BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

BRUCELLA (BRU)

PROFICIENCY TEST 2022/9

CORRECTED VERSION

COMMITTEE OF EXPERTS

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A draft version of this report was submitted to the experts on: 14/12/2022.

The changes in the corrected report are indicated in blue. Changes were made on page 12.

This report replaces the previous version of the global report of 18/01/2023.

> **Authorization of the report:** by Ynse Van de Maele, scheme coordinator
>
>
> **Date of publication: 19/01/2023**

All the reports are also available on our webpage:
- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
- FR: https://www.sciensano.be/fr/evaluation-externe-de-la-qualite/sante-animale-pt-vet
- EN: https://www.sciensano.be/en/external-quality-assessment/animal-health-pt-vet

TABLE OF CONTENTS

1 INTRODUCTION

Details relevant to the proficiency test (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 bacteriology:
- The aim of the **serology part** was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies against *Brucella* in tank milk of cattle.
- The aim of the **bacteriology part** was to evaluate the ability of the participating laboratories to identify the presence of *Brucella* spp. in organs.

From now on, the term *Brucella* will be indicated as 'BRU' or in extension.

3 MATERIALS AND METHODS

3.1 Serology (milk)

3.1.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of BRU serology on milk. The names of the participating laboratories are:
- Sciensano, department of Veterinary bacteriology
- ARSIA
- MCC-Vlaanderen
- Laboratoire de Médecine Vétérinaire de l'Etat (LMVE)
- ANSES - Unité Zoonoses bactériennes (UZB) du laboratoire de santé animale

3.1.2 THE SAMPLES

The samples (frozen milk) were prepared by the National Reference Laboratory (NRL), Service of Veterinary bacteriology, Sciensano.

Information about the **origin** and **preparation** of the samples:
- Sample PM1: positive serum from animal number 3667, experimentally infected with *Brucella*, diluted 1/800 in commercial negative milk batch 12I515
- Sample PM2: positive serum from animal number 3467, experimentally infected with *Brucella*, diluted 1/6400 in commercial negative milk batch 12I515
- Sample PM3: positive serum from animal number 3667, experimentally infected with *Brucella*, diluted 1/1000 in commercial negative milk batch WOO1917
- Sample PM4: positive serum from animal number 1275, experimentally infected with *Brucella*, diluted 1/200 in commercial negative milk batch L351
- Sample NM1: commercial negative milk batch WOO1917
- Sample NM2: commercial negative milk batchW150296

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 freeze-dried vials (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%.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of 20 different samples: 14 positive and 6 negative samples.

Sample ID	Repetition	Status
PT2022BRUSERPM1	4	POS
PT2022BRUSERPM2	2	POS
PT2022BRUSERPM3	5	POS
PT2022BRUSERPM4	3	POS
PT2022BRUSERNM1	4	NEG
PT2022BRUSERNM2	2	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: BRUSER	97504	97507	97511	97516	97517
22-1	NM1	PM3	PM3	PM4	NM2
22-2	NM1	PM2	NM1	PM3	NM1
22-3	PM3	NM1	PM1	PM1	PM4
22-4	PM1	NM2	PM4	PM3	PM3
22-5	PM4	PM1	NM2	PM3	PM4
22-6	PM4	PM3	PM4	NM1	PM3
22-7	PM1	PM4	PM3	NM1	PM1
22-8	PM3	NM1	PM1	PM1	PM2
22-9	PM3	PM3	NM1	PM3	PM3
22-10	NM2	PM1	PM3	NM1	NM1
22-11	PM2	PM4	PM3	PM4	PM3

Sample ID: BRUSER	97504	97507	97511	97516	97517
22-12	PM1	PM3	PM2	PM4	NM1
22-13	PM1	PM4	PM3	PM1	PM1
22-14	NM2	PM1	NM1	PM2	PM1
22-15	PM2	PM1	NM2	PM1	NM1
22-16	PM3	PM3	PM1	NM2	PM2
22-17	PM3	PM2	PM4	PM3	NM2
22-18	NM1	NM2	PM2	NM1	PM4
22-19	NM1	NM1	PM1	PM2	PM1
22-20	PM4	NM1	NM1	NM2	PM3

3.2 Bacteriology (organs)

3.2.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of BRU bacteriology on organs. The names of the participating laboratories are:

- Sciensano, Department of Animal Diseases
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- ANSES - Unité Zoonoses bactériennes (UZB) du laboratoire de santé animale

3.2.2 THE SAMPLES

The samples (frozen smashed organs from cattle) were prepared by the National Reference Laboratory (NRL), Service of Veterinary bacteriology, Sciensano.

