BLTSERNS2
BTSER21-4	PT2021BLTSERPS1	PT2021BLTSERPS5	PT2021BLTSERPS2
BTSER21-5	PT2021BLTSERPS1	PT2021BLTSERPS1	PT2021BLTSERNS2
BTSER21-6	PT2021BLTSERNS1	PT2021BLTSERPS1	PT2021BLTSERNS1
BTSER21-7	PT2021BLTSERPS5	PT2021BLTSERNS1	PT2021BLTSERPS5
BTSER21-8	PT2021BLTSERPS5	PT2021BLTSERPS4	PT2021BLTSERPS4
BTSER21-9	PT2021BLTSERPS2	PT2021BLTSERPS1	PT2021BLTSERPS3
BTSER21-10	PT2021BLTSERPS5	PT2021BLTSERNS2	PT2021BLTSERPS2
BTSER21-11	PT2021BLTSERNS1	PT2021BLTSERPS3	PT2021BLTSERNS2
BTSER21-12	PT2021BLTSERNS2	PT2021BLTSERPS2	PT2021BLTSERPS4
BTSER21-13	PT2021BLTSERPS4	PT2021BLTSERPS4	PT2021BLTSERPS4
BTSER21-14	PT2021BLTSERPS3	PT2021BLTSERPS2	PT2021BLTSERPS1
BTSER21-15	PT2021BLTSERPS4	PT2021BLTSERPS3	PT2021BLTSERPS5
BTSER21-16	PT2021BLTSERPS3	PT2021BLTSERPS4	PT2021BLTSERNS1
BTSER21-17	PT2021BLTSERPS2	PT2021BLTSERNS1	PT2021BLTSERPS5
BTSER21-18	PT2021BLTSERPS4	PT2021BLTSERNS1	PT2021BLTSERPS1
BTSER21-19	PT2021BLTSERPS1	PT2021BLTSERPS5	PT2021BLTSERPS1
BTSER21-20	PT2021BLTSERNS1	PT2021BLTSERNS2	PT2021BLTSERPS3

3.2.2. Reference blood samples

3.2.2.1. Origin of the blood samples

Replicates of 8 reference blood samples of bovidae origin, either free from detectable BTV RNA (n = 2; coded 'PT2021BLTVIRNB1' and 'PT2021BLTVIRNB2') or containing detectable BTV RNA (n = 6; coded 'PT2021BLTVIRPB1', 'PT2021BLTVIRPB2', 'PT2021BLTVIRPB3' 'PT2021BLTVIRPB4', 'PT2021BLTVIRPB6' and 'PT2021BLTVIRPB7') were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference blood samples 'PT2021BLTVIRNB1', 'PT2021BLTVIRPB1', 'PT2021BLTVIRPB4' and 'PT2021BLTVIRPB6' and 2 aliquots of the reference blood samples 'PT2021BLTVIRNB2', 'PT2021BLTVIRPB2', 'PT2021BLTVIRPB3' and 'PT2021BLTVIRPB7'. The positions of the reference blood samples in the blocks sent, were randomized for each participant (Table 4).

For each reference blood sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference blood samples was based on (i) the background of the samples and (ii) the results obtained during pre-verification, hereby using the LSI™ VetMAX™ BTV NS3 RT-qPCR kit from LSI (detecting all BTV serotypes) and 5 different in-house developed BTV RT-qPCR assays: one detecting all BTV serotypes, one detecting only BTV-1, one detecting only BTV-3, one detecting only BTV-4 and one detecting only BTV-8.

Table 3. Overview of the reference blood samples

Sample ID	Origine	Background	Strain(s)	Repetition	Status
PT2021BLTVIRNB1	bovine	uninfected	NA	3	negative
PT2021BLTVIRNB2	bovine	uninfected	NA	2	negative
PT2021BLTVIRPB1	bovine	uninfected/ blood spiked	BTV-3	3	positive
PT2021BLTVIRPB2	bovine	uninfected/ blood spiked	BTV-1	2	positive
PT2021BLTVIRPB3	bovine	uninfected/ blood spiked	BTV-8	2	positive
PT2021BLTVIRPB4	bovine	uninfected/ blood spiked	BTV-8	3	positive
PT2021BLTVIRPB6	bovine	uninfected/ blood spiked	BTV-4	3	positive
PT2021BLTVIRPB7	bovine	uninfected/ blood spiked	BTV-4	2	positive

3.2.2.2. Homogeneity

The homogeneity of the samples was tested by the NRL on 10 replicates of each sample using RT-qPCR method before the survey. The samples were considered as homogeneous.

3.2.2.3. Stability

The stability was determined by comparison of the pre-survey results with the results obtained by the NRL during and after the survey. The samples were considered as stable.

3.2.2.4. The participants

8 laboratories participated to this survey:
Sciensano, ARSIA, DGZ, Lavetan, LNCR (France), LMVE (GD Lux.), IDVET (France), ULG.