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

- Sample PO1: 1:2 diluted crushed ganglion matrix + *Brucella abortus* biovar 3
- Sample PO2: 1:2 diluted crushed ganglion matrix + *B. canis*
- Sample PO3: 1:2 diluted crushed ganglion matrix + *B. abortus* bv 3 + *Pseudomonas aeruginosa*
- Sample PO4: 1:2 diluted crushed ganglion matrix + *B. canis* + *Morganella morganii*
- Sample PO5: 1:2 diluted crushed ganglion matrix + *B. abortus* bv 3 + *Ochrobactrum intermedium*
- Sample NO1: 1:2 diluted crushed ganglion matrix
- Sample NO2: 1:2 diluted crushed ganglion matrix + *P. aeruginosa*
- Sample NO3: 1:2 diluted crushed ganglion matrix + *M. morganii*
- Sample NO4: 1:2 diluted crushed ganglion matrix + *O. intermedium*
- Sample NO5: 1:2 diluted crushed ganglion matrix + *O. intermedium* + *P. aeruginosa* + *M. morganii*

Information about the **preparation** of the samples:

- The sample were prepared according to the material production reference file.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 2 aliquots of each sample before the PT. The samples were considered as homogeneous.

3.2.4 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.

Sample ID	Repetition	Status
PT2022BRUPO1	1	POS
PT2022BRUPO2	1	POS
PT2022BRUPO3	1	POS
PT2022BRUPO4	1	POS
PT2022BRUPO5	1	POS
PT2022BRUNO1	1	NEG
PT2022BRUNO2	1	NEG
PT2022BRUNO3	1	NEG
PT2022BRUNO4	1	NEG
PT2022BRUNO5	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 on 3 aliquots of each sample and was conform (performed on 08/11/2022).

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: BRUBAC	97504	97507	97508	97517
22-1	NO5	NO3	NO5	NO1
22-2	PO1	PO5	PO3	NO4
22-3	NO3	NO4	NO2	NO3
22-4	NO2	PO2	NO1	PO3
22-5	PO2	NO5	PO2	PO1
22-6	PO3	PO3	NO4	NO2
22-7	NO1	PO1	PO5	PO2
22-8	PO5	NO1	PO4	NO5
22-9	NO4	PO4	NO3	PO5
22-10	PO4	NO2	PO1	PO4

4 TIMELINE

Transfer of the samples from NRL to QL: 12/09/2022
Randomization of the samples by QL: 13/09/2022
Sending samples to participants: 26/09/2022

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

Deadline for submitting the results: 21/10/2022
Preliminary report: 07/11/2022

5 RESULTS

5.1 Serology (milk)

5.1.1 RESULTS PER SAMPLE

The panel consisted of 6 different samples. Samples PM2 and NM2 were repeated twice. Sample PM4 was repeated three times. Samples PM1 and NM1 were repeated four times. Sample PM3 was repeated five times. Therefore, in total, the panel consisted of 20 samples (14 positive and 6 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
PM1	POS	4 (20)	20 POS
PM2	POS	2 (10)	10 POS
PM3	POS	5 (25)	25 POS
PM4	POS	3 (15)	15 POS
NM1	NEG	4 (20)	20 NEG
NM2	NEG	2 (10)	10 NEG

(POS = positive; NEG = negative)

5.1.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Indirect	IDEXX - Brucellosis Antibody test kit (Tank milk)	Short	5	25	25	100
TOTAL			5	25	25	100

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

5.1.3 CONCLUSION

In 2022, five laboratories participated in proficiency test of *Brucella* serology (milk) organized by Sciensano. The method Brucellosis Antibody test kit (Tank milk) from IDEXX was selected by all the participants for the detection of antibodies against *Brucella* in milk. This method falls under the indirect format.

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 *Brucella* in tank milk.

5.2 Bacteriology (organs)

5.2.1 RESULTS PER SAMPLE

The panel consisted of 10 different samples (5 positive and 5 negative samples). No repetitions of samples were included in this panel.

Sample ID	Status	Number of repetitions (total results)	Observed result
PO1	POS	1 (4)	4 POS
PO2	POS	1 (4)	3 POS 1 NEG
PO3	POS	1 (4)	4 POS
PO4	POS	1 (4)	4 POS 1 NEG
PO5	POS	1 (4)	4 POS
NO1	NEG	1 (4)	4 NEG
NO2	NEG	1 (4)	4 NEG
NO3	NEG	1 (4)	4 NEG
NO4	NEG	1 (4)	4 NEG
NO5	NEG	1 (4)	4 NEG

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Reagens	Batchnummer	N	NR	NCR	%
1) Farell - home made 2) Oxydase - Sigma 3) Reagent Urease - home made 4) Serum for agglutination anti-S - REMEL 5) Serum for agglutination anti-R - ANSES 6) Negative serum for agglutination - ANSES 7) Merck - H_2O_2 30%	1) PHVAN/22/04 2) MKCC4915 3) PAMIC/22/03 4) PAMIC/22/01 5) PAMIC/22/01 6) PAMIC/22/01 7) K54376510222	1	10	10	100
1) Remel - Agglutination serum B. abortus 2) Sérum Agglutination B. melitensis Remel 3) Oxydase Bactident Sigma Aldrich	1) 3324198 2) 3338873 3) HC297883	1	8	10	80
Homemade medium - BRU22/22	/	1	10	10	100
Anses - homemade	/	1	10	10	100
TOTAL		**4**	**38**	**40**	**95**

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

5.2.3 CONCLUSION

In 2022, four laboratories participated in the proficiency test for *Brucella* bacteriology (organs) organized by Sciensano. Different reagents were selected by the participants for the isolation and identification of *Brucella* in organs.

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 samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Three laboratories succeeded in achieving the maximum score (100%) for this test. Only one laboratory had a score of 80% and did not achieve the 90% standard. This can be explained because the methodology performed in this laboratory does not allow to identify *B. ovis* and/or *B. canis* and the two failed samples were spiked with *B. canis*. Therefore, according to their procedures, it is consistent that they cannot detect or identify *B. canis*.

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 SEROLOGY (MILK)

PT2022BRUSERPM1

Lab number	97504	97507	97511	97516	97517
Method		IDEXX - Brucellosis Antibody test kit (Tank milk)			
OD (REP1)	1,31	1,75	1,39	2,77	1,05
OD (REP2)	1,17	1,67	1,47	2,51	1,17
OD (REP3)	1,27	1,62	1,26	2,56	1,17
OD (REP4)	1,18	1,50	1,33	2,44	1,12
Mean	1,23	1,63	1,36	2,57	1,13
SD	0,07	0,11	0,09	0,14	0,06
CV (%)	5,39	6,53	6,57	5,48	4,90