3.2.2.5. Randomisation and panel composition

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

Table 4. Panel composition virology

Sample ID	97506	97507	97508	97509
BTVIR21-1	PT2021BLTVIRPB6	PT2021BLTVIRPB6	PT2021BLTVIRPB1	PT2021BLTVIRPB4
BTVIR21-2	PT2021BLTVIRPB1	PT2021BLTVIRNB1	PT2021BLTVIRPB7	PT2021BLTVIRPB3
BTVIR21-3	PT2021BLTVIRPB1	PT2021BLTVIRNB2	PT2021BLTVIRPB4	PT2021BLTVIRPB2
BTVIR21-4	PT2021BLTVIRNB1	PT2021BLTVIRPB6	PT2021BLTVIRNB1	PT2021BLTVIRNB2
BTVIR21-5	PT2021BLTVIRNB2	PT2021BLTVIRPB1	PT2021BLTVIRPB6	PT2021BLTVIRPB6
BTVIR21-6	PT2021BLTVIRNB2	PT2021BLTVIRPB3	PT2021BLTVIRPB3	PT2021BLTVIRPB4
BTVIR21-7	PT2021BLTVIRNB1	PT2021BLTVIRPB7	PT2021BLTVIRNB2	PT2021BLTVIRNB1
BTVIR21-8	PT2021BLTVIRNB1	PT2021BLTVIRPB2	PT2021BLTVIRPB1	PT2021BLTVIRPB2
BTVIR21-9	PT2021BLTVIRPB1	PT2021BLTVIRPB3	PT2021BLTVIRNB2	PT2021BLTVIRNB2
BTVIR21-10	PT2021BLTVIRPB6	PT2021BLTVIRNB1	PT2021BLTVIRPB6	PT2021BLTVIRPB4
BTVIR21-11	PT2021BLTVIRPB7	PT2021BLTVIRPB2	PT2021BLTVIRPB1	PT2021BLTVIRNB1
BTVIR21-12	PT2021BLTVIRPB4	PT2021BLTVIRPB4	PT2021BLTVIRNB1	PT2021BLTVIRPB6
BTVIR21-13	PT2021BLTVIRPB2	PT2021BLTVIRPB1	PT2021BLTVIRPB2	PT2021BLTVIRPB6
BTVIR21-14	PT2021BLTVIRPB6	PT2021BLTVIRPB1	PT2021BLTVIRPB4	PT2021BLTVIRPB7
BTVIR21-15	PT2021BLTVIRPB3	PT2021BLTVIRPB4	PT2021BLTVIRPB7	PT2021BLTVIRPB1
BTVIR21-16	PT2021BLTVIRPB4	PT2021BLTVIRNB2	PT2021BLTVIRPB4	PT2021BLTVIRPB7
BTVIR21-17	PT2021BLTVIRPB7	PT2021BLTVIRNB1	PT2021BLTVIRPB3	PT2021BLTVIRPB1
BTVIR21-18	PT2021BLTVIRPB2	PT2021BLTVIRPB7	PT2021BLTVIRPB2	PT2021BLTVIRPB3
BTVIR21-19	PT2021BLTVIRPB3	PT2021BLTVIRPB6	PT2021BLTVIRPB6	PT2021BLTVIRNB1
BTVIR21-20	PT2021BLTVIRPB4	PT2021BLTVIRPB4	PT2021BLTVIRNB1	PT2021BLTVIRPB1
Sample ID	**97510**	**97516**	**97522**	**97527**
BTVIR21-1	PT2021BLTVIRNB1	PT2021BLTVIRPB6	PT2021BLTVIRPB3	PT2021BLTVIRNB1
BTVIR21-2	PT2021BLTVIRNB1	PT2021BLTVIRNB2	PT2021BLTVIRPB4	PT2021BLTVIRPB1
BTVIR21-3	PT2021BLTVIRPB1	PT2021BLTVIRPB7	PT2021BLTVIRNB1	PT2021BLTVIRPB4
BTVIR21-4	PT2021BLTVIRPB6	PT2021BLTVIRPB3	PT2021BLTVIRPB6	PT2021BLTVIRPB1
BTVIR21-5	PT2021BLTVIRPB7	PT2021BLTVIRPB2	PT2021BLTVIRPB6	PT2021BLTVIRPB7
BTVIR21-6	PT2021BLTVIRPB7	PT2021BLTVIRNB2	PT2021BLTVIRPB7	PT2021BLTVIRPB2
BTVIR21-7	PT2021BLTVIRPB3	PT2021BLTVIRPB1	PT2021BLTVIRPB4	PT2021BLTVIRPB7
BTVIR21-8	PT2021BLTVIRPB3	PT2021BLTVIRPB1	PT2021BLTVIRPB1	PT2021BLTVIRPB6
BTVIR21-9	PT2021BLTVIRPB4	PT2021BLTVIRNB1	PT2021BLTVIRPB3	PT2021BLTVIRPB3
BTVIR21-10	PT2021BLTVIRPB2	PT2021BLTVIRPB4	PT2021BLTVIRPB7	PT2021BLTVIRPB2
BTVIR21-11	PT2021BLTVIRNB1	PT2021BLTVIRPB6	PT2021BLTVIRNB2	PT2021BLTVIRPB6
BTVIR21-12	PT2021BLTVIRPB6	PT2021BLTVIRNB1	PT2021BLTVIRNB1	PT2021BLTVIRPB6
BTVIR21-13	PT2021BLTVIRNB2	PT2021BLTVIRPB7	PT2021BLTVIRPB4	PT2021BLTVIRPB4
BTVIR21-14	PT2021BLTVIRPB2	PT2021BLTVIRNB1	PT2021BLTVIRPB1	PT2021BLTVIRNB2
BTVIR21-15	PT2021BLTVIRPB1	PT2021BLTVIRPB3	PT2021BLTVIRNB2	PT2021BLTVIRNB1
BTVIR21-16	PT2021BLTVIRPB6	PT2021BLTVIRPB6	PT2021BLTVIRPB6	PT2021BLTVIRNB2
BTVIR21-17	PT2021BLTVIRNB2	PT2021BLTVIRPB4	PT2021BLTVIRPB1	PT2021BLTVIRPB4
BTVIR21-18	PT2021BLTVIRPB1	PT2021BLTVIRPB1	PT2021BLTVIRPB2	PT2021BLTVIRPB1
BTVIR21-19	PT2021BLTVIRPB4	PT2021BLTVIRPB4	PT2021BLTVIRPB2	PT2021BLTVIRNB1
BTVIR21-20	PT2021BLTVIRPB4	PT2021BLTVIRPB2	PT2021BLTVIRNB1	PT2021BLTVIRPB3

3.3. Classification of results, level of agreement and threshold for qualification

3.3.1. Classification of results

Results provided by the participating laboratories are categorized as success when the reported result matches with the assigned status (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or failure when the reported result does not match with the assigned status (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

3.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of success for each of the 20 aliquots of reference samples used for this PT.

3.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 reference samples is at least 90%.

IV. Survey Timeline

Transfer of the samples from NRL to QL: 27/09/2021
Randomization of the samples by QL: 30/09/2021
sending samples to participants:4/10/2021
Deadline for the results encoding: 18/11/2021
Preliminary report: 06/12/2021

V.

VI. Results

5.1. Serology

5.1.1. Results per sample

The panel consisted of 20 serum samples (6 negative and 14 positive).Seven laboratories encoded one dataset giving 140 results.

Table 5. Results per sample

Sample ID	N	Rep	NR	Expected result	NCR	%
PT2021BLTSERNS1	7	3	21	Negative	21	100
PT2021BLTSERNS2	7	3	21	Negative	21	100
PT2021BLTSERPS1	7	3	21	Positive	21	100
PT2021BLTSERPS2	7	2	14	Positive	14	100
PT2021BLTSERPS3	7	3	21	positive	21	100
PT2021BLTSERPS4	7	3	21	Positive	21	100
PT2021BLTSERPS5	7	3	21	Positive	21	100
Total	7	20	140			

N: number of datasets, Rep: number of repetitions; NR: number of results; NCR: number of correct results
100% of the encoded results were correct.

5.1.2. Results per method

Table 6. Results per method

Method	N	NR	NCR	%
ID.VET - ID SCREEN® BLUETONGUE COMPETITION	7	140	140	100

N: number of datasets, NR: number of encoded results; NCR: Number of correct results, %: percentage of correct results.

5.1.3. Conclusion

All the participants scored 100% of the samples correctly, using the same kit.