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).
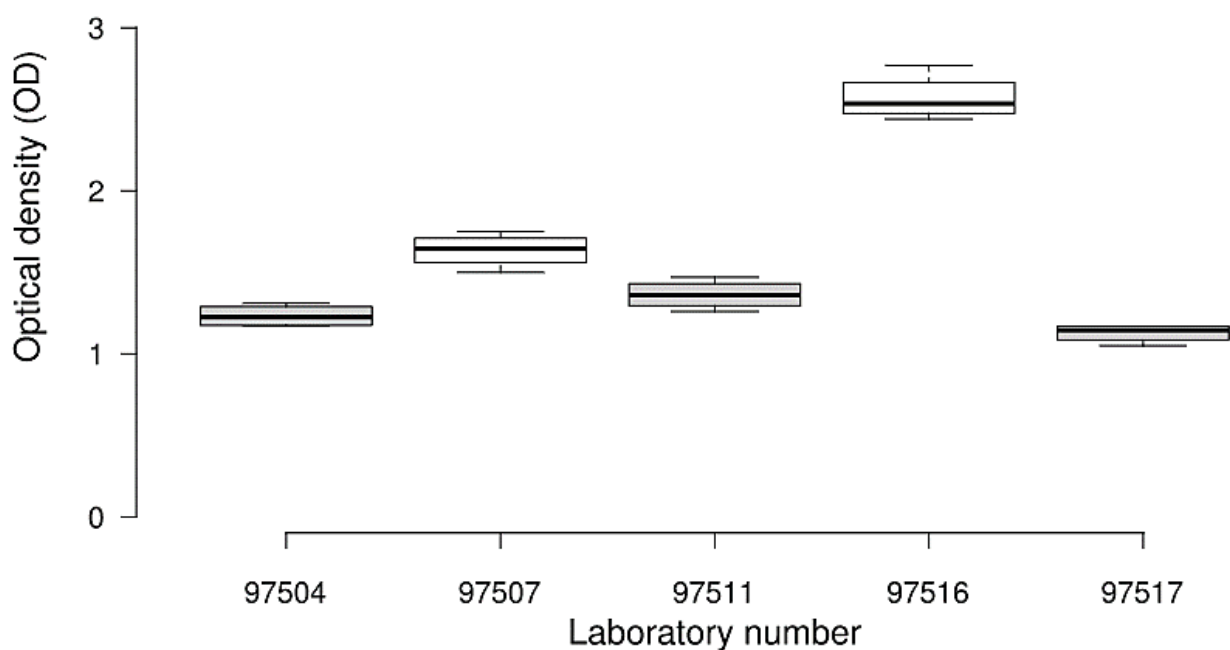

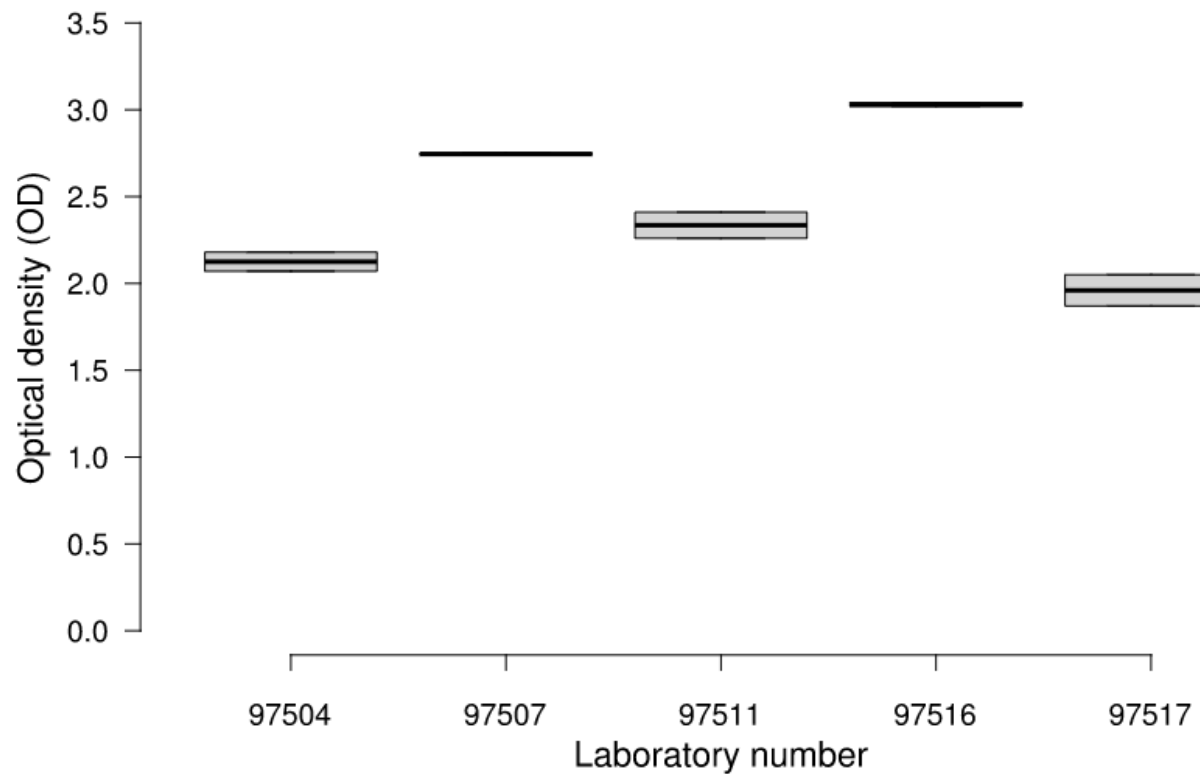*Figure 1. Distribution of the optical densities (box-plots) per laboratory.*

PT2022BRUSERPM2

Lab number	97504	97507	97511	97516	97517
Method	IDEXX - Brucellosis Antibody test kit (Tank milk)				
OD (REP1)	2,07	2,74	2,41	3,02	2,05
OD (REP2)	2,18	2,75	2,26	3,04	1,87
Mean	2,12	2,74	2,33	3,03	1,96
SD	0,08	0,00	0,11	0,02	0,13
CV (%)	3,86	0,08	4,60	0,51	6,56

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

Figure 2. Distribution of the optical densities (box-plots) per laboratory.

PT2022BRUSERPM3

Lab number	97504	97507	97511	97516	97517
Method	IDEXX - Brucellosis Antibody test kit (Tank milk)				
OD (REP1)	1,14	1,87	1,64	2,78	1,25
OD (REP2)	1,15	1,82	1,53	2,64	1,20
OD (REP3)	1,36	1,71	1,40	2,61	1,26
OD (REP4)	1,18	1,64	1,57	2,67	1,16
OD (REP5)	1,24	1,79	1,54	2,79	1,21
Mean	1,21	1,76	1,54	2,70	1,22
SD	0,09	0,09	0,09	0,08	0,04
CV (%)	7,58	5,17	5,64	3,05	3,25

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).
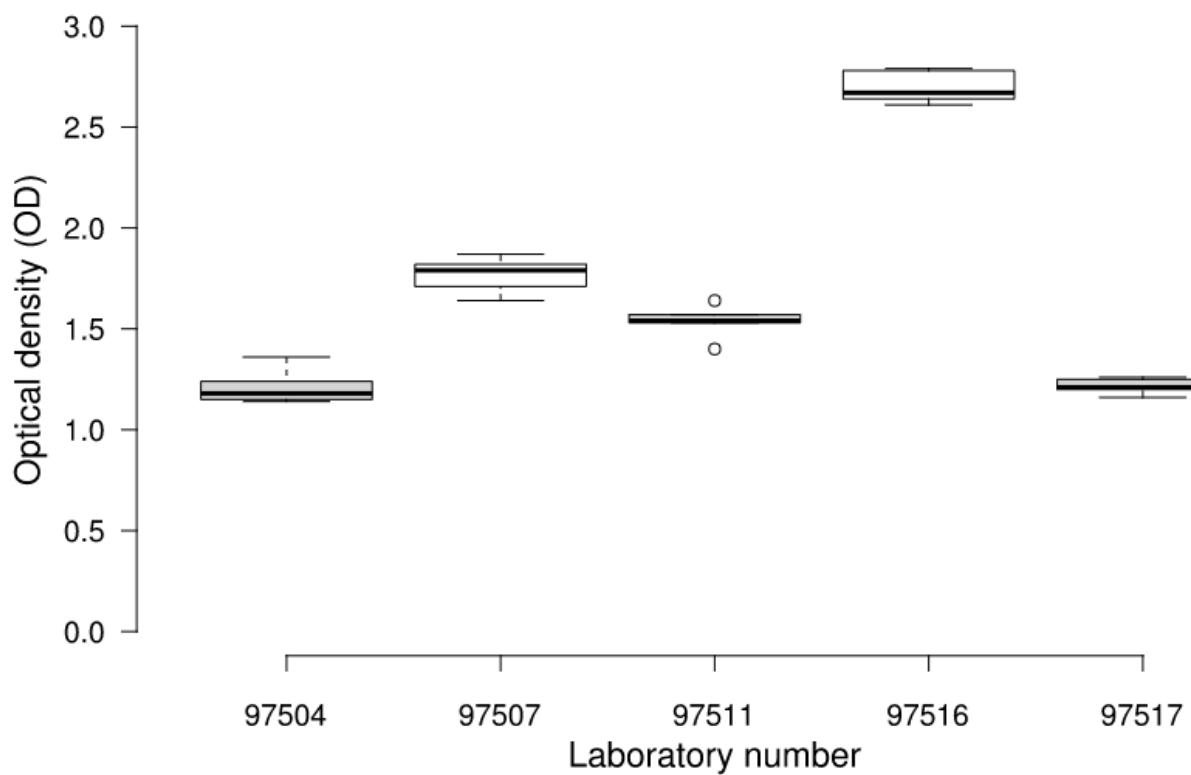