5.2. Virology

5.2.1. Results par sample

A panel consisted of 20 samples (5 negative and 15 positive). Eight laboratories encoded one dataset giving 160 results

Table 7. Results per sample

Sample ID	N	REP	NR	Expected result	NCR	%
PT2021BLTVIRNB1	8	3	24	Negative	24	100
PT2021BLTVIRNB2	8	2	16	Negative	16	100
PT2021BLTVIRPB1	8	3	24	Positive	24	100
PT2021BLTVIRPB2	8	2	16	Positive	16	100
PT2021BLTVIRPB3	8	2	16	Positive	16	100
PT2021BLTVIRPB4	8	3	24	Positive	24	100
PT2021BLTVIRPB6	8	3	24	Positive	24	100
PT2021BLTVIRPB7	8	2	16	Positive	14	87.5
Total	8		160		158	98.75

98.75% of the encoded results were correct. 2 false negative results were encoded by the same laboratory for the sample PB7.

5.2.2. Results per method

Table 8. Results per method

Method	N	NR	NCR	%
Home Made - Home made	2	40	38	95
ADIAGENE - ADIAVET BTV real time	4	80	80	100
Thermofisher - Vetmax Bluetongue Virus NS3-all genotype	1	20	20	100
ID.VET - ID GENE® BLUETONGUE DUPLEX	1	20	20	100
Total	8	160	158	98.75

The laboratory that encoded the 2 false negative results used a home made RT-qPCR method.

5.2.3. Conclusion

All but one of the participants scored 100% of the samples correctly independently of the methods used. One laboratory using a home made method scored 90% of the samples correctly. The sample PB7 can be regarded as a medium positive sample with Ct values greater than 31 for all the participants. Therefore, the false negative results could be due to a lack of sensitivity of the used method.

VII. Annex 1: Quantitative results (not under accreditation)

6.1. Serology

6.1.1. Sample PT2021BTVSERNS1

Table A1: Sample PT2021BTVSERNS1

	97506	97507	97508	97509	97516	97522	97527
rep1	109	114.455	113.41	127.145	118	108	112.47
rep2	114	111.085	112.09	126.095	100	116	100.76
rep3	103	114.075	113.37	131.582	122	114	95.76
average	108.667	113.205	112.957	128.274	113.333	112.667	102.997
SD	5.508	1.846	0.751	2.913	11.719	4.163	8.577
CV	5.1%	1.6%	0.7%	2.3%	10.3%	3.7%	8.3%

Repx: repetition x

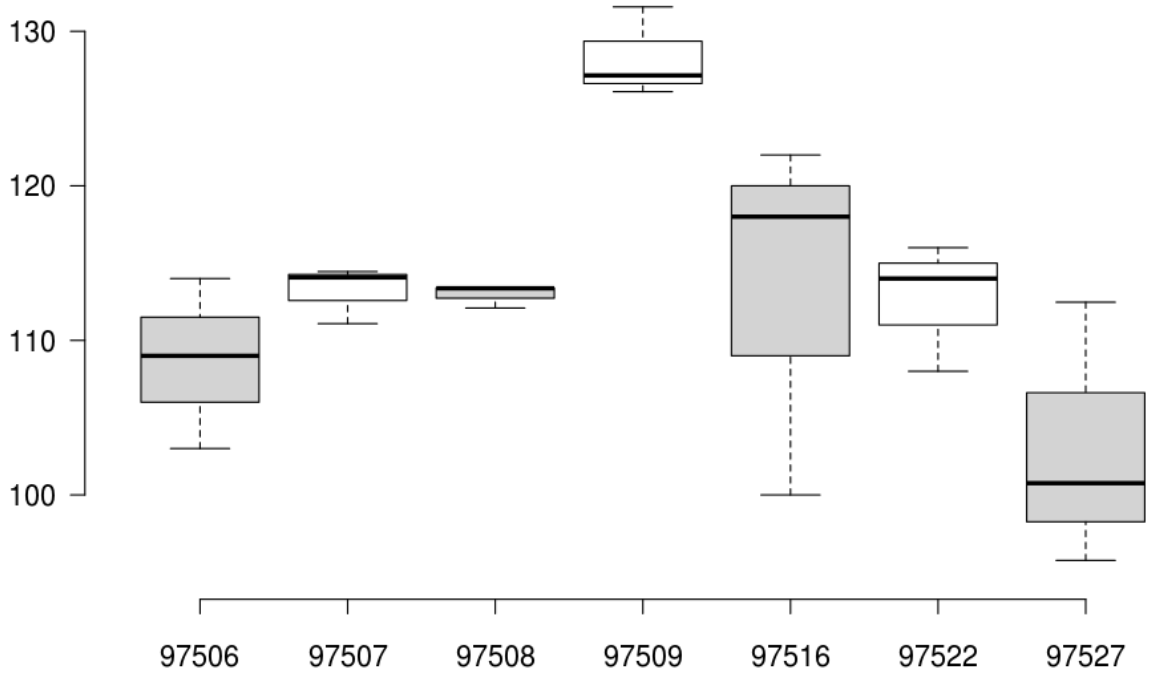

Figure A1. Distribution of the normalized values (box-plot) per laboratory**.**

6.1.2. Sample PT2021BTVSERNS2

Table A2: Sample PT2021BTVSERNS2

	97506	97507	97508	97509	97516	97522	97527
rep1	93	99.929	104.14	104.845	95.7	101	85.2
rep2	100	103.822	107.38	99.299	91.1	97	80.1
rep3	99	107.667	106.18	106.888	111	95	90.71
average	97.333	103.806	105.900	103.677	99.267	97.667	85.337
SD	3.786	3.869	1.638	3.927	10.418	3.055	5.306
CV	3.9%	3.7%	1.5%	3.8%	10.5%	3.1%	6.2%

Repx: repetition x

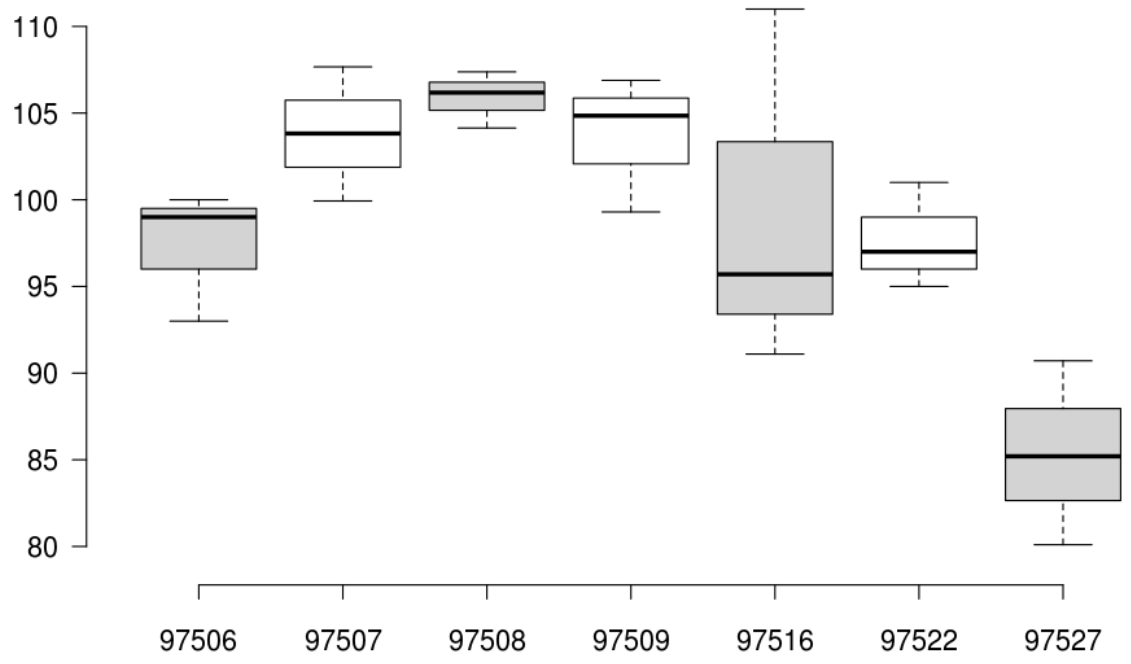

Figure A2. Distribution of the normalized values (box-plot) per laboratory.