Figure 3. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97511	97516	97517
Method	IDEXX - Brucellosis Antibody test kit (Tank milk)				
OD (REP1)	1,02	1,48	1,22	2,49	0,98
OD (REP2)	0,95	1,48	1,33	2,54	1,03
OD (REP3)	0,99	1,46	1,25	2,45	1,03
Mean	0,99	1,47	1,27	2,49	1,01
SD	0,04	0,01	0,06	0,04	0,03
CV (%)	3,83	0,99	4,54	1,79	3,13

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).
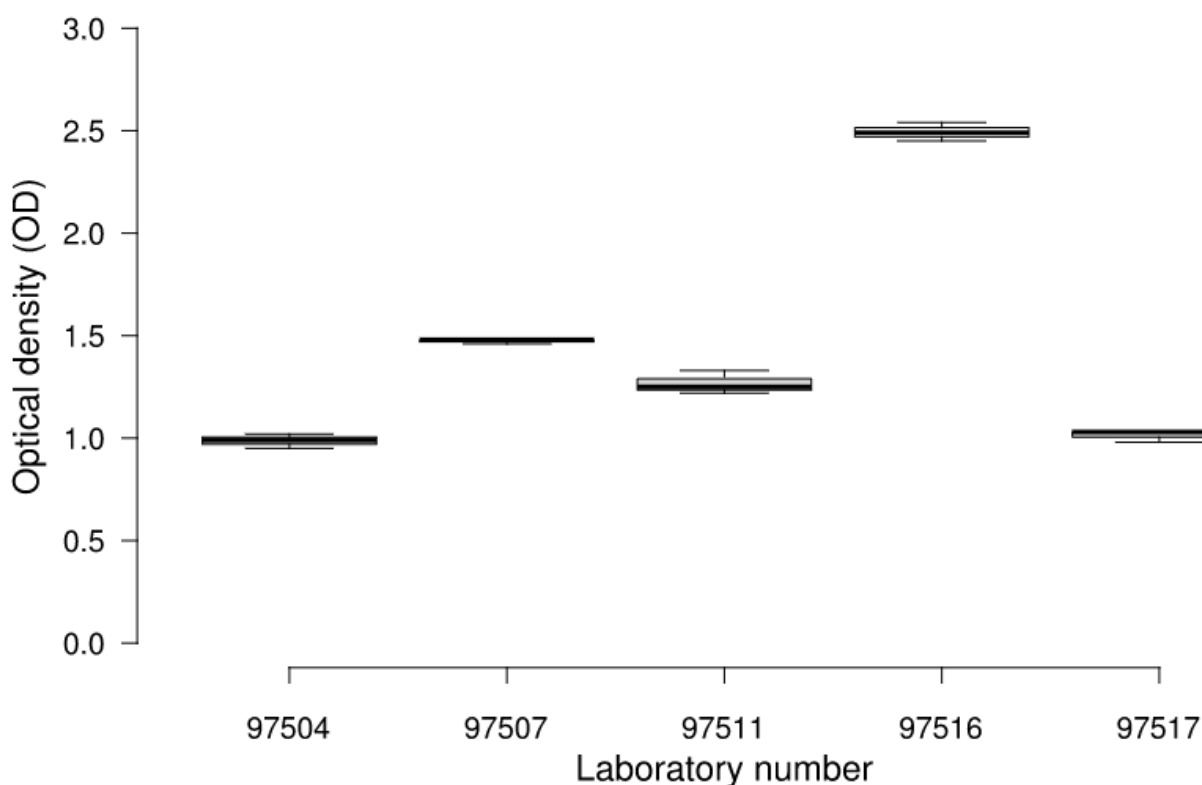

Figure 4. Distribution of the optical densities (box-plots) per laboratory.