6.1.3. Sample PT2021BTVSERPS1

Table A3: Sample PT2021BTVSERPS1

	97506	97507	97508	97509	97516	97522	97527
rep1	5	5.792	7.3964	3.911	9.7	10	7.77
rep2	6	5.554	7.4814	4.845	9.1	10	9.19
rep3	5	5.412	8.5016	5.079	9.1	10	7.47
average	5.333	5.586	7.793	4.612	9.300	10.000	8.143
SD	0.577	0.192	0.615	0.618	0.346	0.000	0.919
CV	10.8%	3.4%	7.9%	13.4%	3.7%	0.0%	11.3%

Repx: repetition x

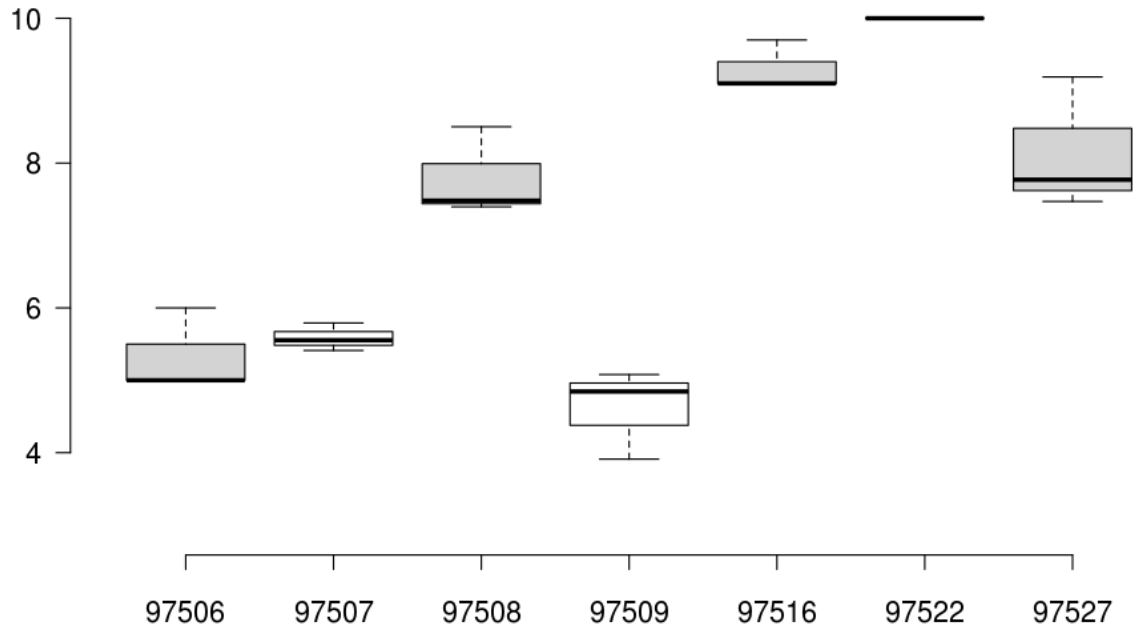

Figure A3. Distribution of the normalized values (box-plot) per laboratory.

6.1.4. Sample PT2021BTVSERPS2

Table A4: Sample PT2021BTVSERPS2

	97506	97507	97508	97509	97516	97522	97527
rep1	19	33.705	35.494	13.952	28.4	30	21.54
rep2	24	33.278	34.772	19.148	31	31	21.54
average	21.500	33.492	35.133	16.550	29.700	30.500	21.540
SD	3.536	0.302	0.511	3.674	1.838	0.707	0.000
CV	16.4%	0.9%	1.5%	22.2%	6.2%	2.3%	0.0%

Repx: repetition x

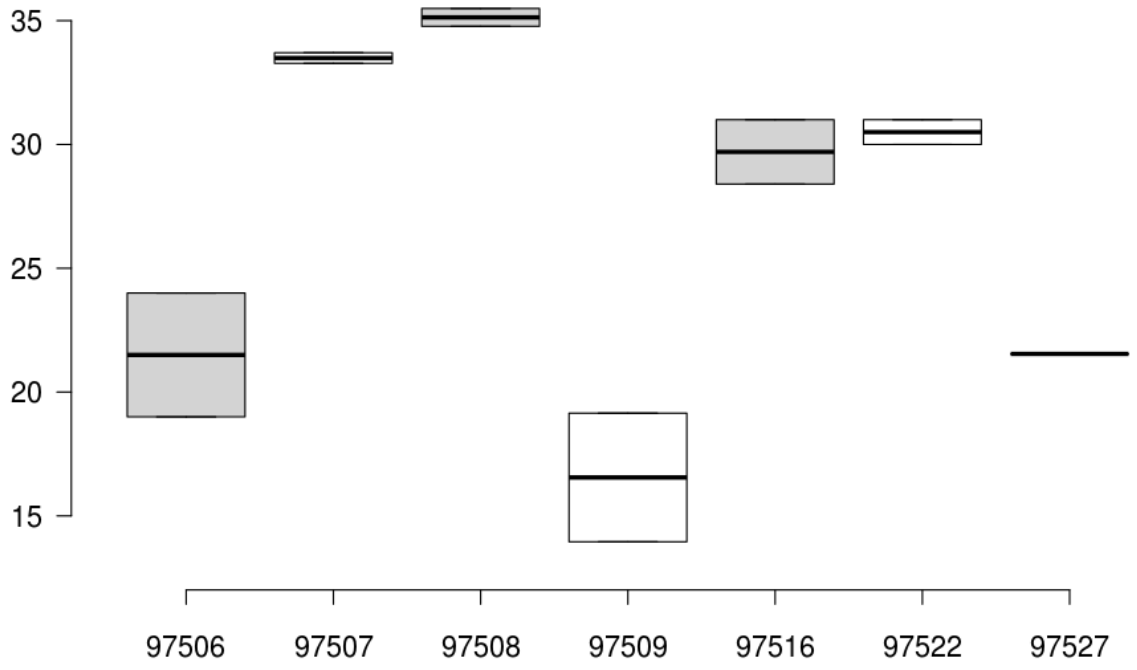

Figure A4. Distribution of the normalized values (box-plot) per laboratory.

6.1.5. Sample PT2021BTVSERPS3

Table A5: Sample PT2021BTVSERPS3

	97506	97507	97508	97509	97516	97522	97527
rep1	5	5.981	7.0138	4.086	9.7	9	6.98
rep2	6	4.747	7.949	4.787	8.2	11	7.16
rep3	7	6.124	7.5664	3.97	8.5	10	8.28
average	6.000	5.617	7.510	4.281	8.800	10.000	7.473
SD	1.000	0.757	0.470	0.442	0.794	1.000	0.704
CV	16.7%	13.5%	6.3%	10.3%	9.0%	10.0%	9.4%

Repx: repetition x

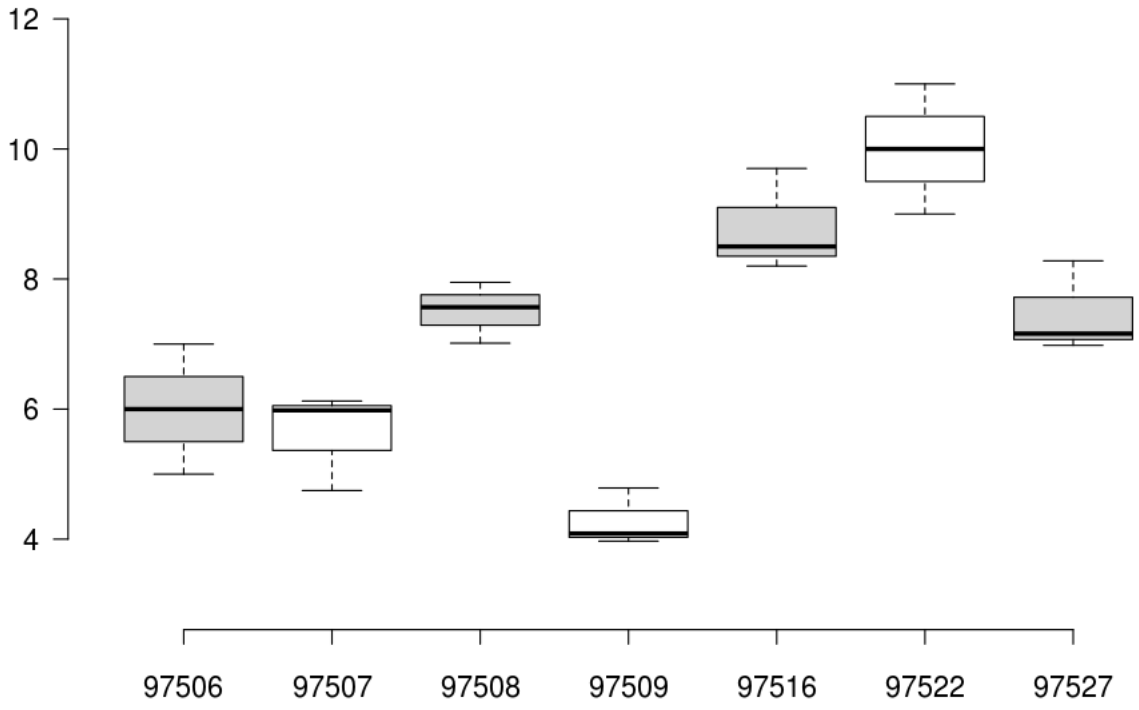

Figure A5. Distribution of the normalized values (box-plot) per laboratory.

6.1.6. Sample PT2021BTVSERPS4

Table A6: Sample PT2021BTVSERPS4

	97506	97507	97508	97509	97516	97522	97527
rep1	5	1.709	4.3783	4.32	5.3	6	4.24
rep2	4	1.899	4.2083	3.327	5.3	7	4.48
rep3	5	1.851	4.7609	4.262	5	6	3.63
average	4.667	1.820	4.449	3.970	5.200	6.333	4.117
SD	0.577	0.099	0.283	0.557	0.173	0.577	0.438
CV	12.4%	5.4%	6.4%	14.0%	3.3%	9.1%	10.6%

repX: repetition number x

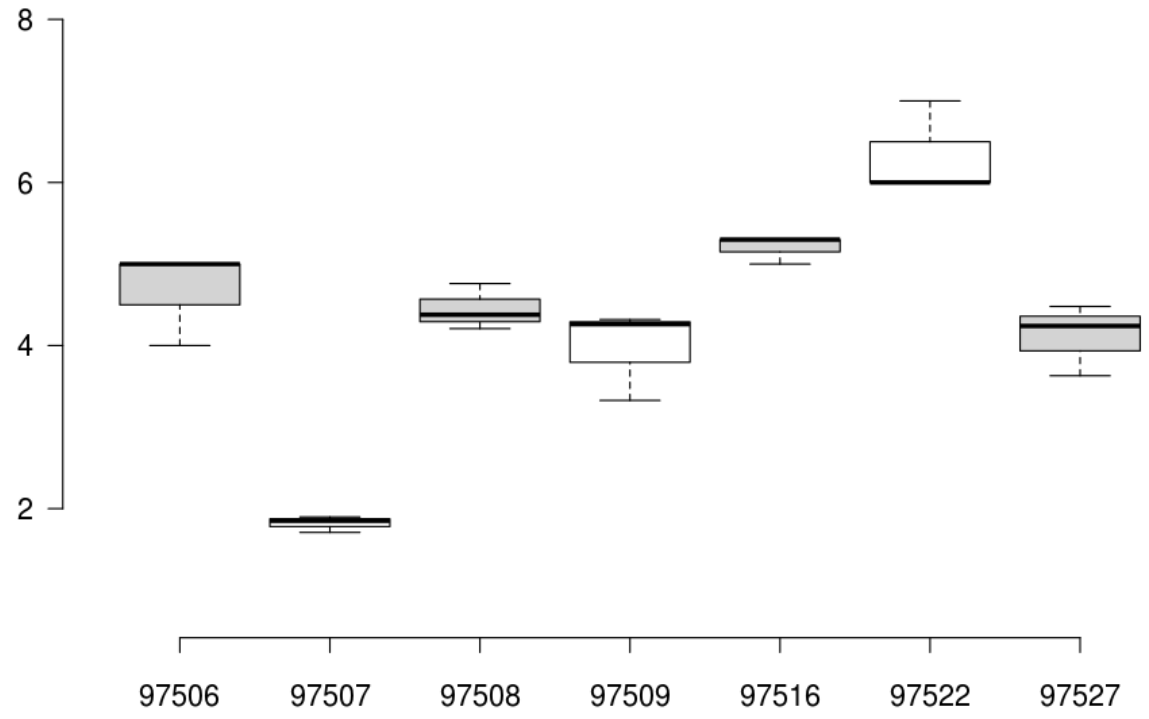

Figure A6. Distribution of the normalized values (box-plot) per laboratory.