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 (**P₂₅**) to percentile 75 (**P₇₅**)
- a central line representing the median of the results (**P₅₀**)
- a lower limit showing the smallest value **x > P₂₅ - 1.5 * (P₇₅ - P₂₅)**
- an upper limit representing the largest value **x < P₇₅ + 1.5 * (P₇₅ - P₂₅)**
- all points outside this interval are represented by a dot.

$O = P_{75} + 3*(P_{75}-P_{25})$

$= P75 + 1.5*(P75-P25)$

LIMITS OF TUKEY

$x < P_{75} + 1.5*(P_{75}-P_{25})$

P_{75}

P_{50}

P_{25}

$= P_{25} - 1.5*(P_{75}-P_{25})$

$x > P_{25} - 1.5*(P_{75}-P_{25})$

value < quantification limit

$O = P_{25} - 3*(P_{75}-P_{25})$

O	I	H	M	H	I	O
M - 4.7s	M - 2.7s	P_{25}		P_{75}	M + 2.7s	M + 4.7s
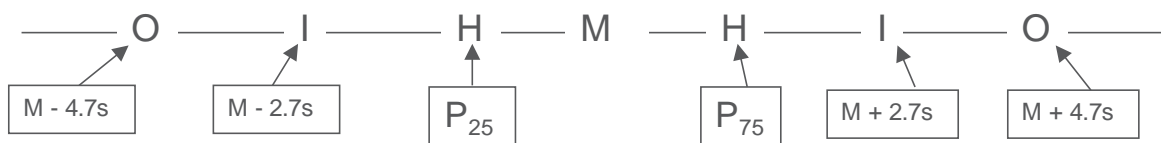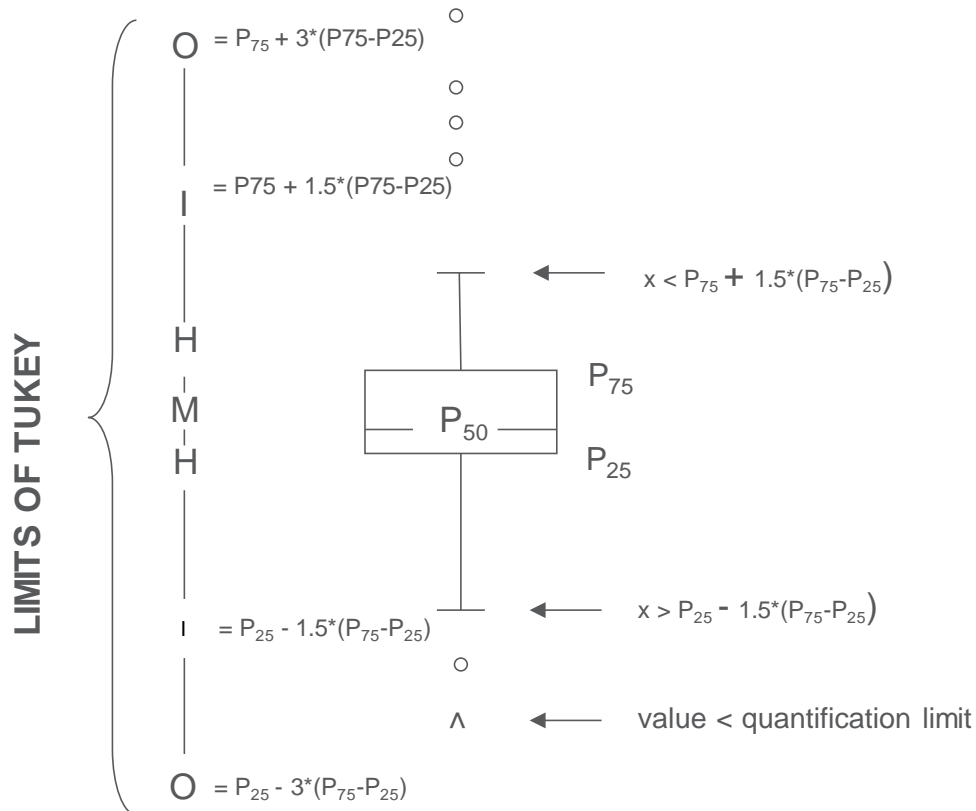
Corresponding limits in case of normal distribution

END