6.1.7. Sample PT2021BTVSERPS5

Table A7: Sample PT2021BTVSERPS5

	97506	97507	97508	97509	97516	97522	97527
rep1	13	20.176	18.066	8.114	18.6	18	14.33
rep2	12	18.039	17.938	8.114	18.3	20	15.58
rep3	14	20.128	20.149	13.777	17.7	21	15.93
average	13.000	19.448	18.718	10.002	18.200	19.667	15.280
SD	1.000	1.220	1.241	3.270	0.458	1.528	0.841
CV	7.7%	6.3%	6.6%	32.7%	2.5%	7.8%	5.5%

repX: repetition number x

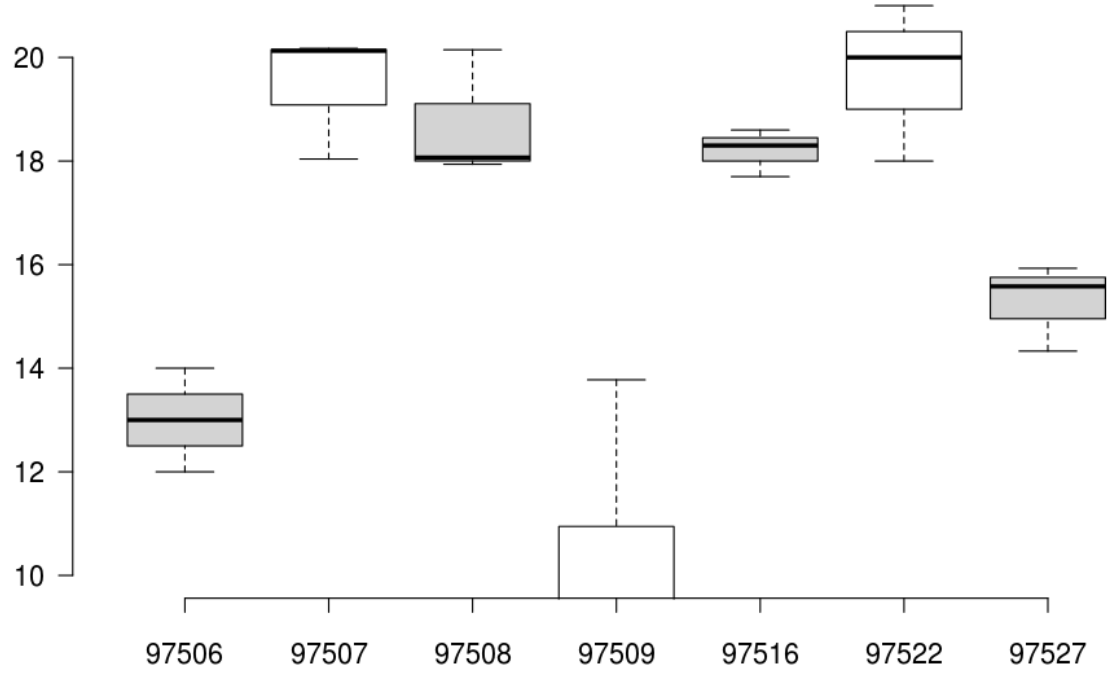

Figure A7. Distribution of the normalized values (box-plot) per laboratory.

6.2. Virology

6.2.1. Sample PT2021BTVIRPB1

Table A8: Sample PT2021BTVIRPB1

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	27.090	25.680	25.220	26.480	25.900	28.010	27.400	28.500
rep2	27.040	25.970	24.900	26.610	25.800	27.950	27.200	27.400
rep3	27.570	26.270	25.070	26.650	25.800	27.990	37.300	28.900
average	27.233	25.973	25.063	26.580	25.833	27.983	30.633	28.267
SD	0.293	0.295	0.160	0.089	0.058	0.031	5.774	0.777
CV	1.1%	1.1%	0.6%	0.3%	0.2%	0.1%	18.8%	2.7%

repX= repetition number X

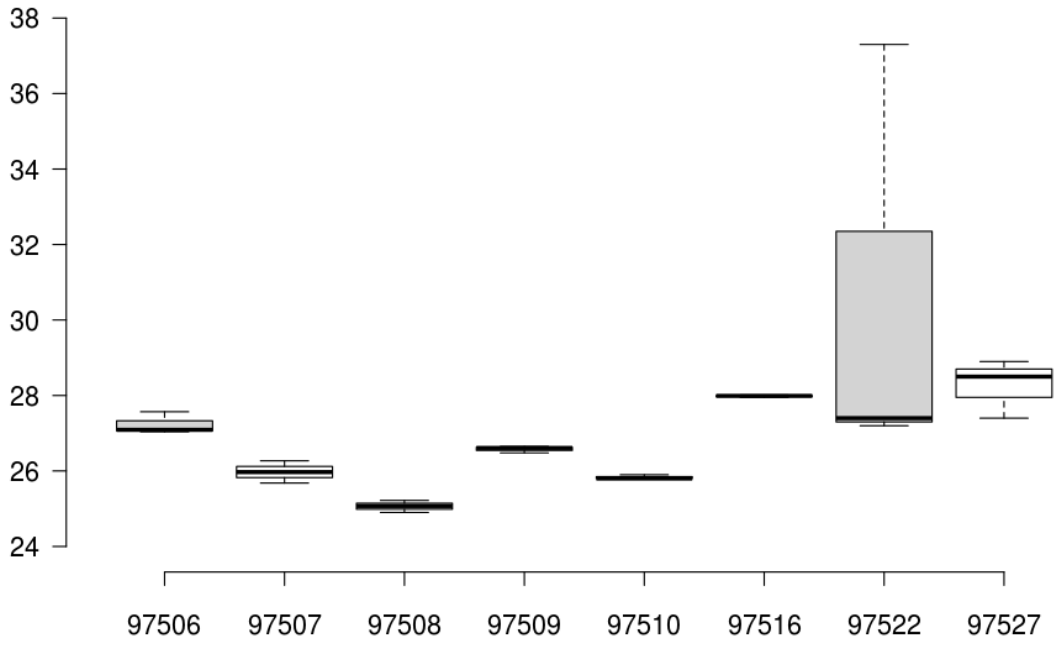

Figure A8. Dispersion of the Ct values (box-plots) per laboratory

6.2.2. Sample PT2021BTVIRPB2

Table A9: Sample PT2021BTVIRPB2

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	30.2	28.92	27.39	29.11	28.9	30.29	29.9	33.3
rep2	30.21	28.53	27.12	29.19	28.6	29.92	30.2	33.4
average	30.205	28.725	27.255	29.150	28.750	30.105	30.050	33.350
SD	0.007	0.276	0.191	0.057	0.212	0.262	0.212	0.071
CV	0.02%	0.96%	0.70%	0.19%	0.74%	0.87%	0.71%	0.21%

repX= repetition number X

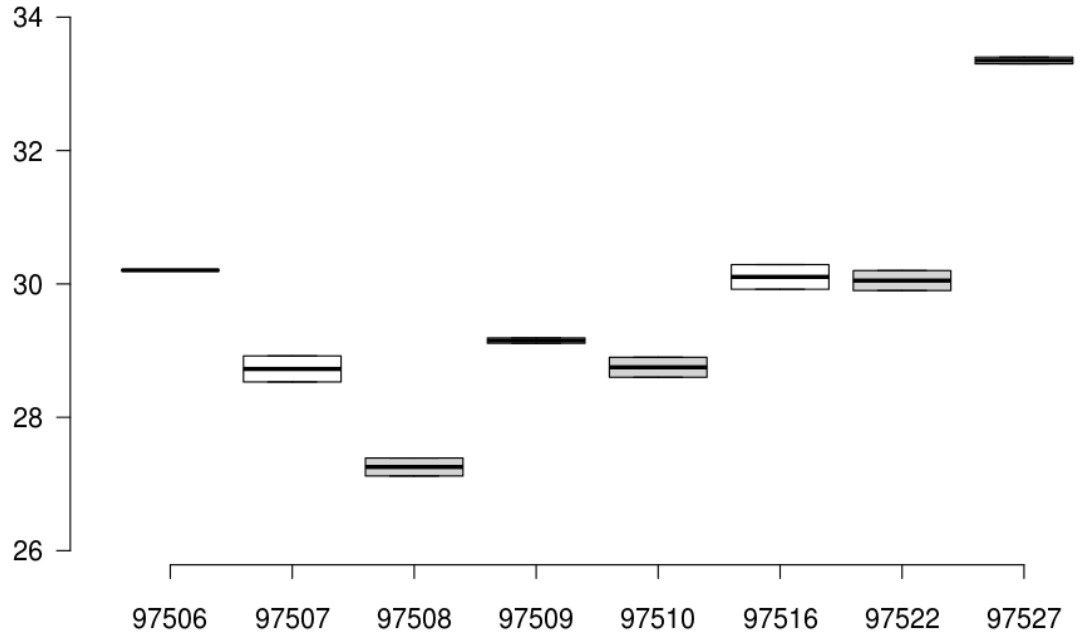

Figure A9:. Dispersion of the Ct values (box-plots) per laboratory

6.2.3. Sample PT2021BTVIRPB3

Table A10: Sample PT2021BTVIRPB3

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	28.84	28.27	27.22	28.9	29.2	30.26	31.3	31.3
rep2	28.58	29.5	27.31	29.02	29.3	30.52	31.5	31.1
average	28.710	28.885	27.265	28.960	29.250	30.390	31.400	31.200
SD	0.184	0.870	0.064	0.085	0.071	0.184	0.141	0.141
CV	0.64%	3.01%	0.23%	0.29%	0.24%	0.60%	0.45%	0.45%

repX= repetition number X

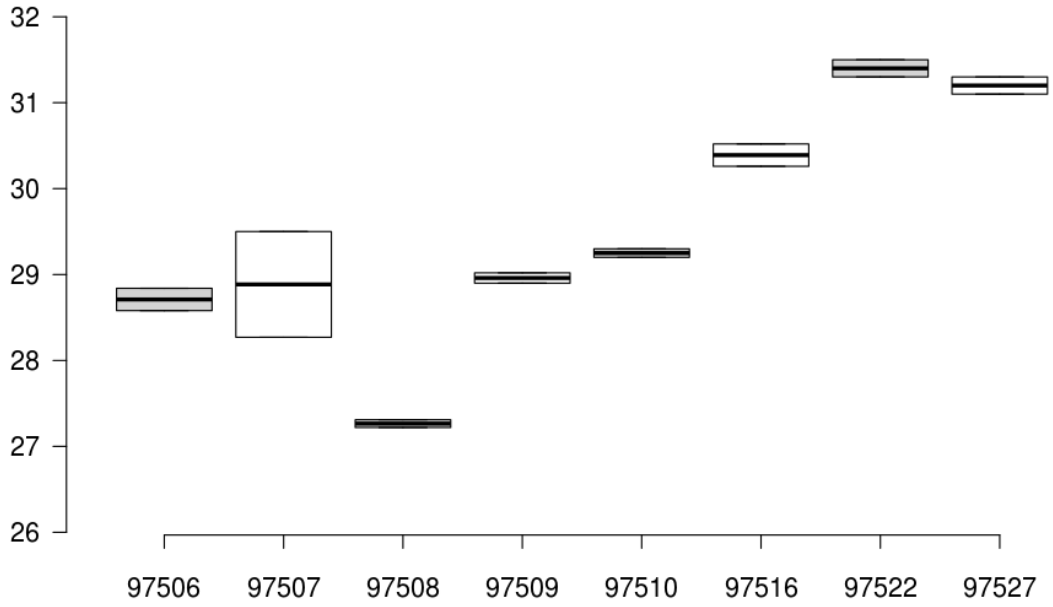

Figure A10. Dispersion of the Ct values (box-plots) per laboratory

6.2.4. Sample PT2021BTVIRPB4

Table A11**:** Sample PT2021BTVIRPB4

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	32.63	31.73	30.11	31.93	31.8	32.59	34.4	35
rep2	31.63	32.27	30.73	31.95	31	32.79	33	34.9
rep3	31.94	32.05	29.99	32.05	31	32.52	33.8	36.1
average	32.067	32.017	30.277	31.977	31.267	32.633	33.733	35.333
SD	0.512	0.272	0.397	0.064	0.462	0.140	0.702	0.666
CV	1.6%	0.8%	1.3%	0.2%	1.5%	0.4%	2.1%	1.9%

repX= repetition number X

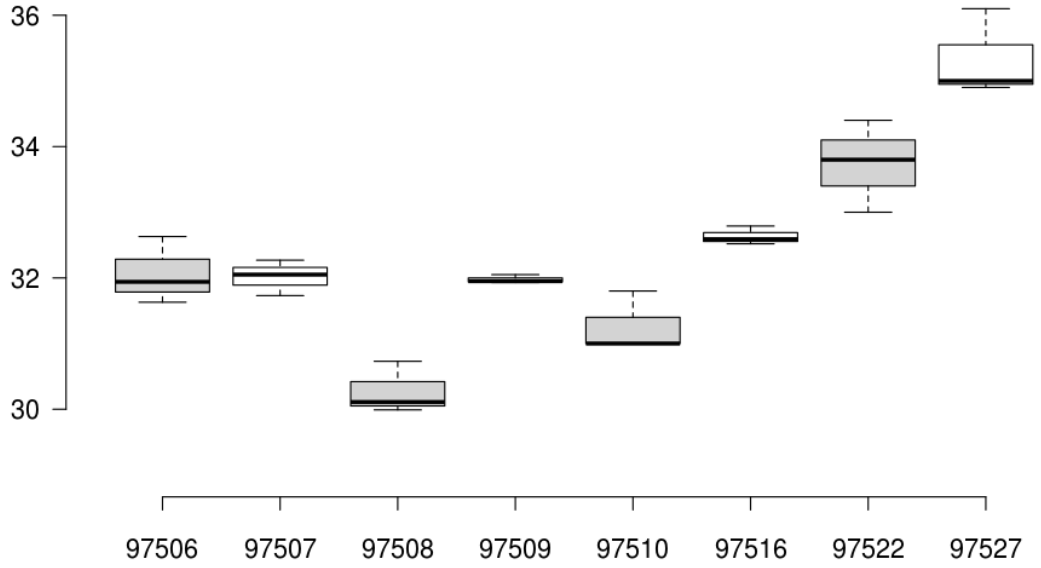

Figure A11. Dispersion of the Ct values (box-plots) per laboratory

6.2.5. Sample PT2021BTVIRPB6

Table A12: Sample PT2021BTVIRPB6

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	28.22	29.39	26.7	28.31	27.5	29.89	28.9	30.3
rep2	29.04	27.93	26.63	27.42	28	29.43	29.1	29.8
rep3	29.09	28.34	26.78	28.44	27.9	29.77	29.2	30.6
average	28.783	28.553	26.703	28.057	27.800	29.697	29.067	30.233
SD	0.489	0.753	0.075	0.555	0.265	0.239	0.153	0.404
CV	1.7%	2.6%	0.3%	2.0%	1.0%	0.8%	0.5%	1.3%

Rep X: repetition number X

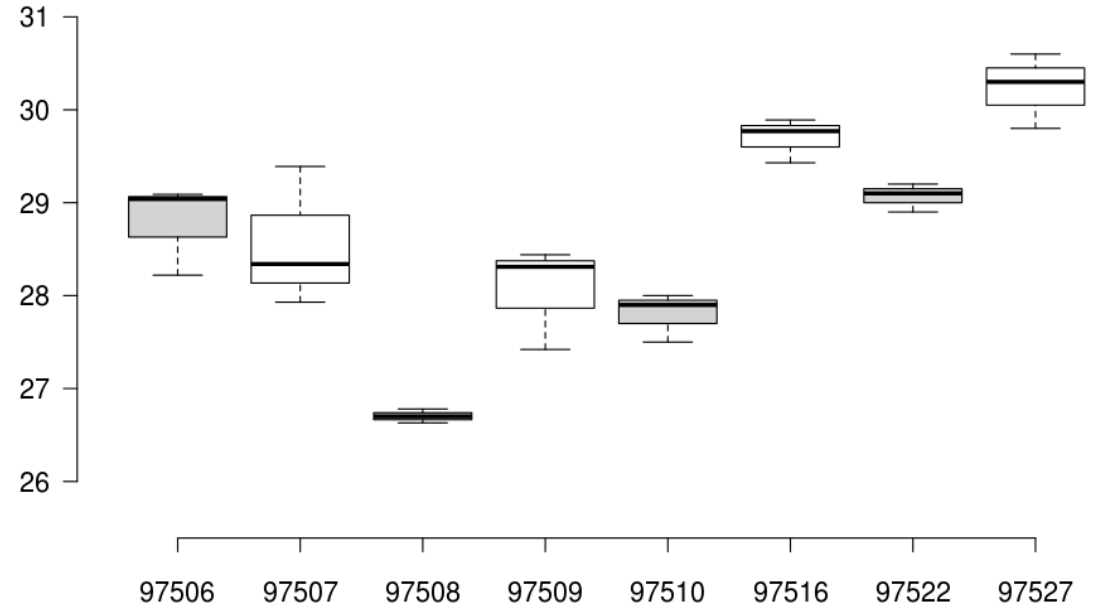

Figure A12. Dispersion of the Ct values (box-plots) per laboratory

6.2.6. Sample PT2021BTVIRPB7

Table A13: Sample PT2021BTVIRPB7

	97506	97507	97508	97509	97510	97516	97522	97527
rep1	36.17	33.18	31.49	33.42	33.7	35.42	34.2	>41
rep2	35.6	34.16	31.18	33.15	31.8	35.05	34.8	>41
average	35.885	33.670	31.335	33.285	32.750	35.235	34.500	ND
SD	0.403	0.693	0.219	0.191	1.344	0.262	0.424	ND
CV	1.1%	2.1%	0.7%	0.6%	4.1%	0.7%	1.2%	ND

repX; repetition number X

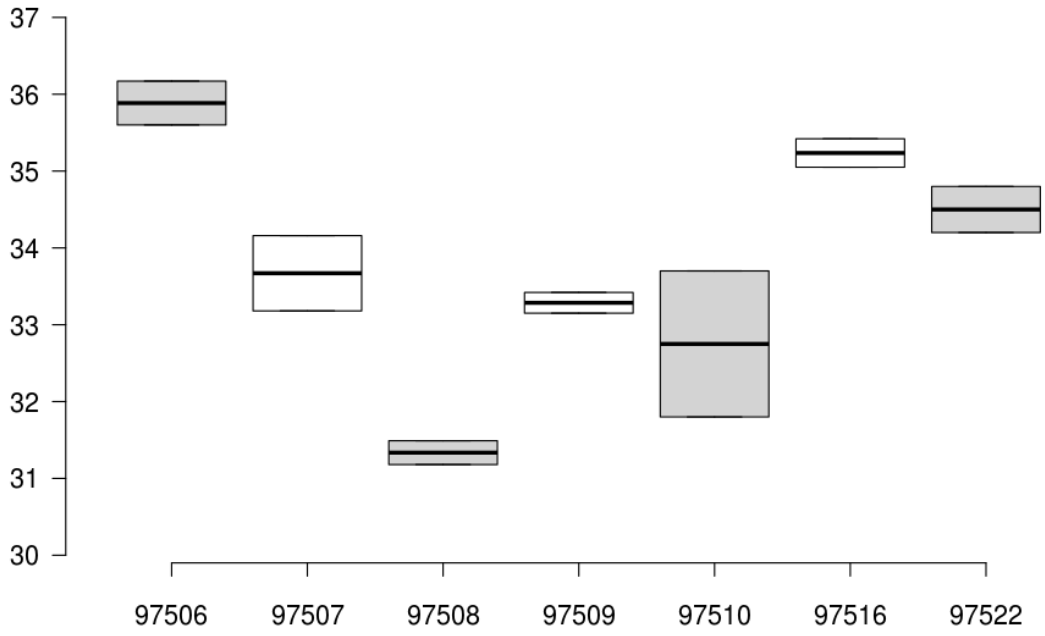

Figure A13. Dispersion of the Ct values (box-plots) per laboratory

VIII. Annex 2 : additional information

PRELIMINARY REPORT

The preliminary report of this survey is available on our website via the following link:

https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

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

The link is:

https://www.wiv-isp.be/QML/activities/external_quality/calendar/calender_PT%20VET/_fr/Calendrier_2021-PT%20VET.htm

END